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FOOD DIAGNOSTICS

NEW RAPID TEST DEVELOPED FOR TOXIC ERGOT ALKALOIDS IN CEREAL GRAINS

Grain processors regularly face the presence of toxic ergot alkaloids (EAs). Europe has new limits since early 2022, but analysis is expensive and time-consuming. The industry therefore needs a faster, cheaper method. Infected batches are then recognised earlier. Thanks to a successful TKI project, led by Wageningen Food Safety Research, this method is on its way.

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Introduction

Ergot alkaloids (EAs) are toxins produced by several species of fungi in the genus *Claviceps* (ergot). In Europe, *Claviceps purpurea* is the most common species and is mainly found in the ears of cereals such as rye, wheat, triticale, barley, millet and oats.

EAs are toxic to humans and animals, they disrupt heart rhythm, blood pressure and temperature regulation. The infection of the plant by fungal spores mainly occurs in the early phase of flowering. Eventually, purple spore heads (sclerotia) appear that can be up to 10 times larger than the grain kernel. The presence of sclerotia in the cereal is therefore already an indication that there may be a food safety risk.

Although sclerotia can be largely removed from the grain by sieving (sclerotia have a lower specific gravity and are different in color than a grain), EAs may still remain in the cereals which is not visually visible. In that case, a lab analysis must determine whether the product is safe for consumption. So far, LC-MS/MS and HPLC-FLD have been the methods of choice for analysing EAs.



Legislation

Since 1 January 2022, new standards have been laid down in European Regulation EU 2021/1399 for both the presence of sclerotia in cereals and the toxins in the cereals or products derived from them. Table 1 gives an overview of the limits. EU 2021/1399 distinguishes between the occurrence of sclerotia and EAs, but also between the different grains and even the different products derived from cereals.

There are a few things that stand out about the new regulation. Rye has a higher EA limit than wheat. The law actually bends along with the practice. Of the grains, rye is most susceptible to *Claviceps* infection and a low EA level is difficult to achieve in rye. The ash content of the flour also influences the limit. This is because the bran, which has a high ash content, tends to absorb the dust from sclerotia and therefore has a higher EA content than the germ/embryo and endosperm contained therein. Concentration of EAs also takes place during the wet processing of wheat grains into a gluten fraction, which is why there is a separate limit for wheat gluten.

Furthermore, the European Commission (EC) expects that the EA content in flour will continue to fall due to process improvement. The EC is already anticipating on this by introducing lower standards in 2024, but it is not inconceivable that the EC will lower the limits even further in the future. The lowest limit applies to infant nutrition. Children and pregnant women appear to be the most sensitive, and children have on average a higher cereal consumption per kg of body weight. Finally, 12 EAs are regulated. This is not without meaning as we shall see later.

Regulation (EU) 2021/1399 has consequences for the monitoring of EAs and the management of contaminated batches of grain by companies. An example: a producer of infant nutrition should not use a batch of wheat with an EA content of more than 20 µg/kg for Brinta whole grain breakfast (limit 20 µg/kg), but perhaps can still use it for self-raising flour (limit 100 µg/kg).

Table 1

Foodstuffs ⁽¹⁾		Maximum Level
2.9	Ergot sclerotia and ergot alkaloids	
2.9.1	Ergot sclerotia	
2.9.1.1	Unprocessed cereals ⁽¹⁸⁾ with the exception of - maize, rye and rice	0,2 g/kg
2.9.1.2	Unprocessed Rye ⁽¹⁸⁾	0,5 g/kg until 30.6.2024 0,2 g/kg as from 1.7.2024
2.9.2	Ergot alkaloids ^(*)	
2.9.2.1	Milling products of barley, wheat, spelt and oats (with an ash content lower than 900mg/100g)	100 µg/kg 50 µg/kg as from 1.7.2024
2.9.2.2	Milling products of barley, wheat, spelt and oats (with an ash content equal or higher than 900mg/100g) Barley, wheat, spelt and oats grains placed on the market for the final consumer	150 µg/kg
2.9.2.3	Rye milling products Rye placed on the market for the final consumer	500µg/kg until 30.6.2024 250 µg/kg as from 1.7.2024
2.9.2.4	Wheat gluten	400 µg/kg
2.9.2.5	Processed cereal based food for infants and young children ^{(3) (29)}	20 µg/kg

(*) The maximum level for ergot alkaloids refers to the lowerbound sum of the following 12 ergot alkaloids: ergocornine/ergocorninine; ergocristine/ergocristinine; ergocryptine/ergocryptinine (α and β-form); ergometrine/ergometrinine; ergosine/ ergosinine; ergotamine/ergotaminine. In the lowerbound sum, the contribution of each non-quantified epimer is set at zero.

In the Netherlands, exceedances of the limit must be reported to the NVWA. Member States are also obliged to report monitoring data to EFSA. The EC wants to monitor whether progress is being made in reducing EAs in cereals, especially rye, so that the EC can further lower the limits in the future. Whether this is feasible remains to be seen. Because of climate change, crops can become waterlogged due to heavy rainfall which can actually exacerbate the fungal problem. The EC therefore wants to maintain an awareness of current trends and the future situation.

New Method for Ergot Alkaloids

It is also crucial for companies to monitor the level of EAs. One of the ways to prevent too much EAs from ending up in the end product is to use a rapid test at the place where grains are stored or processed, or a quick check of ready-to-eat products. Until now, EAs could only be measured with LC-MS/MS or HPLC-FLD. These methods are precise, but also time-consuming and expensive.

In 2019, TKI Agri&food financed a project (LWV19252) that aimed to contribute to the development of a validated ELISA test for EA together with companies. Access to practical samples, especially blank samples with the lowest possible level of EAs, was essential to arrive at a validated ELISA. The Committee of Grain Traders supplied the various grain samples used by WFSR and Eurofins for making QC materials. Radox Food Diagnostics used the QC materials for the first validation of the ELISA they developed. In a second round using field samples, the ELISA test was validated by Radox Food Diagnostics, WFSR and Nutrilab as an independent third party. The collaboration resulted in a useful ELISA test for detecting EA in rye, barley, wheat, oats and spelt.

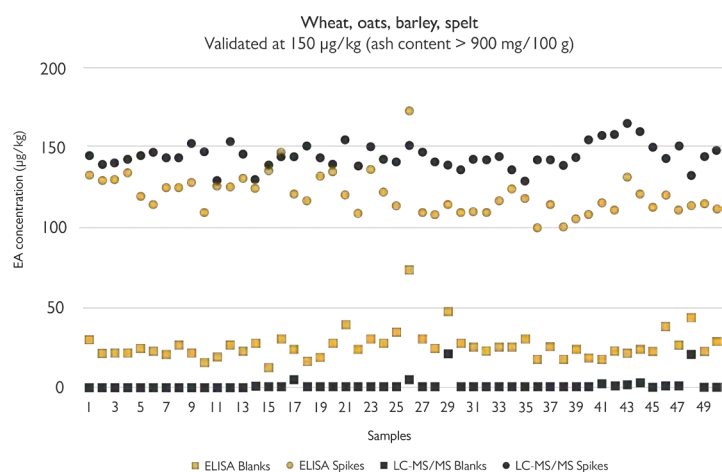
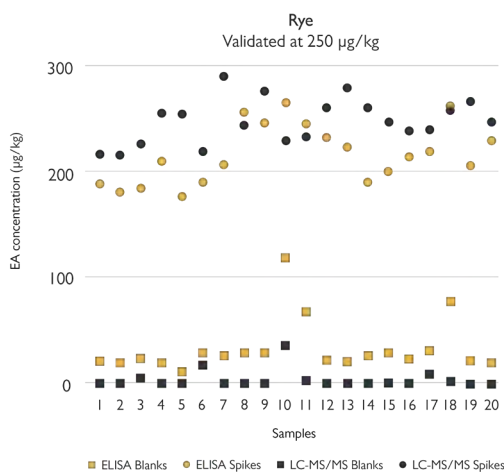
Research Design

The project was divided into two phases. In phase I (2021, see the figure below), the grain traders supplied a total of 70 samples for five different grains. These samples were sanitised by manually removing all sclerotia present. Then the cereals were ground into a powder. EAs were added up to the maximum permitted limit concentrations according to EU 2021/1399. The EA levels were measured with a validated LC-MS/MS method and these data were used for the first validation of the newly developed ELISA. New grain samples were delivered in phase 2 (2022). These samples were ground without cleaning and divided into three portions. Aliquots were analysed independently for the presence of EAs. In this phase, the ELISA was performed by the kit developer (Radox Food Diagnostics) and by an independent laboratory (Nutrilab).



Results

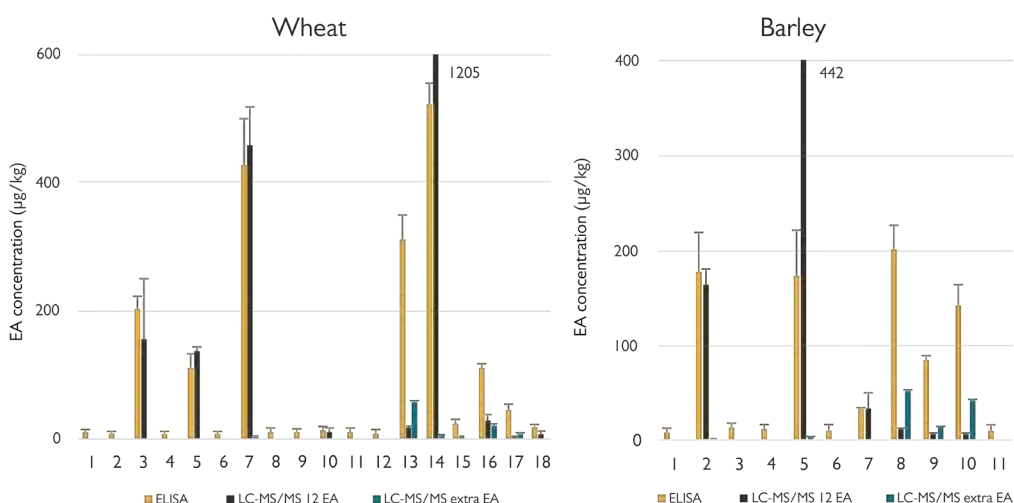
The figure below shows results from phase I. The squares represent the EA content of the cleaned blanks as measured by the LC-MS/MS method and the ELISA. The ELISA gives a slightly higher background than the LC-MS/MS. The dots represent the results of the grain samples spiked with the 12 different EAs in equal amounts to a total of 250 µg/kg for rye or 150 µg/kg for the other four grains. The LC-MS/MS and ELISA results are generally well matched. There are no false negatives, which means that the ELISA can play a valuable role in monitoring the food safety of cereals. The ELISA gives a level higher than 50 µg/kg for four blank samples, apparently a false positive result. But that has to be seen in context. More on that later.



In phase 2, real live samples were measured. For all grains, some samples were found with an EA content higher than the legal limit, both by LC-MS/MS and the ELISA (see, for example, wheat samples 3, 7 and 14 in the figure below). This again illustrates the value of the ELISA, because on the basis of a positive result, the grain trader can, for example, decide to choose a different application for the batch of grain.

Surprisingly, in some samples (wheat 13, 16) the ELISA gives a significantly higher level of EAs than the LC-MS/MS. In contrast to ELISA, LC-MS/MS sees all individual EAs: so in addition to the 12 regulated EAs, also EAs that may be present in the grain, but that are as yet unregulated. The ELISA cannot make that distinction and thus measures the sum of regulated and unregulated EAs. It is striking, and this can be clearly seen from samples 8, 9 and 10 of barley, that the ELISA seems to react more strongly to the presence of unregulated EA. Examples of this are the EAs of the clavine type. The ELISA can therefore more easily give a false positive result for grains containing unregulated EA. In the event of a positive ELISA, the grain trader can still consider having an additional chemical analysis carried out, which may then lead to the conclusion that the grain meets the legal requirements.

In addition, it is important that the grain samples are ground properly and as finely as possible, so that a representative result is obtained. Sclerotia are difficult to grind and distribute homogeneously throughout the test sample.



Summary and Conclusions

- Since 1 January 2022 there are European limits in effect for the presence of EAs in cereal products.
- LC-MS/MS is the most common method for quantitatively measuring EAs. This method is precise, but also time-consuming and expensive. The industry needs a faster, cheaper method, so that contaminated batches can be recognised earlier.
- With the contribution of a consortium of companies led by WFSR, Randox Food Diagnostics has developed an ELISA that is sensitive enough to measure EA around the limit value. The ELISA has been validated for rye, barley, wheat, oats and spelt, and no false negatives were found for the field samples tested.
- The ELISA requires only a simple sample preparation without the need for additional sample clean-up.
- The ELISA seems to be sensitive to some EA that are not regulated, such as the clavine type EA. These EA usually occur in lower concentrations than the twelve regulated ones but can nevertheless lead to false suspect samples.

Acknowledgement

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