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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

Selenised yeast Saccharomyces cerevisiae Y03-0 inactivated

(FAD-2021-0045; CRL/210029)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2021-0045 - CRL/210029**
Name of Product:	***Selenised yeast Saccharomyces cerevisiae Y03-0 inactivated***
Active Agent:	**Selenium**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**María José González de la Huebra** **12/01/2022**
Report approved by: Date:	**Christoph von Holst** **13/01/2022**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *selenised yeast Saccharomyces cerevisiae Y03-0 inactivated* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* for all animal species.

According to the Applicant, the *feed additive* is intended to be marketed as two solid granulated or micro-granulated preparations containing a minimum of 3000 mg of total *selenium* / kg of the preparation. Each preparation contains a minimum of 98 % (w/w) of organic *selenium* and at least of 63 % (w/w) of total *selenium* in each preparation is in the form of *selenomethionine*.

The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* at a proposed maximum dose of 0.50 mg total *selenium* / kg of complete *feedingstuffs*. The proposed levels are in agreement with several Commission Implementing Regulations, namely (EU) 2020/2117, (EU) 2019/804, (EU) 2015/489 and (EU) No 427/2013 for total *selenium* content from selenised yeast in complete *feedingstuffs*.

For the quantification of *selenomethionine* in the *feed additive* the Applicant proposed the single-laboratory validated and verified method based on high performance liquid chromatography and inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) after triple proteolytic digestion. This method has been previously evaluated and recommended by the EURL in the frame of FAD-2009-0010 for the quantification of *selenomethionine* in selenium enriched yeast. In addition, the EURL identified another single-laboratory validated and verified method based on reversed phase high performance liquid chromatography with UV detection (RP-HPLC-UV), which has been also evaluated and recommended for the quantification of *selenomethionine* in the product in the frame of the above mentioned dossier. Furthermore, the two above mentioned methods were further included in Commission Implementing Regulation (EU) 2015/489 authorising the product of FAD-2009-0010. In addition, these two methods were also included in the Commission Implementing Regulations (EU) 2020/2117 and (EU) 2019/804 authorising other similar selenised yeast products.

Based on the overall available data, the EURL considers the two mentioned methods based on HPLC-ICP-MS and HPLC-UV also suitable for official control for the quantification of *selenomethionne* in the *feed additive* of the current application.

For the quantification of total *selenium* in the *feed additive* the Applicant proposed the EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS). This method was tested for feed samples in the ring-trial validation studies for total *selenium* content

ranging from 0.4 to 13.3 mg / kg feed. However, the Applicant did not submit any data demonstrating the applicability of the method for the product of the current application with much higher content of *selenium* (*ca.* 3000 mg / kg).

Meanwhile, the EURL identified two single-laboratory validated and verified methods based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) and ICP-MS, which have been previously evaluated and recommended for official control in the frame of several dossiers for similar products of selenised yeast and further included in several Commission Implementing Regulations authorising the selenised yeast products.

Based on the overall available data, the EURL considers the two mentioned methods based on ICP-AES and ICP-MS also suitable for official control for the quantification of total *selenium* in the *feed additive* of the current application.

For the quantification of total *selenium* in *premixtures* and *feedingstuffs* the Applicant proposed the EN 16159 method based on hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion with 65 % nitric acid and 30 % hydrogen peroxide.

The following performance characteristics are reported in the frame of ring-trial validation studies for the determination of total *selenium* content in *feedingstuffs* ranging from 0.25 to 73.6 mg / kg: a relative standard deviation for repeatability (RSD_r) ranging from 3.4 to 10.1 %; a relative standard deviation for reproducibility (RSD_R) ranging from 14.9 to 23.2 %; and a limit of quantification of 0.125 mg / kg *feedingstuffs*.

This method has been previously evaluated and recommended by the EURL for official control of total *selenium* content in *premixtures* and *feedingstuffs* in the frame of several dossiers for similar selenised yeast products. The method was further included in several Commission Implementing Regulations authorising the selenised yeast products.

Based on the overall available data, the EURL considers the EN 16159 method also suitable for official control for the quantification of total *selenium* in *premixtures* and *feedingstuffs* in the frame of the current application.

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

Selenised yeast Saccharomyces cerevisiae Y03-0 inactivated, *Selenium*, nutritional additives, compounds of trace elements, all animal species.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new *feed additive*) for *selenised yeast Saccharomyces cerevisiae Y03-0 inactivated* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the authorisation is sought for the use of the *feed additive* for all animal species [2].

According to the Applicant, the *feed additive* is intended to be marketed as two solid granulated or micro-granulated preparations containing a minimum of 3000 mg of total *selenium* / kg of the preparation [3]. Each preparation contains a minimum of 98 % (w/w) of organic *selenium* and at least of 63 % (w/w) of total *selenium* in each preparation is in the form of *selenomethionine* [3]. The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* at a proposed maximum dose of 0.50 mg total *selenium* / kg of complete *feedingstuffs* [4]. The proposed levels are in agreement with several Commission Implementing Regulations for total *selenium* content from selenised yeast in complete *feedingstuffs* [5-8].

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 *Selenised yeast Saccharomyces cerevisiae Y03-0 inactivated* 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)

Selenomethionine

For the quantification of *selenomethionine* in the *feed additive* the Applicant proposed the single-laboratory validated and further verified method based on high performance liquid chromatography and inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) after triple proteolytic digestion [9]. This method has been previously evaluated and recommended

by the EURL in the frame of FAD-2009-0010 for the quantification of *selenomethionine* in selenium enriched yeast [10].

In addition, the EURL identified another single-laboratory validated and verified method based on reversed phase high performance liquid chromatography with UV detection (RP-HPLC-UV), which has been also evaluated and recommended for the quantification of *selenomethionine* in the product in the frame of the above mentioned dossier [10].

Furthermore, the two above mentioned methods were further included in Commission Implementing Regulation (EU) 2015/489 [7] authorising the above mentioned product [10]. In addition, these two methods were also included in the Commission Implementing Regulations (EU) 2020/2117 [5] and (EU) 2019/804 [6] authorising other similar selenised yeast products.

According to the HPLC-ICP-MS method, the sample is digested by mixture of proteolytic enzymes for 16 h at 37 ^{o}C and centrifuged. The digestion of the residue is repeated twice, the supernatants combined, treated with dithiothreitol and further analysed by HPLC-ICP-MS. The quantification was performed using matrix free external calibration with the standard solutions of *selenomethionine* [10].

When applying the RP-HPLC-UV method, the samples are subjected to a mild acid digestion to break the proteins down to free amino acids with minimal destruction of *selenomethionine*. After dilution with an internal standard (D-, L-, alpha-aminobutyric acid) and filtration, a small aliquot is used for derivatisation with phenylisothiocyanate. The phenylthiocarbamate derivatives are analysed by reversed phase high performance liquid chromatography with UV detection (RP-HPLC-UV) at 254 nm. The quantification was performed using standard solutions of *selenomethionine* derivatised with the phenylisothiocyanate [10].

Based on the overall available data, the EURL considers the two mentioned methods based on HPLC-ICP-MS and HPLC-UV also suitable for official control for the quantification of *selenomethionne* in the *feed additive* of the current application.

Selenium

For the quantification of total *selenium* in the *feed additive* the Applicant proposed the EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS) [12]. This method was tested for feed samples in the ring-trial validation studies for total *selenium* content ranging from 0.4 to 13.3 mg / kg feed. However, the Applicant did not submit any data demonstrating the applicability of the method for the product of the current application with much higher content of *selenium* (*ca.* 3000 mg / kg).

Meanwhile, the EURL identified two single-laboratory validated and verified methods based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) and ICP-MS, which

have been previously evaluated and recommended for official control in the frame of several dossiers for similar products of selenised yeast [10,13] and further included in several Commission Implementing Regulations authorising the selenised yeast products [5-7].

Following the ICP-AES method, 1 g of homogenised sample is digested in the mixture (10+10 mL) of concentrated nitric and hydrochloric acids. The solution is heated first at 75 ^{o}C for 10 min, then at 150 ^{o}C for 2 h. The remaining liquid is cooled down to room temperature and quantitatively transferred to a 100 ml volumetric flask with deionised water, to produce 100 ml of clear solution with no suspended particles. *Selenium* is then quantified by ICP-AES using an external standard calibration [10].

According to the ICP-MS method, the sample (0.2 g) is mineralised in microwave closed-vessels in the presence of mixture (3+3 ml) of nitric acid and hydrogen peroxide. The digested solution is further diluted and the total *selenium* content is determined by ICP-MS, using standard addition of inorganic selenium standard together with a ^{103}Rh internal standard [13].

Based on the overall available data, the EURL considers the two mentioned methods based on ICP-AES and ICP-MS also suitable for official control for the quantification of total *selenium* in the *feed additive* of the current application.

For the quantification of total *selenium* in *premixtures* and *feedingstuffs* the Applicant proposed the EN 16159 method based on hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion with 65 % nitric acid and 30 % hydrogen peroxide [14].

The following performance characteristics are reported in the frame of ring-trial validation studies for the determination of total *selenium* content in *feedingstuffs* ranging from 0.25 to 73.6 mg / kg: a relative standard deviation for repeatability (RSD_r) ranging from 3.4 to 10.1 %; a relative standard deviation for reproducibility (RSD_R) ranging from 14.9 to 23.2 %; and a limit of quantification of 0.125 mg / kg *feedingstuffs* [14].

This method has been previously evaluated and recommended by the EURL for official control of total *selenium* content in *premixtures* and *feedingstuffs* in the frame of several dossiers for similar selenised yeast products [10,13]. The method was further included in several Commission Implementing Regulations authorising the selenised yeast products [5-8].

Based on the overall available data, the EURL considers the EN 16159 method also suitable for official control for the quantification of total *selenium* in *premixtures* and *feedingstuffs* in the frame of the current 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.

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:

- the two single-laboratory validated and further verified methods based on reversed phase high performance liquid chromatography with UV detection (RP-HPLC-UV) or high performance liquid chromatography and inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) after triple proteolytic digestion for the determination of *selenomethionine* in the *feed additive*;
- the two single-laboratory validated and further verified methods based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS) for the determination of total *selenium* in the *feed additive*;
- the CEN ring-trial validated method based on hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (EN 16159) for the determination of total *selenium* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *selenomethionine* in the *feed additive*:

- reversed phase high performance liquid chromatography with UV detection (RP-HPLC-UV) or
- high performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICP-MS) after triple proteolytic digestion

For the determination of *total selenium* in the *feed additive*:

- inductively coupled plasma atomic emission spectrometry (ICP-AES) or
- inductively coupled plasma-mass spectrometry (ICP-MS)

For the determination of total *selenium* in *premixtures* and *feedingstuffs:*

- hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (EN 16159)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Selenised yeast Saccharomyces cerevisiae Y03-0 inactivated* 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. 1831-0037-2021

[2] *Application, Annex 1 – Submission Number 1616410655139-2930

[3] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition of the additive

[4] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition

[5] Commission Implementing Regulation (EU) 2020/2117 of 16 December 2020 concerning the renewal of the authorisation of selenomethionine produced by Saccharomyces cerevisiae CNCM I-3399 with the new name 'selenised yeast Saccharomyces cerevisiae CNCM I-3399' as a feed additive for all animal species, and repealing Regulation (EC) No 900/2009, OJ L 426, 17.12.2020

[6] Commission Implementing Regulation (EU) 2019/804 of 17 May 2019 concerning the renewal of the authorisation of organic form of selenium produced by Saccharomyces cerevisiae CNCM I-3060 and of selenomethionine produced by Saccharomyces cerevisiae NCYC R397 as feed additives for all animal species and repealing Regulations (EC) No 1750/2006 and (EC) No 634/2007, OJ L 132, 20.5.2019

[7] Commission Implementing Regulation (EU) 2015/489 of 23 March 2015 concerning the authorisation of selenomethionine produced by Saccharomyces cerevisiae NCYC R645 as a feed additive for all animal species, OJ L 78, 24.3.2015

[8] Commission Implementing Regulation (EU) No 427/2013 of 8 May 2013 concerning the authorisation of selenomethionine produced by Saccharomyces cerevisiae NCYC R646 as a feed additive for all animal species and amending Regulations (EC) No 1750/2006, (EC) No 634/2007 and (EC) No 900/2009 as regards the maximum supplementation with selenised yeast, OJ L 127, 9.5.2013

[9] *Technical dossier, Section II – Annex_II_116

[10] EURL Evaluation Report : FAD-2009-0010 https://ec.europa.eu/jrc/sites/default/files/addendum%20Fad-2009-0010-selenosource_supinfdoss_fad-2014-0004.pdf

[11] Supplementary information – m3-fa-3b817

[12] EN 17053:2018 – *Animal feeding stuffs: Methods of sampling and analysis – Determination of trace elements, heavy metals and other elements in feed by ICP-MS (multi-method)*

[13] EURL Evaluation Report : FAD-2009-0029+FAD-2010-0044 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2009-0029%2B2010-0044.pdf

[14] EN 16159:2012 – *Animal feedingstuffs - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65% nitric acid and 30% hydrogen peroxide)*

*Refers to Dossier no: FAD-2021-0045

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)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 – Labore Landwirtschaft, Nossen (DE)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- [1]Wageningen Food Safety Research (WFSR) (NL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)

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