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EXPERT SYSTEM FOR DETERMINATION OF FUMONISINS IN CORN SAMPLES

ABSTRACT: An expert system (ES) to solve the problem of choosing an optimal procedure for the determination of fumonisins in corn samples was developed, having in mind that these toxins most frequently contaminate this particular cereal. In constructing the ES use was made of the deterministic approach starting from the assumption that the experts in the field have a profound knowledge about the problem in question. The ES knowledge base contains the solutions that have been published in the pertinent literature, as well as some solutions and recommendations, which we have developed and introduced. On the basis of this information, as well as on the basis of the demanded method detection limit, available equipment, chemicals, as well as the time the experimenter has at his disposal for the determination, the ES proposes a procedure for solving the given analytical problem, starting from sampling, preparation of all the necessary solutions, the appropriate apparatus, probe preparation, the mode of determining results, calculation of the results, and provides a survey of all the relevant literature references. The base of ES is a shell, which can work under a variety of Microsoft Windows operating systems. In the development of ES and its adaptation for users who are not familiar with computer techniques, different tools, which operate with Microsoft Windows operating systems, as well as the tools within Microsoft Office are used.

KEY WORDS: Corn analysis, expert system, fumonisins, mycotoxins

INTRODUCTION

Expert systems (ES) are a branch of applied artificial intelligence (AI) which began to develop by the AI community in the mid-1960s (L i a o, 2005). The basic idea behind ES is simply that expertise, which is the vast body of task-specific knowledge, is transferred from a human to a computer. This knowledge is then stored in the computer as a software package and users call
upon the computer for specific advice as needed. In the field of analytical chemistry a larger number of ESs have already been developed (A br a m o v ić et al., 1996; N a m i e s n i k, 2000; B o n a s t r e et al., 2001; P e r i s, 2002).

Although, the replacement of the human expert by advanced computer-aided systems in chemical analysis laboratories in the food industry has been perhaps slower than in other fields of analytical chemistry (P e r i s, 2002), ESs are straightforward with important advantages, namely:

1. ESs can work 24 hours over the year without stopping, but chemists cannot.
2. ESs can work in difficult working conditions, but chemists cannot.
3. Copies of an ES can easily be made for as many locations as needed, while training new analysts is expensive and time-consuming.
4. ESs do not forget even the large number of parameters, which often have to be taken in account during the analyses, but chemists may.
5. ESs make comparable recommendations for like situations, similar cases being handled in the same way, whereas, humans are influenced by primacy effects (early information dominates the judgment) and contemporary effects (most recent information having a disproportionate impact on judgment).
6. Mistakes and errors can be prevented.
7. In the real-time systems information needed for decision-making is available sooner than from humans and even from different and sometimes very distant locations.
8. The knowledge of multiple human experts and even from different fields of science can be combined to give a system more breadth than a single person is likely to achieve.

However, ESs are still no match for chemists in food analysis in terms of:

1. Sensory experience: Human experts have available to them a wide range of sensory experience, whilst ESs are currently very often dependent only on symbolic input.
2. Degradation: ESs are very often not good at recognizing when no answer exists or when the problem is outside their area of expertise.
3. Learning: Human experts automatically adapt to changing environments and unlike them, ESs must be explicitly updated. ES combined with database, case-based reasoning and neural networks are methods that can incorporate learning.
4. Creativity: Analysts can respond creatively to unusual situations; ESs cannot.

Up to now, no one ES was developed in the field of mycotoxin analysis. In view of this, the aim of the present work was to develop an ES for solving the problem of choosing an analytical procedure for the determination of fumonisins in corn.
BASIC PRINCIPLES IN THE DEVELOPMENT
OF THE EXPERT SYSTEM

In developing the ES the guiding idea was that it has to satisfy several
goals, namely:

1) To be modular and easy to expand;
2) That new information and knowledge can be easily updated in the ES,
without, or with a minimal change in the formal logic system;
3) That the acquisition and preparation of data and procedures can be
conveniently carried out by experts to their final implementation into the ES,
so that the communication between the expert and system engineer is made
easier, as well as that a fast implementation is ensured;
4) That the once developed ES can be easily translated into other languages,
with minimum change of the inference engine and that its updating in that
language should be as simple as possible;
5) That the developed ES and its upgrades easily can be downloaded over
the Internet.

With the aim of fulfilling these requirements we designed the ES with of-
ten use of graphical parameters. The system of formal logic has mostly been
designed to operate with symbolic parameters, which are independent of the
precise content of its graphical presentation. In this way, we were able to de-
velop a system of formal logic, which is intelligible and easy for checking,
modification, or expansion. In the graphical presentation of the parameters a
full freedom is ensured in choosing the text, language, tables, mathematical or
chemical formulas, pictures or drawings.

In designing the knowledge base it was extremely important to carry out
formalization, organization and parameterization of the knowledge base in a
way, which will be user-friendly, and its usage utmost freed of possible ambi-
guities. The inference engine was made so that it ensures the shortest way in
obtaining an answer, which ought to be clear and unambiguous, and provides
the user full information about the procedure to be applied. Also in the de-
veloping of the present ES we have chosen the certain criteria, which then were
used for designing the hierarchical structure and in the making the branching
choice.

In developing of the ES and adapting it to wide variety of the users even
to those, which are not familiar with the computer technique, beside ES shell,
some of Microsoft Office tools and some of the software tools operating with
the Microsoft Windows operating systems were used. The ES was constructed
having constantly in mind that its application should be maximally simplified
and easy to use for specialists in chemistry but also with those who are not.

BASIC CHARACTERISTICS OF FUMONISINS

Fumonisins, secondary metabolites of fungi from the genera Fusarium,
are mycotoxins, of importance to human and animal health (WHO, 2000).
They are the most frequently found as natural contaminants in corn and
corn-based products worldwide (Shepard et al., 1996). Fumonisins have experimentally been shown to be a causative agent of equine leukoencephalomalacia, porcine pulmonary edema syndrome and to produce liver cancer in rats (WHO, 2000). Acute fumonisin toxicity in humans was not confirmed, but the presence of fumonisins in corn was statistically associated with high incidence of esophageal cancer in people from South Africa (Sydenham et al., 1990) and China (Chu and Li, 1994). International Agency of Cancer Research has classified Fusarium moniliforme toxins as potential carcinogens for humans (class 2B carcinogens), similar to ochratoxin A (IARC, 1993). An official tolerance value for dry corn products (1 µg/g) has been issued only in Switzerland, while only recommendations have been issued in the United States and France (Solfrizzo et al., 2001). To date, European Commission has recommended, but not legislated, maximum levels for combinations of fumonisins B₁ and B₂ which range from 2000 µg/kg for unprocessed corn to 100 µg/kg for infant food (EMAN, 2000). The tolerance level for fumonisins in feed and groceries has not yet been set in Serbia and Montenegro.

![Diagram of method for determination of fumonisins]

Fig. 1. Presentation of methods for determination of fumonisins. (IMA — immunoaffinity column; SAX — column with strong anion exchange; C₁₈ — column with C₁₈ reverse phase; ELISA — enzyme-linked immunosorbent assay; LC-ESP-MS — liquid chromatography with electrospray mass spectrometry; TLC — thin-layer chromatography; LC — liquid chromatography; GC — gas chromatography; DL — detection limit)
By their chemical structure, fumonisins are polar organic compounds with a long hydrocarbon chain. According to their structure, there are four series: A, B, C and P. The most attention is devoted to the toxins from the B series, since they are the most toxic. These toxins are diesters of propane-1,2,3-tricarboxylic acid and 2-amino-12,16-dimethylpolyhydroxyeicosanes. Many analytical procedures have been developed for determining fumonisins in corn and corn-based foods and feeds (Jaksic, 2004). Besides the fact that methods for fumonisins B series determination differ in detection limit (Fig. 1), there is a significant difference in the price of analysis, as well as in duration, which was also considered in creating the ES.

**BASIC HIERARCHICAL ORGANIZATION OF THE EXPERT SYSTEM**

To get an impression about the system as a whole and of its parts let’s have a look at the following basic flow-chart. (Fig. 2). First of all, the user obtains some basic information about the ES (Fig. 2, Q2 and Q3) and then, if the user so desires, basic information on fumonisins (production and presence in nature, chemical structure of fumonisins, toxic effects of fumonisins, carcinogenicity, legislative regulations, as well as the detection limits for various methods, Fig. 2, Q5—Q10). This information on fumonisins can be later omitted. Parameter Q11 is introduced to enable simpler spreading, i.e. branching of ES parts, as well as for easier control. After that, the user has to choose with what sensitivity, i.e. detection limit (DL) of the method he wants to determine fumonisins (Fig. 2, Q12). In this ES, methods are divided into four groups according to DL. Liquid chromatography has the lowest DL (0.01 µg/g), followed by capillary zone electrophoresis (0.05 µg/g), gas chromatography and thin-layer chromatography (0.1 µg/g), and enzyme-linked immunosorbent assay (ELISA) and fluorometry (0.25 µg/g). If the user wishes to apply the method with the lowest DL, i.e. liquid chromatography (LC), he should answer the question about the availability of the equipment. If the answer is affirmative (Fig. 2, Q13, 1), the next question is whether cartridges are available (immunoaffinity column, column with strong anion exchange, and/or C_{18} reverse phase), or not (Fig. 2, Q15). If the answer is affirmative, seven options are available, depending on whether the user has only one column at disposal (Fig. 2, Q16, 1—3), two columns (any two) (Fig. 2, Q16, 4—6) or all three columns (Fig. 2, Q16, 7). If only one column is at disposal, ES gives him an appropriate procedure (choices 2—4). However, if the ES user has more than one cartridge at his disposal, a more appropriate procedure is suggested, providing a chromatogram with fewer interfering peaks. If, however, the user does not have cartridges at his disposal (Fig. 2, Q15, 2), individual cartridges are then suggested within the HELP, along with additional information about their advantages, i.e. disadvantages (Fig. 2, Q22—Q24).

As a result of the consultation, the user gets basic information about the technique (in this case about LC), along with a detailed determination procedure, starting from sampling, necessary chemicals as well as preparation of all
Fig. 2. Flow chart of one part of ES for determination of fumonisins
APARATURA

Immunoafinitetna kolona (FumoniTest™, Vicam, Watertown, MA, USA). Po deklaraciji kolona je za jednokratnu upotrebu, a kapacitet kolone je ≥ 10 µg fumonizina B₁ i B₂. Međutim, prema našim istraživanjima kolona se može primeniti 10 puta nakon regeneracije kako je opisano kasnije.

Izgled FumoniTest™ kolone i faze prečišćavanja ekstrakta pomoću IMA-kolona.

ANALITIČKI POSTUPAK (nastavak)

Kalibraciona kriva

Pripremi se kalibraciona kriva pomoću serije standardnih rastvora. Ovi rastvori obuhvataju opseg koncentracija od 0,125 – 2,000 µg/g FB₁ i 0,0625 – 1,000 µg/g FB₂. Kalibracionu krivu treba snimiti pre LC analize i proveriti linearnost.

Kalibracione krive za određivanje FB₁ i FB₂

Kalibracione krive, koeficijenti korelacije i rezidualne standardne devijacije za određivanje FB₁ i FB₂

<table>
<thead>
<tr>
<th>Mikotoksin</th>
<th>Opseg koncentracija (ng/µl)</th>
<th>Kalibraciona kriva</th>
<th>Koeficijent korelacije</th>
<th>Rezidualna standardna devijacija</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB₁</td>
<td>0,125 – 2,000</td>
<td>ε = 11,64·10⁵·c - 0,57·10⁵</td>
<td>0,999</td>
<td>0,37</td>
</tr>
<tr>
<td>FB₂</td>
<td>0,0625 – 1,000</td>
<td>ε = 8,30·10⁴·c - 0,29·10⁵</td>
<td>0,999</td>
<td>0,18</td>
</tr>
</tbody>
</table>

Linearnost je data prema jednačini: ε = m·x + b (m = nagib, b = odsečak na y-osi).
Linearnost za FB₁ i FB₂ je određena kalibracijom pomoću pet tačaka.

Fig. 3. Graphic presentation of a few parts of the results of consultation
RULES:
------------------------------------------

RULE NUMBER: 1
IF:
   Ekran 2 OK
   AND Ekran 3 OK
   AND Da li zelite dodatne informacije o fumonizinima? da
   AND info01 OK
   AND info02 OK
   AND info03 OK
   AND info04 OK
   AND info05 OK
   AND info06 OK
THEN:
   A1 1

RULE NUMBER: 5
IF:
   A1 1
   AND Odaberite granicu detekcije metode kojom zelite da detektujete i odredite sadržaj fumonizina?
   = 0.01
   AND Za ovo određivanje Vam je potreban tečni hromatograf sa fluorescentnim detektom. Da li raspolazete sa potrebnom opremom?
   DA
THEN:
   A2 1
ELSE:
   A2 0
------------------------------------------

RULE NUMBER: 8
IF:
   A2 1
   OR: A16 1
   AND Za preciscavanje sirovog ekstrakta kukuruza mogu se primeniti IMA, SAX i RP C18 kolone. Da li raspolazete nekom od njih?
   Da
   AND Kojom od navedenih kolona raspolazete? IMA AND SAX AND RP C18
   AND Da li zelite da primenite kolonu IMA?
   Da
THEN:
   Postupak Choice02 - Confidence=10/10
   and REPORT(IMA01.out)
   and REPORT(IMA02.out)
   and REPORT(IMA03.out)
   and REPORT(IMA04.out)
   and REPORT(IMA05.out)
   and REPORT(IMA06.out)
   and REPORT(IMA07.out)
   and REPORT(IMA08.out)
   and REPORT(IMA09.out)
------------------------------------------

Fig. 4. Few rules for defining one of the consultation results
the necessary solutions, the appropriate apparatus, probe preparation, the mode of determining results, calculation of the results, and provides a survey of all the relevant literature references. Some of the screens are presented in Fig. 3 for the sake of illustration, and the way of coming to one such solution, i.e. rules which ensure it are presented in Fig. 4.

If, however, an LC is not available to the user, he should define whether a higher DL is acceptable for his determination of fumonisins in corn (Fig. 2, Q14). If the answer is YES (number 1), then branching is provided toward branch B. If, however, the answer is NO (number 2), the user gets the information that by using the other technique the desired DL cannot be achieved and that the consultation is ending (Fig. 2, choice 1).

If the user defined a higher DL at the beginning of the job, the choice of methods is certainly higher (Fig. 2, branching toward branches B—D). For example, if the DL of 0.25 μg/g is acceptable, the user can apply, aside from LC, capillary zone electrophoresis, gas chromatography, thin-layer chromatography, as well as ELISA and fluorometry. To narrow down the choice, a possibility of choosing a method is offered to the user on the basis of analysis cost, as well as its duration (not shown in Fig. 2).

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ЕКСПЕРТНЫЙ СИСТЕМ ЗА ОДРЕЖИВАНИЕ ФУМОНИЗИНА
У КУКУРУЗУ

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Резиме

Експертини системи се последњих петнаестак година примењују у све већем обиму у различитим областима људске делатности као изванредно средство за решавање комплексних проблема који захтевају знање и искуство одговарајућих експерата. Једна од тих области је и хемијска анализа у којој је већ развијен значајан број експертних система.

Циљ овог рада је био да се реализује експертни систем за решавање проблема избора оптималног поступка одређивања фумонизина. У првој фази развоја експертног система определили смо се за одређивање фумонизина у кукурузу имајући у виду да ови токсини најчешће контаминирају управо ову житарицу. При изради експертног система прихваћен је детерминистички приступ који пох за претпоставке добор познавања наведене проблематике од стране експерата из дате области. Системом су обухваћена решења која су досада објављена у литератури за наведену област, као и наша искуства до којих смо дошли током испитивања појединих метода. На основу тих информација, као и на основу захтевање границе детекције методе, расположиве опреме, хемикалија, као и времена које експериментатор има на располагању за одређивање, експертни систем
даје предлог оптималног поступка одређивања фумонизина у кукурузу почевши од узимања узорка за анализу, припремања свих потребних раствора, поступка одређивања, израчунавања резултата, па до прегледа релевантне литература. Основа експертног система је љуска која може да ради под разним верзијама Windows оперативног система. У његовом потпуном уобличавању и прилагођавању корисницима који рачунарску технику не познају долоично коришћен је алат обухваћен унутар Microsoft Windows и Microsoft Office. При изради експертног система водило се рачуна да његова примена буде максимално поједностављена за кориснике.