Agilent case study: ProteoAnalyzer system

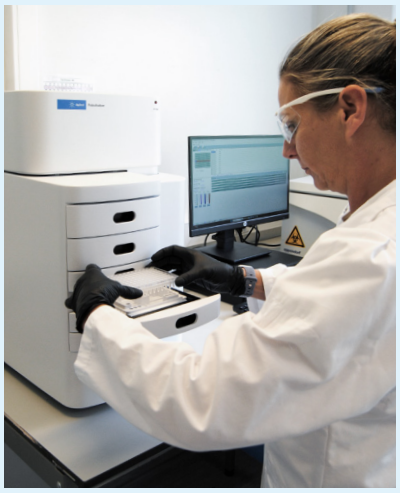
A New Story for Myoglobin—Producing Yeast-Generated Alternative Proteins at Paleo

Precision fermentation drives alternative protein production

The rising popularity of plant-based meat alternatives has driven food scientists to explore new and innovative technologies to meet consumer needs. Recent research highlights precision fermentation as a promising method. The technique involves modifying the metabolic pathways of safe microorganisms, followed by scaled-up fermentation and processing steps, to produce food ingredients using abundant and cost-effective sources.[1] Paleo applies this approach with the aim to meet this increasing demand.

The Belgium-based company produces various heme proteins (myoglobins) using a specific yeast known as *Pichia pastoris* as a production host during precision fermentation. The scientists integrate a DNA sequence from cow, chicken, and fish to create the different flavors of meat. During this process, myoglobin is externally secreted from the yeast cell. Once isolated, Paleo food scientists test these purified extracellular myoglobin proteins for use in various alternative meat and fish products. Rigorous efficacy testing based on common plant-based food challenges, such as taste, texture, aroma, and nutrition, ensures that the company's products are of optimal quality.

As a finalist for one of the world's most sustainable companies of 2024, Paleo's eco-friendly solution for alternative meat production hasn't gone unnoticed. With such success, implementing one efficient, high-throughput protein analysis workflow was essential for the company's research team to advance the development of their innovative products.



Paleo lab technician Tatiana Cools with the Agilent ProteoAnalyzer system

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An increasing workload requires a faster method to assess proteins

Engineering yeast to generate specific alternative proteins of interest requires constant experimentation to single-out top-producing strains. Paleo's Chief Scientific Officer Elsa Lauwers, PhD, mentioned that having to test so many strains can be challenging. "Searching for an optimal strain that allows us to make a lot of myoglobin at a very low cost requires generating and testing dozens of strains every week."

So many different yeast strains equate to a large amount of myoglobin to assess, and traditional methods of protein analysis like SDS-PAGE were slowing down the lab's fast-paced research. Running gels would take multiple hours: requiring overnight staining, destaining, and then gel imaging. Those who use this method understand how much time and labor it effectively takes. Recognizing the need to streamline this process for faster time to results, they introduced the Agilent ProteoAnalyzer system as a key component in creating a high-throughput workflow. The system is a capillary electrophoresis instrument that automates the analysis of 12 protein samples in parallel in just 30 minutes.

Automating analysis for improved reliability and turnaround time

Victor De Pillecyn, MSc, molecular biology scientist and bioinformatician at Paleo, along with colleague Tatiana Cools, BSc, lab technician, helped smoothly integrate the ProteoAnalyzer system into the lab. Victor noted how simple it was to operate the instrument. "One of the advantages of the ProteoAnalyzer is that you don't need a lot of background knowledge of electrophoresis to start using it and generate results."

In addition to reducing the turnaround time from introducing a potential new yeast strain to testing its protein production efficiency, the system also eliminates the need for time-consuming and error-prone staining steps. "Increasing reliability was important. We were initially testing all the strains manually, which was a lot of hands-on work, and we were not always confident in the data used to make decisions about the strains."

In the past, those generating new strains had to individually perform the SDS-PAGE and data analysis. Now, all the samples are combined and then run on the ProteoAnalyzer without burden. Since applying automated analysis, the team can run full batches and have the data analyzed in 45 minutes, saving time and effort for the researchers. "You can prepare a bunch of samples at once, load them, start a run, and walk away. Then come back after a brief period and look at your data."

Developing one high-throughput protein analysis workflow

A key factor in the lab's improved ability to test more new strains of *Pichia* was developing a streamlined workflow. The Paleo team uses the ProteoAnalyzer system during the entire precision fermentation process, from start to finish and all phases in between, for protein analysis.

By developing their own internal standard protocol and training materials for their workflow, the R&D scientists are completing tasks in the same way, and the automated system minimizes potential chances for human error. "One big plus point is that the ProteoAnalyzer removes the subjectivity of accurately integrating peaks, and based on this, determining protein purity," Tatiana said. "That means samples analyzed by different people give the same results. It's not easy to attain this with other methods."

Moreover, the system's digital data analysis enables fast, accurate protein assessment. The reduced time to results allows for increased amounts of data available to Paleo food scientists for efficacy testing in the plant-based meat products. Victor highlighted the intuitiveness of the Agilent ProSize data analysis software, and how it enables users to choose how to automate peak and baseline settings and makes it easier to see what data to export. "Using the software to do the smear analysis, you don't have to always select peaks manually. The analysis is done in a rigorous way, and you can automate it as much as possible." This allows the team to analyze their strains quickly, while maintaining high data accuracy to ensure the myoglobins are of optimal quality.

Lessening the environmental impact—in and out of the lab

Paleo's achievements in sustainable precision fermentation and cost-effective myoglobin production have made strides in reducing the environmental impact of the commercial meat industry. A single, high-throughput automated protein analysis workflow has supported the team's growing success.

The company is dedicated to preserving the planet's biodiversity through not only their environmentally conscious products, but also by being mindful of waste generated while performing standard lab tasks. These efforts help induce a positive impact both in and out of the lab.[2]

Future perspectives

Looking forward, Dr. Lauwers is excited for the potential to expand their research into other variations of the myoglobin protein or even look at other types of proteins. The ability of the Agilent ProteoAnalyzer system to assess multiple, varied protein types will be a strong asset for the company.

Read more about Paleo's mission: **Paleo | Same Taste. Different Story**.

Explore the Agilent ProteoAnalyzer system: **Automated CE-SDS Protein Analysis, ProteoAnalyzer | Agilent**.

References

1. Hilgendorf, K.; Wang, Y.; Miller, M. J.; Jin, Y.-S. Precision Fermentation for Improving the Quality, Flavor, Safety, and Sustainability of Foods. *Current Opinion in Biotechnology* **2024**, *86*, 103084–103084. https://doi.org/10.1016/j.copbio.2024.103084

2. *Paleo | Impact*. https://paleo.bio/impact/ (accessed 2024-07-18).

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