Bioproduction

The value of peptones for enhancing biopharmaceutical productivity

Keywords
Gibco peptones, consistency, reliability, productivity

Introduction

Peptones—protein hydrolysates derived from various sources like yeast, plants, and animals—have been widely used in bioproduction for decades. Available in both animal-origin (AO) and animal origin–free (AOF) formulations, these media additives offer a critical advantage over fetal bovine serum (FBS), enabling operators to eliminate key contamination risks like bovine spongiform encephalopathy (BSE). The ethical and practical advantages of peptones, which are highly nutritionally diverse and can offer improved lot-to-lot consistency, make these feed enhancements a critical tool for optimization for many biopharmaceutical applications.

Despite the potential performance gains that peptones can offer an application, there exist ongoing concerns surrounding their own variability and impact on the consistency of processes. Yet for some of the most popular modalities in the biopharmaceutical pipeline today, such as monoclonal antibodies (mAbs), mammalian and microbial vaccines, and even cultured meat applications, incorporating peptones in a process can result in considerable, consistent gains in titer and other key product attributes.

Recent advancements in cell culture, media feeds, supplements, cell lines, and production processes have converged to improve biomanufacturing's consistency and productivity in the last decade. This is likewise true for peptones, which have undergone significant optimization that has rendered them much more consistent and high-quality than those produced in the past or manufactured for other industries. With tighter and more relevant specifications around bioburden and endotoxins, coupled with improved analytical capabilities supporting their characterization and enhanced monitoring for key components, peptones have become a well-standardized material for use in bioprocessing. While all raw materials possess inherent variability, having a good understanding of additives like peptones is key to ensuring that a biopharmaceutical process is well controlled and thus is primed for optimization.

In this case study, we evaluated the performance of six Gibco™ peptones:

- Difco™ TC Yeastolate UF
- Difco™ Phytone™ Supplement UF
- Cotton Peptone 200 UF
- Bacto™ Proteose Peptone No. 3
- Difco™ Soytone
- Bacto™ Yeast Extract

Biological variation is widely considered to account for roughly 10% of potential variability in a process; once operators account for additional variability introduced by either an instrument or workflow, a coefficient of variation (CV) threshold of 15% can be considered well within the range of acceptable variability. By evaluating three or four distinct lots of each peptone across key cell culture metrics, we were able to demonstrate a CV of less than 15%—and in most cases, less than 5%—for each of the selected peptones.

The power of peptones: establishing consistency across a portfolio of products

In the biopharmaceutical space, there is increasing demand to produce high-quality mAbs with both high titers and desirable protein quality profiles. Chinese hamster ovary (CHO) cells are the most common workhorse leveraged for this purpose, and media optimization with supplements like peptones can play a crucial role in achieving an application's productivity goals. There are myriad potential benefits peptones can offer a cell culture process, including improved cell growth and cell-specific productivity (Qp), enhanced viability, delayed apoptosis, and better pH stability.

In our evaluation, we aimed to assess how different peptones impacted cell culture performance and protein quality profiles using CHO-K1 cells expressing immunoglobulin G (IgG) molecules, with the critical success criterion of maintaining a CV below 15% across all lots for all measured parameters.

Materials and methods

This evaluation was performed in shake flasks; all peptones in this study, excluding Bacto Yeast Extract and Cotton Peptone 200 UF, were made as 100 g/L stocks, and were added to a final concentration of 6 g/L to GBS Panel Medium 6 on day 0. Bacto Yeast Extract and Cotton Peptone 200 UF were dissolved as powders in GBS Panel Medium 6 at a final working concentration of 6 g/L and filter-sterilized prior to use. This study was run in simple fed-batch mode utilizing only glucose feeding.

The following cell culture conditions were used in support of the study:

- Cells were grown in GBS Panel Medium 6 supplemented with L-glutamine and an anti-clumping agent.
- Cells were maintained at 37°C with 8% CO_2 and 125 rpm shaking.
- Cell density and viability were measured using a Vi-CELL™ counter (Beckman Coulter).
- Metabolites (glucose, ammonia, lactate, glutamine) and IgG were measured using a Cedex™ BioHT Analyzer (Roche).
- CHO-K1 cells were seeded at 0.3 x 10^6 cells/mL.
- Glucose feeding was performed when the concentration dropped below 3 g/L.
- The experiment was terminated if viability dropped below 60%.

Additionally, we employed a comprehensive analytical strategy comprising multiple assays to perform the evaluation. Charge variance analysis of the mAb was performed on a cation exchange column using a high-performance liquid chromatography (HPLC) system coupled to an ultraviolet (UV) detector. N-glycan analysis was based on the reaction between a mAb-released glycan and 2-aminobenzamide (2-AB) labeling reagent; 2-AB–labeled N-glycans were separated by hydrophilic interaction liquid chromatography (HILIC) and ultra high-performance liquid chromatography (UHPLC), and detected by a fluorescence detector. Trace metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Amino acid samples were run on a liquid chromatography with tandem mass spectrometry (LC-MS/MS) system with HILIC separation.

Results

Gibco peptones performed consistently across the various lots that were tested. Figures 1 through 6 show the highly consistent terminal IgG titers for the peptones evaluated.

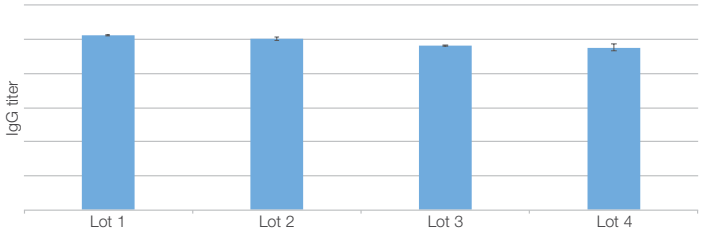

Figure 1. Terminal IgG titers of 4 lots of Difco TC Yeastolate UF. Each lot was run in duplicate.

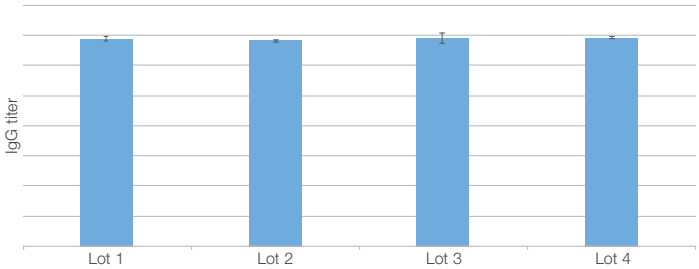

Figure 2. Terminal IgG titers of 4 lots of Difco Phytone Supplement UF. Each lot was run in duplicate.

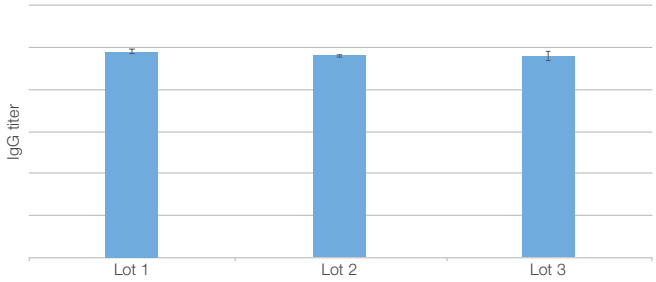

Figure 3. Terminal IgG titers of 3 lots of Cotton Peptone 200 UF. Each lot was run in duplicate.

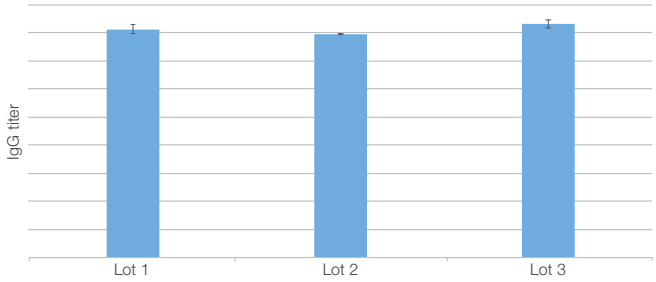

Figure 4. Terminal IgG titers of 3 lots of Bacto Proteose Peptone No. 3. Each lot was run in duplicate.

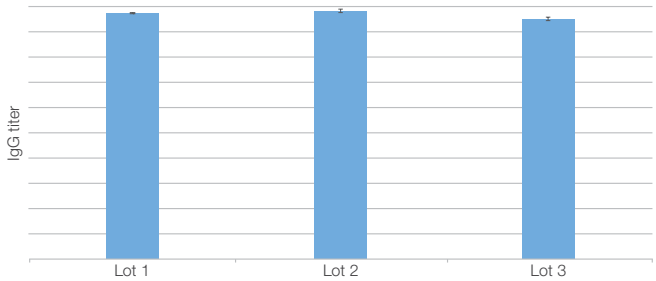

Figure 5. Terminal IgG titers of 3 lots of Bacto Yeast Peptone. Each lot was run in duplicate.

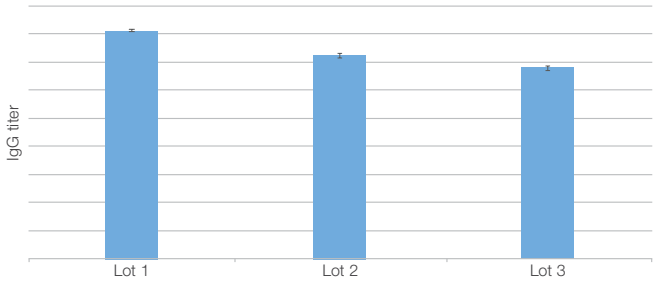

Figure 6. Terminal IgG titers of 3 lots of Difco Soytone. Each lot was run in duplicate.

Figures 7 and 8 show CVs of less than 10% for both terminal IgG titer and viable cell density obtained using the six peptones.

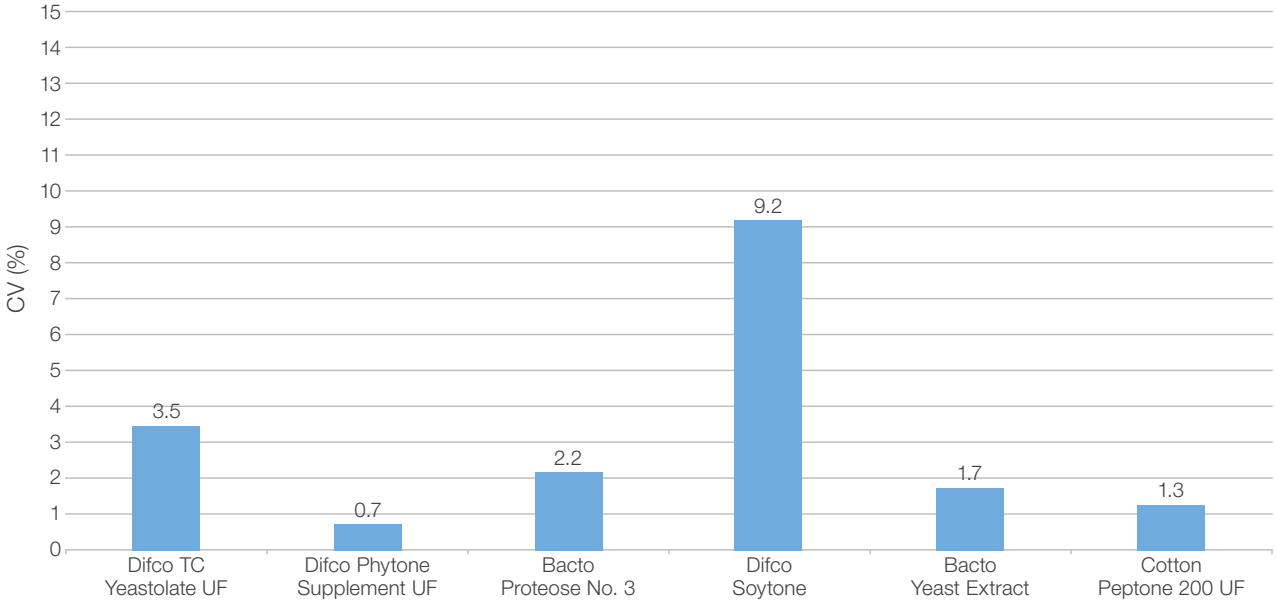

Figure 7. CVs of terminal IgG titer obtained using different lots of the selected Gibco AO and AOF peptones.

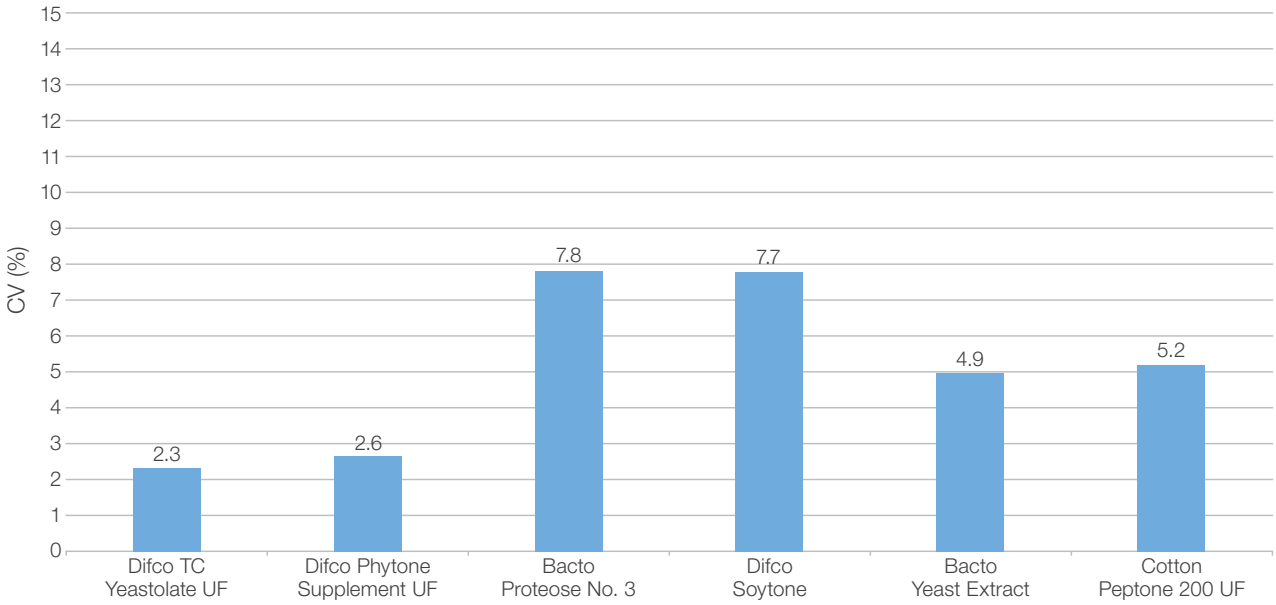

Figure 8. CVs of viable cell density obtained using different lots of the selected Gibco AO and AOF peptones.

In our analytical assessment, we evaluated the charge variants and glycan profiles of four of the six selected peptones (Figures 9, 10).

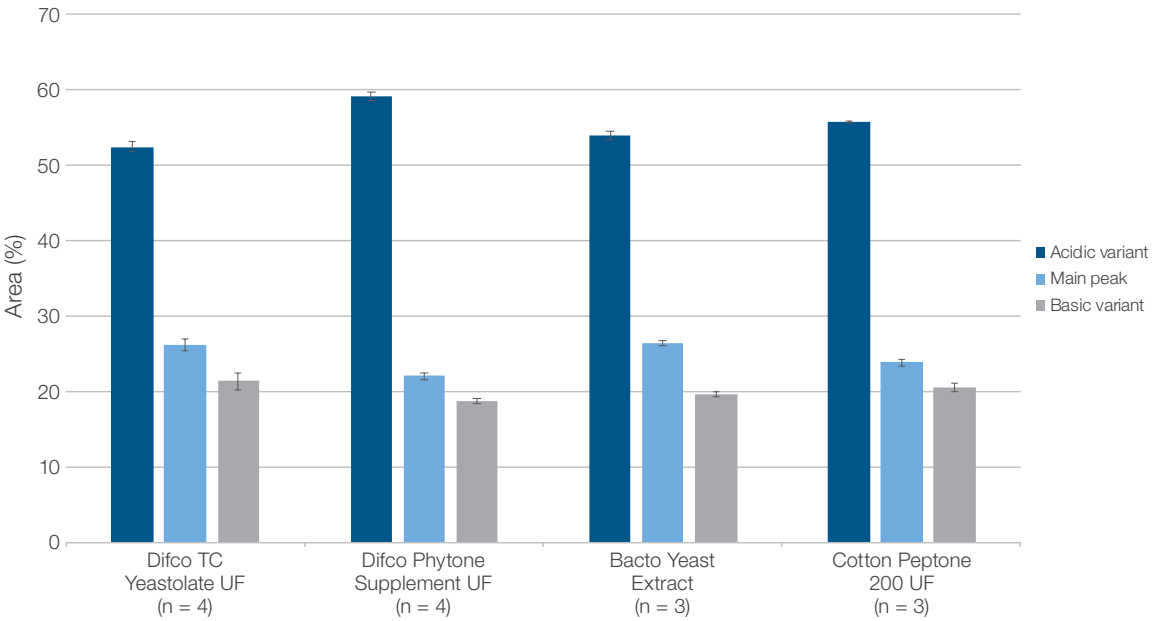

Figure 9. Charge variants for various lots of selected Gibco AOF peptones.

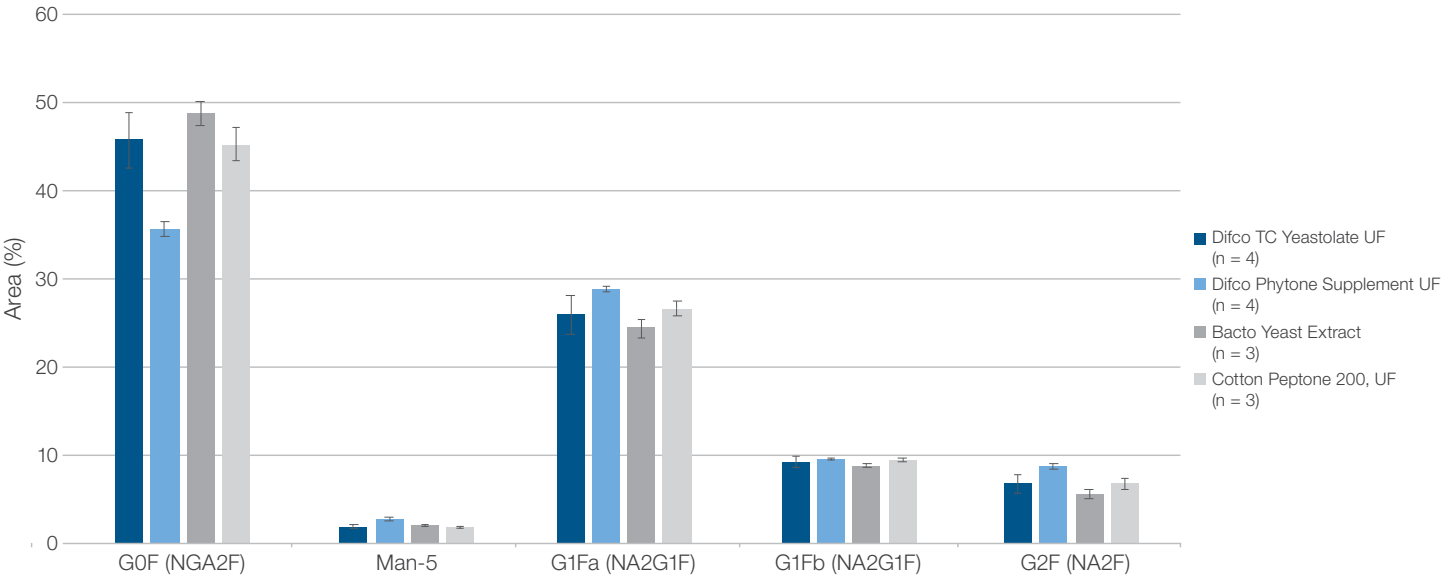

Figure 10. Glycan profiles for various lots of selected Gibco AOF peptones.

In a separate study, we established a CV profile for selected amino acids and trace metals across 12 lots of Difco TC Yeastolate UF (Figures 11, 12).

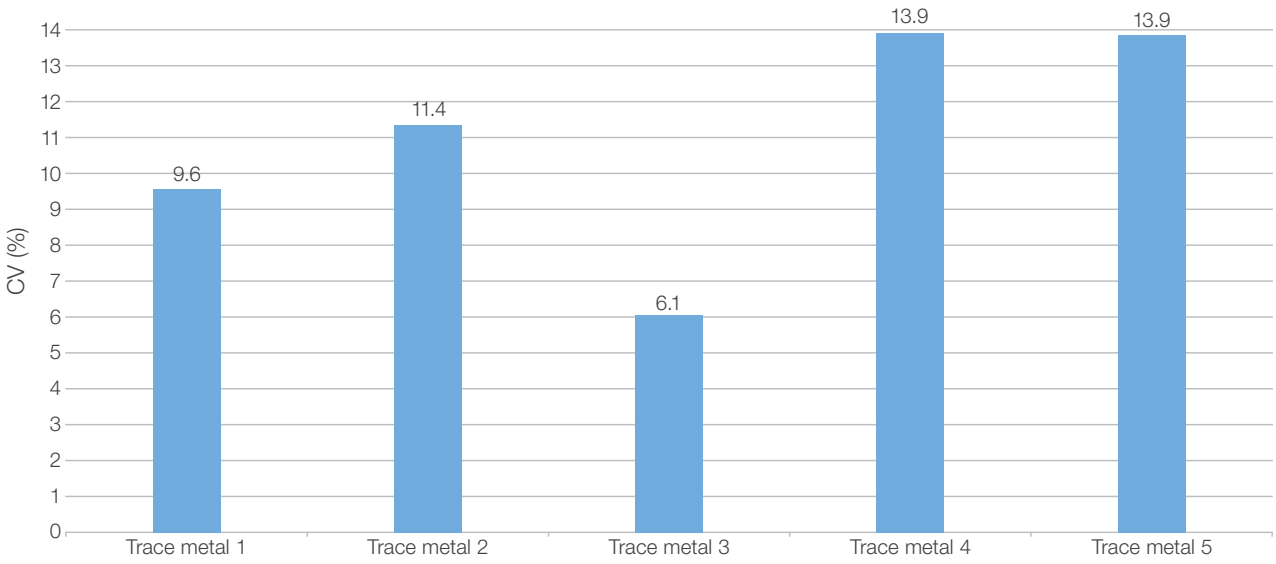

Figure 11. CVs for trace metals across 12 lots of Difco TC Yeastolate UF.

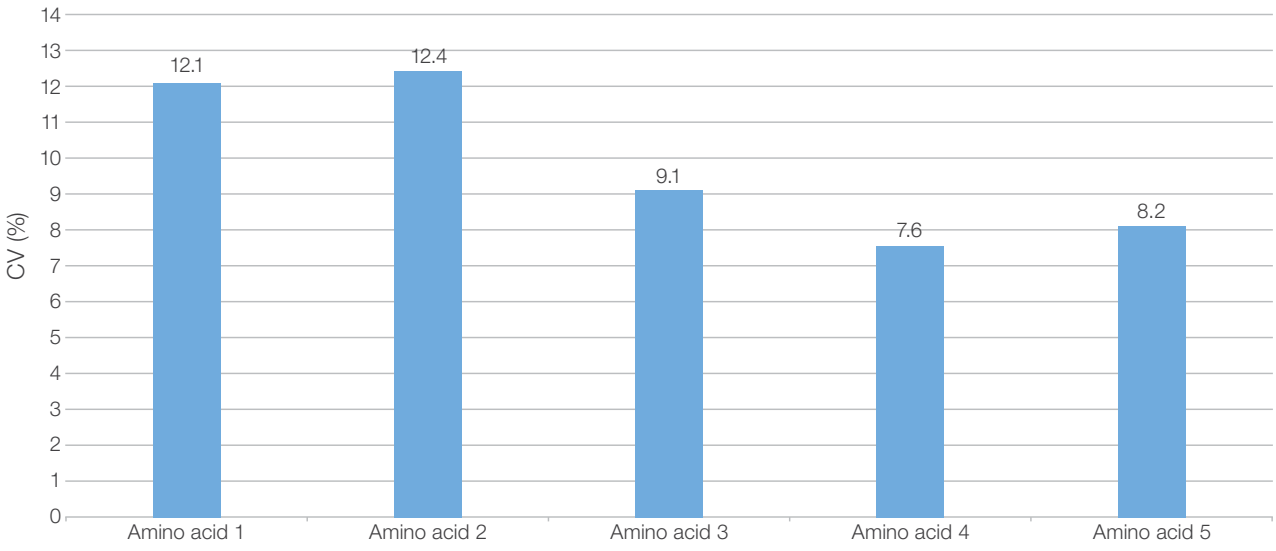

Figure 12. CVs for amino acids across 12 lots of Difco TC Yeastolate UF.

Discussion

Supporting media optimization for better bioproduction

The results of this case study highlight the consistent performance of various Gibco peptones across multiple lots, indicating their suitability for supporting reliable and predictable cell culture performance, a critical factor for achieving consistent and high-quality biotherapeutic production. Although this study focused on the performance of selected peptones using CHO-K1 cells, the data generated can serve as a valuable interim reference for vaccine and microbial production customers.

Though peptones have been significantly optimized for use in biopharmaceutical applications, their value for specific applications hinges on developers' ability to appropriately incorporate them in a media formulation strategy. Peptones possess thousands of components that have the potential to introduce some degree of variability, and operators that experience more significant variability after introducing a peptone into their workflow may require additional help identifying which of those thousands of analytes may be most impactful to a given process. For those requiring greater insight in order to optimally leverage peptones to boost their process's productivity, Thermo Fisher Scientific has established the **Key Driver Identification (KDI) program**, which helps customers by performing in-depth peptone characterization to help improve performance consistency.

Thermo Fisher has long-ranging and far-reaching experience in peptones; Gibco peptones are currently used in the manufacturing processes of more than 150 commercial drugs, 15 of which are blockbusters, each with annual sales in excess of $1 billion. Additionally, we draw on more than a century of experience through our history with Difco, the first company to manufacture peptones for industrial use. As one of the only companies manufacturing peptones specifically for biopharmaceutical applications, as well as the only peptone producer that also offers a full range of bioproduction media products, Thermo Fisher is uniquely positioned to support media optimization across the development space.

Conclusions

Ultimately, the advantages of peptones, particularly their consistency and reliability, make them a critical consideration for optimal bioproduction. Our study demonstrates the consistent performance of many popular Gibco peptones, cementing them as a powerful tool for predictable and high-quality biotherapeutics production. While further research is needed for specific modalities, the data presented here provide valuable insights for a wide range of biopharmaceutical applications. As the demand for high-quality biotherapeutics continues to grow, peptones, with their comparatively defined composition and more consistent performance, can undoubtedly play an increasingly vital role in the biomanufacturing landscape.

Authors

Ashwin Gurunathan and Stacy Holdread, Thermo Fisher Scientific

Explore now at **thermofisher.com/peptones**

For Research Use or Further Manufacturing. Not for diagnostic use or direct administration into humans or animals.
© 2024 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Cedex is a trademark of F. Hoffmann-La Roche Ltd. Difco is a trademark of Becton, Dickinson and Company and is used under license. Vi-CELL is a trademark of Beckman Coulter, Inc. **TCN-8897122 1124**