Vitamins: Too Much or Not Enough?

With so much choice, which method should I choose: ELISA, HPLC or Microbiological Assay?

Introduction
An increasing number of food products are now being enriched with vitamins. The vitamin content of these foods is monitored by manufacturers as well as by private laboratories and control authorities in order to comply with European Community Directives 2002/46/EC and 90/496/EEC, which regulate the use and labelling of nutritional supplements in food products.

Vitamin analysis
Microbiological assays, ELISA systems and HPLC techniques can all be used for vitamin determination. ELISA tests are excellent systems for process control since they provide test results within 3 hours. Immunoaffinity columns are often used in conjunction with HPLC for analysis of more difficult and coloured food matrices, providing sample purification, which helps to reduce matrix effects and improve sensitivity.

Novel microbiological assays for vitamins in test kit format (VitaFast®) have been marketed by R-Biopharm since January 2006. VitaFast® kits can be used in place of traditional microbiological assays and have a much shorter hands-on time. The microorganisms used with VitaFast® do not have to be cultivated and stored; furthermore, each test kit comes ready-to-use medium and standard reagents. Further information on the different test systems are summarized in Table 1.

Test kits give the user the advantage of performing quality control tests with official reference standards whilst ensuring the high analytical performance of the system. The standards required to run the tests are included in the kit.

Microbiological test systems
VitaFast® test kits provide a fast and easy method of determining the content of vitamins in food products, animal feed and pharmaceutical products. The microtitre plates for these microbiological assays are coated with specific microorganisms, which grow according to the presence/absence of vitamins in the sample. After placing sample, standard and assay medium into the wells, the plates are incubated and subsequently evaluated using a microtitre plate reader. The use of a clean bench is recommended. Unlike traditional microbiological assays, all VitaFast® kit reagents are ready for use. The entire assay can be run using single unit dose materials, thus minimising the risk of contamination during testing.

The microtitre plate format allows a high degree of automation, thus achieving a significant reduction of labour while increasing precision.

Table 1: Summary of techniques for vitamin analysis

<table>
<thead>
<tr>
<th>ELISA</th>
<th>HPLC</th>
<th>Traditional Microbiology</th>
<th>VitaFast®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underlying principle</td>
<td>Antigen-antibody reaction</td>
<td>Purification with immunoaffinity columns containing vitamin specific antibodies and detection by HPLC</td>
<td>Measurement of metabolic activity of microorganisms</td>
</tr>
<tr>
<td>Equipment required</td>
<td>ELISA photometer</td>
<td>HPLC system and accessories</td>
<td>Microbiological lab, clean bench, photometer</td>
</tr>
<tr>
<td>Personnel costs</td>
<td>Low</td>
<td>High</td>
<td>High: Time-consuming culture and storage of microorganisms (MO) and MO suspensions</td>
</tr>
<tr>
<td>Test duration</td>
<td>3 hours</td>
<td>2 hours</td>
<td>5 days</td>
</tr>
<tr>
<td>Recommended use</td>
<td>Production control of internally validated matrices and vitamin-enriched foods</td>
<td>Universal method for analysis of added vitamins and/or natural vitamins in difficult or coloured food</td>
<td>Universal method</td>
</tr>
</tbody>
</table>
Some individuals develop potentially life-threatening allergic reactions after consuming even trace amounts of certain foods, such as peanut, hazelnut, egg, and gliadin/gluten. The risk of a food allergy sufferer consuming a trigger substance increases significantly when food products contain hidden allergens. The fact that food products can be unintentionally contaminated with food allergens during the manufacturing or storage process is of particular concern.

In accordance with Directive 2003/89/EC (amending Directive 2000/13/EC) on the indication of ingredients in food, food manufacturers have been required to specify ingredients that may trigger food allergies on the product label as of 25 Nov. 2005. The new directive dispenses with the so-called “25 % rule”. According to the new ingredient listing rules, compound ingredients that make up more than 2 % of the finished product must be listed individually on the label.

In addition, the new regulations require that potential allergens such as gluten/gliadin, egg, peanut, hazelnut, almond, soya, sulfite, milk and lactose be listed on the product label. Until now, no limits for allergens have been established, with the exception of sulfite (10 mg/kg).

Listing all ingredients on the label is a measure designed to provide transparency and increase consumer confidence.

In order to fulfill their obligations under the new food labelling regulations, food manufacturers and regulatory agencies must have analytical techniques capable of detecting these allergens.

The many advantages of VitaFast® test kits include:

- Ready-to-use reagents and standards for 96 determinations, including standards (three runs possible)
- Method in conformity with official guidelines (Section 64 of the German Food & Feed Act (LFGB), AOAC, etc.)
- High degree of accuracy and precision (CV < 10 %)
- Single unit dose materials can be used
- Uniform test procedure for entire VitaFast® product line
- Results available within 24 to 48 hours

Test systems for vitamin analysis supplied by R-Biopharm:

- Microtitre plate-based microbiological assays
  - P1001 - VitaFast® Folsäure (Folic Acid)
  - P1002 - VitaFast® Vitamin B12 (Cyanocobalamin)
  - P1003 - VitaFast® Vitamin B6 (Biotin)
  - P1004 - VitaFast® Vitamin B1 (Niacin)
  - P1005 - VitaFast® Pantothenic Acid
  - P1006 - VitaFast® Vitamin B7 (Thiamine)
  - P1007 - VitaFast® Vitamin B12 (Riboflavin)
  - P1008 - VitaFast® Vitamin B8 (Pyridoxine)
- ELISAs
  - R2002 - RIDASCREEN®FAST Folic Acid
  - R2201 - RIDASCREEN® Biotin
  - R2012 - RIDASCREEN® FAST Vitamin B12
- Immunoaffinity column for HPLC
  - RBRP808 - Easi Extract™ Vitamin B12
- Enzymatic test systems
  - 10409677035 - L-Ascorbic Acid

### Allergens / Labelling Regulations and Analysis

Table 2: Allergen tests supplied by R-Biopharm (including catalog numbers)

<table>
<thead>
<tr>
<th>ELISA Kits</th>
<th>Test Strips</th>
<th>PCR-ELISAs</th>
<th>real-time PCRs</th>
<th>Enzymatic Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliadin (Gluten)</td>
<td>R/7001</td>
<td>R7002</td>
<td>S3106</td>
<td></td>
</tr>
<tr>
<td>FAST Gliadin</td>
<td>R6401</td>
<td>R6201</td>
<td>S3003</td>
<td>S3103</td>
</tr>
<tr>
<td>RIDA®QUICK Gliadin</td>
<td>R6202</td>
<td>R6901</td>
<td>S3004</td>
<td>S3104</td>
</tr>
<tr>
<td>FAST Almond</td>
<td>R6802</td>
<td>R3002</td>
<td>S3102</td>
<td>S3102</td>
</tr>
<tr>
<td>FAST Hazelnut</td>
<td>R4901</td>
<td></td>
<td>S3107</td>
<td></td>
</tr>
<tr>
<td>Walnut</td>
<td>R1604</td>
<td>R1603</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-Lactoglobulin</td>
<td></td>
<td></td>
<td></td>
<td>10176303035</td>
</tr>
<tr>
<td>Lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Histamine</td>
<td></td>
<td></td>
<td></td>
<td>10176303035</td>
</tr>
<tr>
<td>RIDA®QUICK Histamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celery</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Soya</td>
<td></td>
<td></td>
<td></td>
<td>10273845035</td>
</tr>
<tr>
<td>Sesame</td>
<td></td>
<td></td>
<td></td>
<td>10139092035</td>
</tr>
<tr>
<td>Mustard</td>
<td></td>
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<td></td>
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<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfite</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Glutamic acid</td>
<td></td>
<td></td>
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</tbody>
</table>

The new directive dispenses with the so-called “25 % rule”. According to the new ingredient listing rules, compound ingredients that make up more than 2 % of the finished product must be listed individually on the label.

Immunological analysis techniques such as ELISA are very effective tools for determination of allergens. PCR and enzymatic test systems for vitamin analysis supplied by R-Biopharm include:

- Microtitre plate-based microbiological assays
- ELISAs
- Immunoaffinity column for HPLC
- Enzymatic test systems

Test Strips and PCR-ELISAs are available. For a complete list of allergens and labelling regulations, see Table 2.
systems are also used. The allergen tests marketed by R-Biopharm are user-friendly and internationally proven:

- RIDASCREEN® FAST Peanut: AOAC-RI approval
- RIDASCREEN® Peanut: Section 64 of the German Food & Feed Act (LFGB)
- RIDASCREEN® Gliadin: International ring test by Prolamin Working Group
- RIDASCREEN® FAST Hazelnut: Section 64 LFGB (in progress)

Validation reports for some RIDASCREEN® allergen tests (Gliadin, Peanut, Almond, FAST Hazelnut and Egg / Egg Protein) are available on request.

RIDASCREEN® β-Lactoglobulin – New Applications

In response to increased customer demand, R-Biopharm extended the applications for its β-Lactoglobulin test in the Fourth Quarter of 2005.

Current legislation requires that all food-stuffs containing more than 0.9 % GMOs be labelled accordingly. To comply with these rules, highly sensitive methods such as PCR are needed to ensure that GMOs are reliably detected even in highly processed food products. The responsible testing authorities, institutes and private laboratories are therefore in need of simple yet sensitive and reliable test systems.

SureFood® is a modular test system with which sample preparation and determination methods can be variably combined depending on the specific sample characteristics and available laboratory facilities.

SureFood®: Complete modular test system for determination of genetically modified organisms (GMOs)

1. DNA preparation
SureFood® PREP-Plant is used to extract and purify DNA from raw materials and food-stuffs of mainly plant origin.

2. Amplification
SureFood® GMO ensures sensitive and highly specific amplification of the target DNA sequence

3. Detection
- SureFood® GMO ensures the reliable detection of amplification products through the use of DNA hybridization probes
- Highly purified DNA is prepared rapidly without organic solvents
- Easy-to-use column technology ensures high DNA yields
- High sensitivity and specificity of detection
- Specific detection of RoundUp Ready Soya, Bt176 Maize, Bt11 Maize, T25 Maize, MON 810 Maize, 35 S Maize, and LibertyLink Canola plus screening for the 35S promoter and NOS terminator
- Modules for detection of other GMOs available on request
- Qualitative analysis of amplification using DNA hybridization probes with subsequent optical detection on microtitre plates (DNA-ELISA)
- Quantitative analysis of GMO content using hybridization probes and “real-time” technology

If you are interested in our products, please contact your local distributor for more information.

RIDASCREEN® β-Lactoglobulin, a competitive immunoassay in a 96-well microtitre plate format, is calibrated with native β-lactoglobulin. The test can be used for determination of native and processed forms of the protein and its fragments (partial denaturation of native β-lactoglobulin occurs when milk is heated).

Thus, it is now possible to perform qualitative analyses of β-lactoglobulin in beverages and foods such as yoghurt, biscuits, sausages, etc. in addition to the usual quantitative determinations of β-lactoglobulin in hypoallergenic food products, especially powdered milk.

In both cases (quantitative and qualitative analysis), samples must be homogenized and extracted. The preparation time required for 10 samples is around 30 minutes. For quantitative testing, the recovery rate in powdered milk is approx. 130 % and the detection limit 5 mg/kg (ppm). In qualitative analyses, the results are ranked < 0.2 ppm or > 0.2 ppm β-lactoglobulin (equivalent to 2 ppm total milk protein).
Workshop Dates in Austria:

<table>
<thead>
<tr>
<th>Workshop &quot;Mycotoxin&quot;</th>
<th>Time</th>
<th>Fee in €</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Linz, AGES CC Cluster-Chemie Company Together with AGES (Austrian Agency for Health and Food Safety) and the International Association for Cereal Science and Technology (ICC)</td>
<td>Day 1 (theory) 05. – 06. April, 2006 8:45 a.m. – 4:30 p.m.</td>
<td>150.–</td>
</tr>
<tr>
<td></td>
<td>Day 1 &amp; Tag 2 05. – 06. April, 2006 8:45 a.m. – 4:30 p.m.</td>
<td>300.–</td>
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<tr>
<td></td>
<td>Day 2: (practice) 9:00 a.m. – 4:30 p.m.</td>
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</table>

Enzymatic BioAnalysis Workshop

| Venue: Seminarzentrum Grambach Teslastrasse 2, A-8074 Grambach/Steiermark Held in collaboration with ANALYTICUM Laboratory for Food Testing – University Professor Dr. Werner Pfannhauser GmbH, the GÖCH Task Force on Food Chemistry and Technology, and Technical University of Graz Institute of Food Chemistry and Technology | 22. June, 2006 9:00 a.m. – 5:00 p.m. | 150.– |

For further information or to register for the workshops in Austria, please contact:
Ms. Christine M. Gutschelhofer, Tel: +43 (0) 664 135 21 22, Fax: +43 (0) 1 / 768 80 57 or E-mail: c.gutschelhofer@r-biopharm.de

Trade Fairs and Meetings

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<thead>
<tr>
<th>Date</th>
<th>Event</th>
<th>Time and Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>04. – 06.05.2006</td>
<td>INGESAA 2006 International Grain Trading Workshop In Salzburg, Austria WFI (Trade Promotion Institute) of the Salzburg Chamber of Commerce Representative: R-Biopharm AG</td>
<td></td>
</tr>
<tr>
<td>16. – 18.05.2006</td>
<td>VIV Europe Meat Safety (from Feed to Meat) In Utrecht, The Netherlands Representative: R-Biopharm AG</td>
<td></td>
</tr>
<tr>
<td>16. – 19.05.2006</td>
<td>Symposium on Hormone and Veterinary Drug Residue Analysis In Antwerp, The Netherlands Representative: R-Biopharm AG</td>
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The next R-Biopharm news will be published during the II. quarter 2006

R-Biopharm news is edited by

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