

Improve enzyme standardization in food production with automated analysis

Authors: Alexander Rohde, Global Business Development Manager, SternEnzym, and Gary He, Product Marketing Manager, Thermo Fisher Scientific

arohde@sternenzym.de
gary.he@thermofisher.com

Enzymes are the unsung heroes of the food and drink industry. These biocatalysts play a key role in many production processes, including standardizing flour quality, giving relief from food intolerances, and modifying the properties of food products. The versatility and efficacy of enzymes make them integral components in production process standardization. Yet, achieving standardization requires consistent enzyme activity, which must be determined analytically.

Traditional, manual spectroscopic techniques to analyze enzyme activity, however, pose significant challenges. Notably, they are time-consuming, resource-intensive, and often yield poorly reproducible results. So, how can enzyme activity be better determined for product uniformity?

Automated discrete analyzers offer a promising solution to enzyme analysis. These advanced systems hold the potential to revolutionize enzyme analysis, offering laboratories the ability to automate their analysis procedures. In doing so, automated discrete analyzers promise more consistent, efficient, and reproducible results, paving the way for improved standardization in the food and drink industry.

Enzymes: Catalyzing improved production processes

Historically, the standardization of food products often relied on oxidizing agents. A growing awareness of health and safety concerns, however, led to the banning of many such chemicals. For instance, manufacturers routinely used potassium bromate to improve the quality of flour, but its use was phased out after research linked it to an increased cancer risk in consumers. In contrast, standardization using enzymes presents an alternative that is safe for consumers.

Enzymes, which catalyze or speed up



chemical reactions, play a pivotal role in converting raw materials into other products. Crucially, as enzymes can only transform complementary substrates, they enable highly specific reactions. Enzymes can therefore be carefully selected for a given production process, and provide multiple benefits:

- Speed up manufacturing
- Improve product quality
- Reduce waste
- Optimize product yield

However, enzyme use is not without its complexities. Most notably, enzyme activity can vary based on many external factors, which makes their behaviors difficult to predict. To ensure consistency and effectiveness in preparing food and drink products, it is essential to analyze enzyme activity.

Expanded choice for milk consumers

Enzymes are indispensable in enhancing the properties of food staples. Take the dairy industry, for example. Given that approximately 70% of the global adult population is unable to effectively digest lactose, the production of lactose-free milk is vital. Enzymes such as lactase (a type of galactosidase) play a pivotal role, as they convert lactose into glucose and galactose — thereby creating lactose-free milk.

But the benefits of this enzymatic process extend beyond lactose removal. The resulting glucose is sweeter than lactose, which enhances the flavor of the milk. Moreover, changes in solubility brought about by the enzymatic reaction ensure a smoother mouth feel in high dry matter dairy products, such as ice cream.

However, different enzyme activity is needed



depending on the product. For example, milk, yogurt, fermented dairy products, and milk drinks require a much higher dosage of lactase than ice cream and sweetened condensed milk. Producers must therefore precisely control enzyme activity, or residual lactose may remain in the products — leading to potential digestive issues for consumers.

This example underscores the critical need for effective, accurate, and consistent enzyme activity, which is obtained through careful monitoring and control of enzyme activity.

Better bread properties

In the baking industry, enzymes play a significant role in optimizing production processes and improving the final product. Traditionally, bread standardization was achieved by adjusting equipment settings. While the method worked, it proved to be both time- and resource-intensive.

The addition of enzymes has emerged as a more efficient and effective alternative. Grains naturally contain enzymes that can impact the dough-making process, so additional enzymes — such as amylase — can be added to ensure consistency in bread quality across different batches. Other enzymes, such as glucose-oxidase and xylanase, are also used to standardize the flour's characteristics, including water absorption and surface dryness.

As well as dough properties, enzymes can also enhance bread properties. For instance, lipases can be used instead of emulsifiers to increase bread volume, while maltogenic amylases can help prolong shelf life.

The benefits of enzymes in bread production are clear. But to use them effectively, it is crucial to know the exact quantities of enzymes required as

their activity can vary. For example, an excessive amount of amylase can make the dough too moist and sticky. Precise knowledge of enzyme activity is therefore essential for consistently achieving the desired outcomes in food production, regardless of enzyme application.

Enzyme activity analysis: A tricky endeavor

Enzyme assays, an integral part of enzyme analysis, are typically performed to measure enzyme activity. So, how are they developed?

Traditional enzyme assay development is manual (Figure 1), which is effective, but often tedious and time-consuming. The process can be broken down into three key steps:

1 Identification — Analysts identify the key method variables that influence enzyme activity through carefully designed experiments. As numerous variables can impact enzyme activity, analysts must investigate many sets of samples across these variables.

2 Analysis — After the experiment is conducted, researchers collect and interpret data to determine the optimal experimental conditions.

3 Validation — Once the best method is determined, it needs validating to determine if it is suitable and reproducible.

Measuring enzyme activity is also notoriously difficult. Crucially, activity is influenced by many variables including temperature, pH, buffer type, and ionic strength. For instance, a temperature fluctuation of even one degree can lead to a 4-8% variation in enzyme activity. Therefore, to achieve reproducible results in enzyme assays, these variables must be tightly controlled.

Current options to measure enzyme activity

include spectroscopic techniques such as a spectrophotometric assay. In this method, analysts follow the reaction course by measuring changes in the intensity of light absorbed or scattered by the analyzed solution. While it is low-cost and simple, it is also a manual process, leading to inconsistent results and limited throughput.

Enzyme assays based on photometry or fluorometry serve as a higher-throughput alternative to spectrophotometers. However, these assays face their own limitations, including limited incubation temperature, temperature stability, and precision due to the edging effect caused by evaporation.

Simply put, existing methods to measure enzyme activity often lack precision and reproducibility. As such, the development of new, improved methods for enzyme analysis is a necessity in the pursuit of consistent and high-quality food and beverage production processes.

Automate enzyme analysis for greater productivity

Automated discrete analyzers represent a significant advancement in enzyme analysis. These comprehensive platforms provide two parallel measurement techniques — photometric (which includes colorimetric and enzymatic methods) and electrochemical (allowing for pH and conductivity analysis). New systems are specifically engineered to facilitate a broad array of enzyme assay applications such as method development, routine analysis, and quality assurance/control (QA/QC). These instruments offer:

- Automation — Systems have innovative software and pre-prepared reagents to enable fully automated reagent additions, incubations, and measurements
- High capacity — Some systems are capable of several hundred photometric tests per hour, with direct-read analysis
- Superior temperature control — Samples are run from the lowest to the highest temperature requirement, while the instrument prevents samples from overheating
- Disposable low volume cuvettes — Single use cuvettes remove the risk of cross contamination in your real-time kinetic measurements and enzyme activity determinations

Additionally, new, advanced systems offer enhanced workflow features. After loading cuvettes, samples, and reagents, the automated systems can run samples, including building or importing sample series. They can also perform fully automated enzyme analysis with precise control of parameters to maximize reproducibility of experimental conditions. And finally, they generate consolidated reports that can be exported in PDF, LIMS export, or spreadsheet formats.

Overall, newer automated systems offer considerable advantages for enzyme analysis:

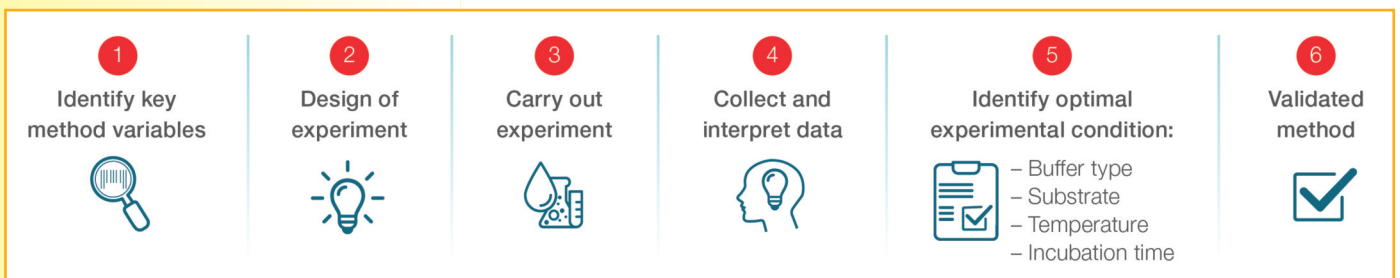


Figure 1: Manual method development for enzyme analysis.

- Higher confidence in results, due to no edge effects
- Increased valuable personnel time through automated procedures
- Reproducible results, as automated procedures eliminate manual errors
- Improved method robustness owing to high accuracy and precision

Discrete enzyme analysis in action

At SternEnzym, we produce tailor made customer products — functional blends of enzymes that offer different enzyme activities. As part of our processes, we analyze raw enzymes for product production. We also perform QA measurements across our global sites, so results must be comparable and as error-free as possible.

Traditionally, we have used spectrophotometric assays for our processes. While these work well, there are opportunities for errors to be introduced. First, there are many manual steps involved in these assays, such as substrate addition. Manual addition in this manner must be measured by a stopwatch, which can add to the overall measurement error. Additionally, substrates are added manually via pipette, further increasing the risk of inconsistencies through variations in both technique and operator.

To make our processes and results more reliable, we have begun implementing automated discrete enzyme analyzers into workflows in our

German site. The transfer of our manual assays to the automated system has been rapid and seamless, ensuring minimal disruption to our workflows. Although a significant number of our assays are now automated, we still perform smaller assays — that is, assays that have less than three samples per run — manually.

Automating the critical steps of our assays has brought numerous advantages to our processes:

- Data is more reliable, with smaller variance between measurements
- Staff require less training to perform the assays
- Assay transfer to abroad production staff is simpler

Based on the benefits discrete enzyme analysis has brought to our site in Germany, we are looking to implement further instruments in our production facilities in North America, the Middle East and Africa, and Asia. By expanding its use into our different sites, we will remove the need to send samples to Germany — thereby bringing cost and time savings to our processes.

More productive processes through automation

Enzymes play a pivotal role in the food and drink industry, making food fresher and more consistent between batches. However, the use of enzymes in the industry is not without its challenges. Their activity can vary, and many factors can influence it, making their behaviors

difficult to predict. The standardization of enzyme activity is therefore critical to ensuring consistent, high-quality food and drink products.

Automated discrete analyzers, with their dual measurement techniques, high capacity, and wide temperature range, offer a promising solution to the challenges of enzyme standardization. These systems not only save time and eliminate manual errors, but they also provide a robust method for enzyme analysis. Ultimately, the precision, reproducibility, and traceability offered by this new automation era leads to higher confidence in your results, enabling manufacturers to consistently achieve the best from their food and drink products.

