Introduction

As the global population continues to rise, commensurate food production will dramatically alter the food industry. Under the current paradigm, which relies heavily on industrial farming, a greater food demand will require more industrial farming output and continue to exacerbate our environmental footprint with increased greenhouse gas formation. Therefore, new strategies must be implemented to satisfy rising food production demand, while simultaneously reducing its environmental impact.

Sustainable food sources will no doubt be fundamental in solving this global food production crisis. The top considerations when selecting new food sources include excellent flavor profiles, wide availability, sustainable sourcing, low cost, and optimal nutrient compositions. In this whitepaper, we will discuss sustainable food sources that meet these points and the key analytical innovations needed to bring them to market.

The Global Food Production Crisis

According to the United Nations, world population is projected to reach 9.8 billion by 2050 and 11.2 billion by 2100.\(^1\) It is intuitive to assume that to feed this anticipated growth in population, governments around the world will need to preemptively increase their food resources to satisfy growing demand.

However, the issue arises when one looks at the current food production paradigm and realizes that agriculture, specifically industrial farming, is responsible for one of the largest environmental burdens through the release of greenhouse gases, i.e., methane. Agricultural commodities used for feed require significant water supplies, and as water resources become scarcer in certain parts of the globe, these feed commodities, if not already, will become environmentally overbearing. Additionally, this reliance on livestock as a key food product is forcing more feed commodities into development, which in turn is removing significant regions of rainforest and other carbon sink ecosystems, driving global warming even further. Thus, the global food production crisis will require an alternative method outside of what our current food production paradigm offers.\(^1\)
The Sustainable Food Solutions

As industrial livestock operations continue to demonstrate negative outcomes for the environment, sustainable food sources must be established in order to meet growing food demands. In doing so, food manufacturers around the globe are considering a variety of protein sources that are:

- Environmentally sustainable
- Have optimal nutrient composition
- Cultivated in a socially responsible way

Currently, there are several promising sustainable food sources derived from insects, cultured meat, fungi, algae and plants.

Insect Protein Sources

Insect protein sources provide significant protein to mass ratios, making them ideal selections for alternative protein development. In addition to a direct food source, insects are being developed as an animal feed source as well. Amongst the variety of insects currently under consideration for mass protein development, there are 5 varieties that position themselves as top options.

Figure 1 shows some of the key insect sources being considered for mass production.

Insect Processing, Formulation and Analytical Considerations

To prepare insects for mass distribution, manufacturers will require a number of processing and formulation steps to reach the desired end product. The production process involves identifying the key insect species and relevant developmental age, analyzing endogenously produced compounds that may be of concern and assess any physical/ecological risks pertaining to mass production and harvesting of each species.

After selected species are determined, insect varieties are farmed when rearing conditions and feeding optimizations are determined for maximum yields. Next, insects are separated from frass and decaying animals, harvested, and then killed. Processing is now possible, and manufacturers are now able to carry out microbiological assessments, process contaminants and determine stability of end products.

Insect Safety Considerations

The preparation of insect food products will require a detailed qualitative and quantitative characterization of the main components within the insect tissue matrix. Additionally, careful safety assessments must be applied throughout the entire processing lifecycle to ensure allergen and unwanted material, such as chitin, is removed from the final product.

Nutritional information via protein quantification, protein quality, starch analysis, lipid analysis, fiber analysis, macronutrients analysis, micronutrients analysis, and antinutritional factors must be characterized and quantified as well. Additional factors to address include vitamins, minerals, impact of feed pertaining to bioaccumulation and cross-contamination, stability, and processing contaminants.

Cultured Meat

Food products derived from cultured cells provide a food source that is sustainable in its development while offering similar nutritional profiles, textures and tastes as traditional animal derived food products. Cultured meat is manufactured by isolating stem cells from a livestock animal and placing them in a growth medium. Myotubule formation is developed with the resulting end product being muscle tissue. This enables developers to generate tastes and textures of traditional meat products.

Key elements of characterization that must be considered with cultured meat products include:

- Identification of impurities, by-products, and antimicrobial/mycotoxin residues
- Nutritional profiles
- Safety profiling of various biohazards including viruses, contaminants, and BSE/TSE
- Comprehensive analysis targeting analytes which will vary based on stem cell source and production process

Figure 1. Top inspect protein sources.
Sustainable Solutions for the Global Food Crisis

Cultured Meat Processing, Formulation and Analytical Considerations

The cultured meat production process involves cell treatment, modification, and immortalization of stem cells. Therefore, added consideration must be placed on the raw materials and starting substances being used in production. Key considerations include growth medium, substrate, growth factors and hormones, antimicrobials, culturing parameters, and analytical equipment being utilized.\(^5\)

Cultured Meat Safety Considerations

Comprehensive safety testing using analytical instrumentation is necessary to determine impurities, microbiological toxins, and allergens. In addition to the compositional data afforded from analytical testing, bioinformatic data analysis is helpful to analyze genetic and proteomic considerations in more detail. Key ‘omics’ of interest in cultured meat safety and production include genomics, proteomics, metabolomics and transcriptomics.\(^5\)

Plant-Based Foods

Plant-based foods are already recognized as a leading sustainable food source worldwide due to their well-established global manufacturing infrastructure and sustainable production potential.\(^6\) Figure 2 shows some of the key plant sources being utilized for mass production.

Each plant source differs greatly in their protein, carbohydrate, starch, nutrient and anti-nutrient profile; thus, comprehensive analyses are needed to determine the compositional profiles of different plant varieties. Additionally, further analysis is needed to evaluate protein digestibility and nutrient bioavailability.\(^6\)

Plant Processing, Formulation and Analytical Considerations

Plant food sources will require processing multiple forms, such as the whole plants, grains, seeds, leaves and roots. Processing output will yield protein-based powders, protein isolates/concentrations, and other products (i.e., fermented protein mixtures). The ability to match textures, tastes and scents to traditional meat sources will be a critical parameter for plant-based foods adoption in the food market. Some plant proteins have stronger flavors than others, complicating the formulation requirements needed to bring a competitive product to market. Therefore, comprehensive formulation analysis is needed to develop market ready products.\(^6\)

Plant-Based Food Safety Considerations

Plant-based foods contain a variety of microbial, chemical and antinutritional compounds that need to be assessed prior to market release.\(^7\)

Key safety analyses include:

- Contaminant testing (includes analysis of primary and secondary metabolites, process enzymes, heavy metals, and residues of cultivation conditions)
- Microbiological testing (includes analysis of microbial counts and relevant toxins)
- Process contaminants (includes thermal processing, maillard reaction products, and acrylamide)
- Macro and micronutrients
- Antinutritional factors
- Allergens

Figure 2. Key plant sources.

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Algae

It’s estimated that single cells, such as algae, may meet up to 20% of conventional crop-based animal feed protein demand by 2050. With some species of algae consisting of nearly 70% protein, i.e., spirulina, algae offers an efficient starting organism for alternative protein development. In addition to their large protein content, algae grow rapidly and do not rely on land use making them an ideal environmentally-friendly food source.8,9

There are more than 300,000 known algae species with several currently being considered for food development including:

- *Arthrospira platensis*
- A. fusiformis
- A. maxima
- *Laminaria digitata*
- *Galdieria sulphuraria*
- *Schizochytrium sp.*
- *Phaeodactylum tricornutum*
- *Tetraselmis chui*

Algae Processing, Formulation and Analytical Considerations

After a species is selected, the production process begins by developing the fermentation and cultivation conditions such as temperature, time, pH, light quantities, and utilization of open or closed systems. Additionally, concentration parameters must be assessed as well as detection procedures for cell viability.8,9

Algae Safety Considerations

The following safety considerations must be analyzed prior to development and formulation steps:

- Nutritional composition
- Algae toxins and heavy metal analysis
- Particle size distributions
- Stability testing
- Allergen testing

Fungi

Fungi have been known food sources since 16,700 BC, where we see the first pieces of evidence of human consumption of Boletales Mushrooms. Fungi’s versatility, diversity, nutritional profiles, texture and flavor potential, and environmental sustainability make it an ideal candidate for sustainable food development. As nature’s decomposers, fungi are considered excellent option for circular and precision manufacturing applications, opening a wide variety of new applications for fungal biomass production.10
Key Analytical Technologies for Sustainable Food Analysis

Using the appropriate analytical technology can offer valuable aspects on a variety of important developmental parameters. Analytical analysis will set the operational limits and key parameters of the production process ensuring quality. Companies that take advantage of emerging innovations in analytical technology will no doubt secure a place in this alternative food landscape. See Table 1 for an analytical technology overview of key food testing instrumentation.\(^\text{11}\)

Key Analytical Technologies to Consider Include:

NIR (Near Infrared) Analyzers
PerkinElmer’s NIR at-line or in-process analyzers provide by-product analysis and ensure consistency in the production process, verifying ingredients and finished product quality, and enabling real-time monitoring of key nutritional parameters such as fat, moisture, protein, collagen and salt. Using NIR instrumentation offers the advantage of obtaining results in seconds instead of hours, enabling efficient production monitoring and optimization.\(^\text{12}\)

Process and at-line NIR analyzers, like our DA 7350™, DA 7440™, and DA 7250™, are demonstrating their value in alternative proteins processing optimization by maximizing profit through increasing yield, reducing waste, and improving raw material usage. Typically, in-line and on-line NIR instruments are used to manage control drying, control stream blending, control additions, segregate different fractions, and monitor product quality.

Key Distinguishing Factors Between PerkinElmer’s Process NIR Analyzers Include:

The DA 7350™ analyzes parameters in direct contact with the product and is designed to measure bulk products, pastes and slurries in a processing line. Additionally, the DA 7350™ contains a built-in camera allowing flow visualization and color measurements typical of grain, flour, and feed processing. Examples of applications that utilize the DA 7350™ include controlling blending of wheat streams to optimize protein content before milling, optimize ash content in flour production, and optimizing grain yield by controlling the separation of gluten and starch during milling.

The DA 7440™ is an on-line NIR instrument that measures a product on a conveyor belt or similar transportation system. The real-time measurement enables users to automatically or manually control the process. Examples of applications that utilize the DA 7440™ include controlling flavoring-salt addition to plant-based foods to save on expensive ingredients, control milling to optimize the mill settings and increase yield, and scanning of plant-based products to monitor moisture, protein and fat content.

NIR instrumentation has demonstrated its effectiveness in conducting meat analysis, making it suitable for both cultured meat and traditional animal meat applications. As demonstrated in this application note, where 5,000 meat samples made up of raw and processed beef, poultry, and pork products were collected and successfully analyzed. After homogenizing, samples were analyzed on multiple DA 7250™ instruments using open faced dishes. Several regression techniques were evaluated for calibration development, including ANN and Honig’s Regression™, a proprietary regression technique developed by PerkinElmer to handle large data sets with a wide range of product variability.\(^\text{12}\)

It was concluded that the best performance was achieved using Honig’s Regression, which made it possible to combine all of the various types of samples into one global calibration without performance loss. Additionally, the DA 7250™ achieved similar reproducibility results of the respective reference method, delivering accurate analysis of multiple parameters in seconds using one calibration for all sample types. Figure 4 demonstrates the reproducibility and accuracy the DA 7250™ using the Honig’s Regression.\(^\text{12}\)

FTIR Spectroscopy

Alternative protein formulation solutions will no doubt rely on the utilization of FTIR spectroscopy, such as PerkinElmer’s FT 9700™ FT-NIR analyzer for rapid determination of amino acids.

Another key application of FTIR will be quantifying and ensuring the removal of unwanted and difficult to digest proteinaceous material, such as insect chitin, from end product material.


### Table 1. Analytical technology overview of key food testing instrumentation\(^\text{17}\)

<table>
<thead>
<tr>
<th>What to test?</th>
<th>How to test?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality Standards</td>
<td>Chemical analysis, gravimetric, titrimetric, chromatography</td>
</tr>
<tr>
<td>Metal Contaminants</td>
<td>Chemical, AA, ICP-OES, ICP-MS</td>
</tr>
<tr>
<td>Pesticides</td>
<td>GC, GC/MS, LC/MS/MS, HPLC</td>
</tr>
<tr>
<td>Veterinary Drugs</td>
<td>HPLC, LC/MS/MS</td>
</tr>
<tr>
<td>Additives</td>
<td>Chemical, HPLC, GC, GC/MS, LC/MS/MS</td>
</tr>
<tr>
<td>Nutritional Parameters</td>
<td>Chemical, HPLC, GC, ELISA, LC/MS/MS, AA, ICP-MS</td>
</tr>
<tr>
<td>Microbiology</td>
<td>Conventional, ELISA, PCR</td>
</tr>
<tr>
<td>Adulterants</td>
<td>Chemical, GC, HPLC, TLC, LC/MS/MS</td>
</tr>
<tr>
<td>Allergens/Mycotoxins</td>
<td>ELISA, HPLC, LC/MS</td>
</tr>
<tr>
<td>Flavors</td>
<td>GC, GC/MS</td>
</tr>
<tr>
<td>Food Packaging</td>
<td>FT-IR, GC/MS, GC-HS</td>
</tr>
<tr>
<td>Food Labeling</td>
<td>CHNO, GC, GC/MS, HPLC, LC/MS, AA, ICP-OES</td>
</tr>
<tr>
<td>Quality Analysis</td>
<td>NIR, FT-NIR</td>
</tr>
<tr>
<td>Out of Lab Solution</td>
<td>Portable GC/MS, FT-NIR</td>
</tr>
</tbody>
</table>
Performance Analyzers

Performance analyzers are essential for ingredient analysis. Instruments such as our Rapid Visco-Analyzer (RVA) is ideal to evaluate textural changes to ensure efficient product development, process control and quality assurance.

Sustainable food product developers and manufacturers target a specific set of performance characteristics. This can present challenges, because the performance of sustainable food ingredients are impacted by their extraction and processing conditions (shear, pH, filtration technique, extraction reagents, etc.)

PerkinElmer’s RVA is widely used in the food industry and is a descriptive way to characterize ingredient performance and quantify processing effects by measuring hydration, shear-thinning, cooking, and gelling performance. This makes it easier and faster for the alternative proteins industry to reformulate, establish quality control parameters for incoming ingredients, and evaluate the performance of new ingredients. Measuring ingredient performance improves product quality and consistency, while reducing the time-to-market for new products.

UHPLC/MS/MS and LC/MS/MS

Chromatography systems coupled to tandem MS/MS configurations offer the ultra-sensitive detection capabilities ideally suited for mycotoxin, pesticide and other contaminating compounds. All five alternative protein sources will benefit significantly from these systems as impurity analysis will be critical for commercial development.\(^\text{13}\)

The advantages of multiclass-multianalyte method development for mycotoxins in foods is demonstrated in the following application note Mycotoxins in Food by IAC. Using PerkinElmer’s QSight\textsuperscript{®} LC/MS/MS systems, researchers developed and validated the robust method for the reliable confirmation and quantification of twelve mycotoxins in various food matrices. All of the analyzed mycotoxins, which consisted of a diverse range of physicochemical properties, can be determined simultaneously in a single chromatographic run in eleven minutes. Tables 2 and 3 demonstrate the application notes results of the validated method in eight different food matrices (maize, wheat, soybean, oat cereal, almond, peanut better, red chili and black pepper) with good sensitivity, selectivity, accuracy and precision for all the analyte/matrix combinations.\(^\text{13}\)

GC and GC/MS

Gas Chromatography (GC) is a particularly useful analytical technology for detecting pesticides, additives, nutritional analysis, adulterants, food labeling and packaging and flavor analysis. Additionally, as discussed in this application note GC 2400\textsuperscript{™} System is an excellent analytical tool for evaluating fatty acids isolated from edible oils for food products, which can help to determine both the quality of a sustainable food product and the detection of potential adulterations.\(^\text{14}\)

ICP-MS and ICP-OES

Another key analytical technology in alternative protein development will be inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) to determine elemental and mineral analyses. This is critical for both nutritional and toxic element profiles. Therefore, it is paramount that instrumentation possesses ultra-trace detection capabilities for the determination of trace contaminants and nutritional elements. PerkinElmer’s NexION\textsuperscript{®} ICP-MS series has delivered accurate and validated data, demonstrated in several application notes that overcome traditional elemental analysis obstacles such as the analysis of complex sample matrices, high levels of dissolved solids, and interferences in protein samples.\(^\text{15}\)

In our application note focused on analysis of major and trace elements in plant-based foods researchers were able to demonstrate the ability of PerkinElmer’s NexION\textsuperscript{®} 2000 ICP-MS to effectively measure both the major and the trace elements in the same analytical run.\(^\text{15}\)

Atomic Absorption (AA)

Flame atomic absorption (AA) instrumentation offers another option for the analysis of micronutrients and minerals. While ICP-OES is generally favored in a multi-element analytical environment, the cost savings, simplicity, and speed of operation of a flame atomic absorption (AA) system presents
Table 2. Mycotoxin recovery from food samples at spiking level one.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Spiked (µg/kg)</th>
<th>Method Accuracy or Analyte Recovery from Sample Matrix (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn</td>
<td>Wheat</td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>1</td>
<td>94.5</td>
</tr>
<tr>
<td>Aflatoxin B2</td>
<td>1</td>
<td>85.5</td>
</tr>
<tr>
<td>Aflatoxin G1</td>
<td>1</td>
<td>96.2</td>
</tr>
<tr>
<td>Aflatoxin G2</td>
<td>1</td>
<td>89.9</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>Fumonisin B1</td>
<td>100</td>
<td>103</td>
</tr>
<tr>
<td>Fumonisin B2</td>
<td>100</td>
<td>104</td>
</tr>
<tr>
<td>Fumonisin B3</td>
<td>100</td>
<td>101</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>100</td>
<td>76.6</td>
</tr>
<tr>
<td>Zeearalenone</td>
<td>30</td>
<td>110</td>
</tr>
<tr>
<td>HT-2 Toxin</td>
<td>100</td>
<td>86.9</td>
</tr>
<tr>
<td>T-2 Toxin</td>
<td>10</td>
<td>92.3</td>
</tr>
</tbody>
</table>

Table 3. Mycotoxin recovery from food samples at spiking level two.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Spiked (µg/kg)</th>
<th>Method Accuracy or Analyte Recovery from Sample Matrix (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn</td>
<td>Wheat</td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>5</td>
<td>98</td>
</tr>
<tr>
<td>Aflatoxin B2</td>
<td>5</td>
<td>93.3</td>
</tr>
<tr>
<td>Aflatoxin G1</td>
<td>5</td>
<td>104</td>
</tr>
<tr>
<td>Aflatoxin G2</td>
<td>5</td>
<td>91.2</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>10</td>
<td>97.6</td>
</tr>
<tr>
<td>Fumonisin B1</td>
<td>250</td>
<td>90.6</td>
</tr>
<tr>
<td>Fumonisin B2</td>
<td>250</td>
<td>107</td>
</tr>
<tr>
<td>Fumonisin B3</td>
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<td>98</td>
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<tr>
<td>Deoxynivalenol</td>
<td>250</td>
<td>98.5</td>
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<tr>
<td>Zeearalenone</td>
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<td>101</td>
</tr>
<tr>
<td>HT-2 Toxin</td>
<td>250</td>
<td>85.4</td>
</tr>
<tr>
<td>T-2 Toxin</td>
<td>25</td>
<td>90.4</td>
</tr>
</tbody>
</table>

A favorable alternative solution depending laboratory needs. Measuring multiple elements by flame AA requires each sample to be analyzed individually for each element, increasing the time of analysis for flame AA applications. To account for this, a high-throughput sample automation system can be utilized for robust analysis of sustainable food ingredients. 16

The Future of Alternative Proteins

The future of sustainable foods will require yield and after optimization production improvements in both R&D and analytical research. A recent R&D breakthrough demonstrates this by combining development of fungal species with microalgae development. Researchers have discovered an optimized method of utilizing fungal mycelium as a living scaffold for attachment and proliferation of microalgae cells. In doing so, this co-culturing method can enhance both fungal and algae biomass production beyond what would be expected if performed separately.

Analytical technology is constantly evolving and driving improvements in product development and production optimization by leveraging increasingly versatile, rapid and sensitive equipment. While the global food crisis poses challenges for the future, it is also setting the stage for significant opportunities in the food industry. Companies that take advantage of emerging innovations in analytical technology will no doubt secure a place in this alternative food landscape.
References:


