Defining Hydrolysates: Generation of a Chemically Defined Alternative

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Abstract

Protein hydrolysates are commonly utilized in cell culture processes either as a component of a complete medium formulation or as part of a fed-batch bioreactor process. It is well documented that hydrolysates can have a substantial positive impact on cell growth and/or protein production. Given the undefined nature and the lot-to-lot variability associated with hydrolysates, there exists a need to mitigate these risks with a chemically defined (CD) alternative that can maintain the desired performance. In order to create a CD alternative to protein hydrolysates, a two-pronged approach was taken to capture the associated effects. The first experimental path utilized standard analytical techniques to identify the nutritive components supplied by hydrolysates (i.e., amino acids, vitamins). A “basal” supplement was designed to supply these nutrients, as this is a significant portion of the functionality of hydrolysates. The second path was based upon Reverse Phase HPLC fractionation of multiple different hydrolysate types. Cell culture screening with Chinese Hamster Ovary (CHO) cells led to the identification of “biactive” fractions. Subsequent identification of the components contained within the fractions led to a greater understanding of the effects of hydrolysates. The learnings from both approaches were utilized to generate a chemically defined supplement, EX-CELL® CD Hydrolysate Fusion, which is capable of replacing the functions of hydrolysates in many CHO cell systems.

Materials and Methods

Sigma-Aldrich Corporation (St. Louis, MO, USA) supplied all chemicals, media, and reagents unless otherwise stated.

Cell Lines

Cell lines CHO-IGG1 and CHO-IGG2 are proprietary Chinese Hamster Ovary (CHO) clones expressing recombinant antibodies.

Culture Media

The media used in this study are all proprietary SAFC formulations that are chemically defined or prepared without hydrolysates. SAFC’s IMEDIATE ADVANTAGE™ Small Volume Media Program prepared all media.

Cell Growth and Recombinant Protein Production Assays

The cells were routinely cultured in suspension in shaker flasks and were used to seed experiments conducted in duplicate 50 mL (30 mL working volume) disposable TPU Bioreactor tubes (Techno Plastic Products AG, Switzerland). Initial cell density was 200,000 viable cells/mL. The cells were cultured in a Multitron incubator (Infor HT, Switzerland) at 37 °C, 5% CO₂, and 200 rpm shaker speed. Assays were counted using a Vi-CELL® SR (Beckman Coulter, CA, USA). Spent medium samples were collected for the analysis of nutrients/metabolites and IgG concentration. The secreted IgG was measured by an Octet QK (ForteBio, Inc., CA, USA). The primary chromatography method (TFA Separation) consisted of a binary gradient with high pressure mixing and a Shimadzu SPD-M6 AV detector. A preparative C18 column (2.5 cm x 22.5 cm) was used for the separation of the hydrolysates. Data collection and processing were performed using the Class-VP Chromatography Data System.

Discussion

The process developed for this project is outlined in Figure 1. Four different hydrolysate types were selected for analysis (soy, wheat gluten, yeast extract, and meat). The goal was to identify the unknown active components contained within these hydrolysates, and Reverse Phase HPLC fractionation was used to aid with the separation of these compounds. In Figure 2, two overlaid chromatograms (at 280 nm) demonstrate the reproducibility of the separation. Fractions with improved growth or productivity responses were identified by each hydrolysate. Examples of four fractions from a Yeast Extract are shown in Figures 3 & 4. Significant stimulatory effects are seen with addition on these fractions. Fractions that gave a positive growth and/or productivity response were subjected to further analytical analysis to determine the components contained within. Orthogonal separation techniques and mass spectrometry methods were employed for this analysis (details and data not shown). Chemically defined versions of the identified compounds were subsequently screened for activity using the same CHO cell culture assay outlined above. An example of a compound that was identified to have a positive effect is shown in Figures 5 & 6.

Conclusions

• By analyzing the amino acid, vitamin, and metal composition of several protein hydrolysates and with subsequent optimization, a hydrolysate “Base” supplement was designed to encompass much of the nutritive functions of hydrolysates. The use of this supplement helped to elucidate the unknown positive effectors contained within hydrolysates.

• Reverse Phase HPLC fractionation of four hydrolysate types and subsequent screening with CHO cells led to the identification of active fractions. Further analytical analysis of these fractions led to the discovery of important factors that contribute to the effect seen with hydrolysates.

• A new supplement was created based on the work above, EX-CELL CD Hydrolysate Fusion. It is supplied as a 20x liquid (product # 14700C) or powder (product # 24700C). It can be used as an alternative to hydrolysates in any part of a CHO cell culture process. It also has application with other cell lines, such as NS0 and SP2/0 (data not shown).

• EX-CELL CD Hydrolysate Fusion is designed to perform equivalently as compared to undefined hydrolysates. In most instances this goal is met or exceeded.