

Partial Replacement of Chemically Defined CHO Media with Plant-derived Protein Hydrolysates: Part I – Growth, Viability and Protein Production

James F. Babcock, Amy Antosh and Thea Hassan, Sheffield Center for Cell Culture Technology, 283 Langmuir Lab, 95 Brown Road, Ithaca, NY 14850 - james.babcock@kerry.com - http://www.SheffieldBioScience.com

Introduction

Protein hydrolysates are routinely employed to enhance the overall performance of many biopharmaceutical production systems. The manifestation of this enhancement is subject to the additive effect of the native hydrolysate components on the final composition of the supplemented basal medium. Consequently, it is necessary to experimentally determine the proper hydrolysate dosage for a given hydrolysate-medium combination which provides the desired optimization effect, be it better growth promotion, enhanced cell viability or increased target protein production, or a combination of all three, as determined by the requirements for a particular production system.

Though largely undefined, hydrolysates are known to contain many components already found in standard basal media including, but not limited to, peptides, free amino acids, and carbohydrates. Many microbial systems routinely employ hydrolysates, or combinations of hydrolysates, as principal components of their production media.

In mammalian systems, hydrolysates have been used in combination with a variety of other supplements to help reduce or eliminate serum requirements in systems utilizing traditional minimal basal media. Today, many high-performing, richly formulated chemically defined media have become available as stand-alone substrates for biopharmaceutical production.

In this report, we demonstrate that these rationally designed chemically defined media can benefit from the addition of hydrolysates. We also demonstrate that in some cases, plant-derived hydrolysates can partially replace a significant portion of the active ingredients of these rich media. Our results show re-enrichment of these diluted media, as well as the full-strength formulation supplemented with hydrolysates, can yield CHO cell performance that equals or surpasses that of the original full-strength formulation.

Background

The contribution of protein hydrolysates to the overall performance of a biopharmaceutical production system can be influenced by a number of factors including the specific cell line being employed, the raw material used to manufacture the hydrolysate, the hydrolysate dosage, and the composition of the basal growth medium.

Since both chemically defined media and hydrolysates share a number of common components, the additive effects of these components may negatively impact the performance of a given system as a result of unintended "over-dosing." In certain instances, this "over-dosing" may potentially create, or exacerbate, any perceived variability in performance among different lots of a given hydrolysate supplement.

In order to test this hypothesis, we are conducting a series of experiments comparing the performance of CHO cell cultures cultivated in full-strength chemically defined media, full-strength chemically defined media supplement with plant-derived hydrolysates, and diluted chemically defined media re-enriched with plant-derived hydrolysates.

In preliminary experiments, a single chemically defined medium, diluted to 80% strength with phosphate buffered saline, was re-enriched using various soy, wheat and cottonseed hydrolysates at a range of dosages. Interestingly, not all of the hydrolysates were able to fully overcome the medium dilution with respect to overall performance in cell culture (data not shown). However, particularly interesting results were obtained using a cottonseed-derived hydrolysate (CSH).

Further experiments determined the optimum dilution/dosage schemes to be 8 g/l of CSH in 80% CDM, and 10 g/l CSH in 60% CDM. Each dilution/dosage scheme provided positive, although dissimilar results.

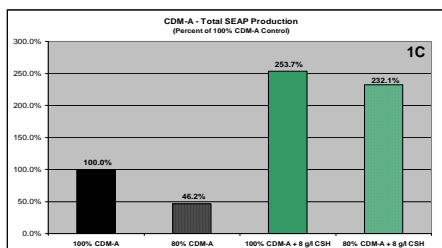
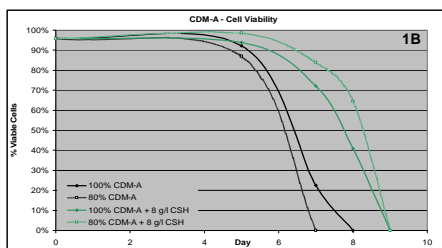
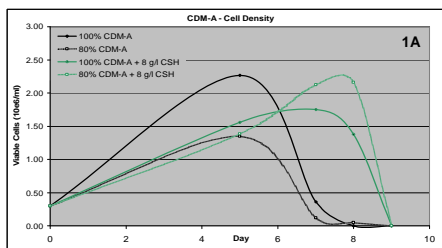
The data presented here are the results obtained using three different commercially available chemically defined media, specifically designed for CHO cell culture. For clarity purposes, only the data from the dilution scheme yielding the best overall results for a given chemically defined medium are included.

Materials and Methods

Sheffield™ Clone B.1 is a transfected CHO-K1 line engineered to constitutively express secreted embryonic alkaline phosphatase (SEAP) by means of a modified human cytomegalovirus (HCMV) promoter. A sub-clone (KCC-010) of the parent line, which has been adapted to suspension culture in serum-free medium, was used in these experiments. Cultures were grown in 125 ml shake-flasks containing a final medium volume of 25 ml. The various basal media were supplemented with 1 mg/ml G-418. Triplicate cultures were seeded at 3×10^5 cells/ml, and incubated at 37°C in 5% CO₂ at 130 rpm for 12 days. Hydrolysate supplementation was achieved via the use of filter-sterilized 100 g/l stock solutions prepared in each respective basal medium.

Screening Protocol

At days 5, 7, 8 and 9, 200 µl of the culture supernatants were removed for assessing cell counts and viability. Cells were counted using a NucleoCounter fluorescence-based automated cell counter. At Day 12, 200 µl of the culture supernatants were removed for SEAP analysis. Levels of functional SEAP in the supernatants were measured using an absorbance-based activity assay.



Chemically Defined Medium – A

Figures 1A, 1B & 1C

The most dramatic effects of medium dilution and re-enrichment were seen in cultures grown in CDM-A. Both cell density and SEAP production were severely reduced in the 80% medium. However, re-enrichment with 8 g/l CSH resulted in a protracted growth curve, which achieved an equivalent maximum cell density as the 100% medium control, and an over two-fold improvement in SEAP production.

The 100% CDM supplemented with 8 g/l CSH did not achieve a comparable maximum cell density as the 100% medium control, yet SEAP production was more than 2.5 times that of the control.

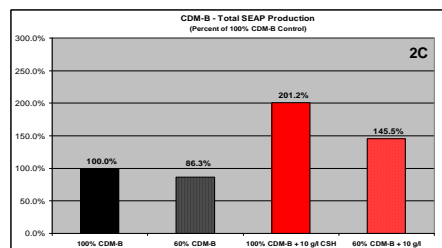
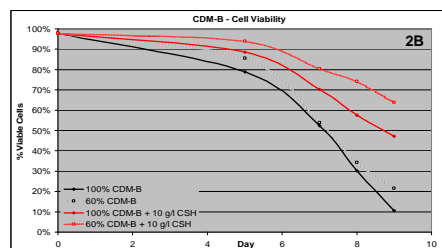
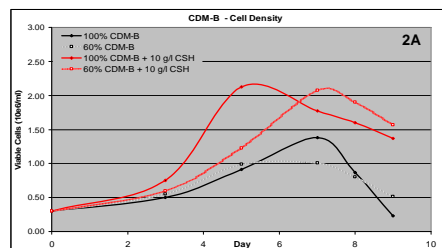
Cell viability was considerably extended in both hydrolysate supplemented treatments.

These data demonstrate that the supplementation of chemically defined media with plant-derived protein hydrolysates can enhance various aspects of cell culture performance in a medium- and dosage-dependent manner. In addition, it is shown that supplementation of diluted chemically defined media can achieve equivalent or enhanced cell culture performance, providing a cost-effective alternative to full-strength chemically defined media.

In the course of these experiments, additional data have been collected which illustrate that cells grown in hydrolysate-supplemented media undergo a significant metabolic change as opposed to cells grown in un-supplemented chemically defined media. These data will be presented in a subsequent poster.

All the media employed in this study were exogenously supplemented with glutamine per the manufacturers instructions. Further experiments are underway which will explore whether similar or improved performance can be obtained using high-glutamine hydrolysates as the sole source of glutamine in diluted chemically defined media.

In addition, we will examine whether the "over-dosing" effect of adding hydrolysates to full-strength chemically defined media contributes to real or perceived inter-lot performance variability of plant-derived hydrolysates, and if this can be overcome through the use of diluted, re-enriched, chemically defined media.



Chemically Defined Medium – B

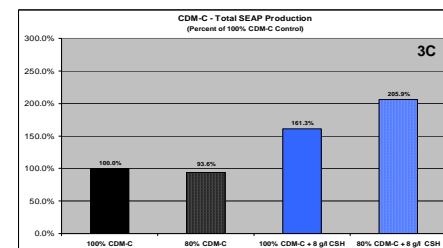
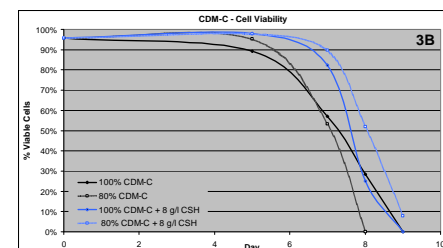
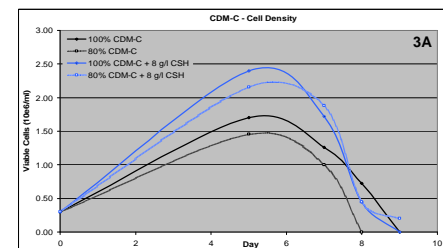
Figures 2A, 2B & 2C

In contrast with the CDM-A and CDM-C experiments, the 60% CDM/10 g/l dilution scheme yielded the best overall results in cultures grown in CDM-B. In this case, the 100% CDM-B supplemented with 10 g/l CSH yielded a growth curve that peaked earlier, and was significantly sustained when compared with the 100% medium control. A two-fold increase in SEAP production was also seen then the 100%/10 g/l treatment.

When the CDM-B medium was diluted to 60%, and re-enriched with 10g/l CSH, the growth curve was similar to the 100% control, but a significantly higher maximum cell density was achieved. SEAP production was also improved by 40% over the 100% medium control.

Once again, cell viability was considerably extended in both hydrolysate supplemented treatments.

Summary



Chemically Defined Medium – C

Figures 3A, 3B & 3C

There appeared to be a minimal dilution effect in cultures grown in CDM-C. The growth curve, cell viability, and SEAP production for the 80% medium control were minimally altered as compared with 100% medium control. This is noticeably different from the results obtained with CDM-A and CDM-B.

The hydrolysate-supplemented samples were also very similar with respect to overall performance. While the 100% CDM-B/8 g/l CSH sample achieved a slightly higher maximum cell density than the 80%/8 g/l sample, the opposite was true for SEAP production. This is a good illustration of the inverse relationship between cell growth and target protein production which is characteristic of hydrolysate supplemented cultures.

Both of the hydrolysate-supplemented samples out-performed the 100% medium control in growth, viability and SEAP production.