

Partial Replacement of Chemically Defined CHO Media with Plant-derived Protein Hydrolysates: Part II - Metabolic Effects of Hydrolysates

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Introduction

Protein hydrolysates are routinely employed to enhance the overall performance of many biopharmaceutical production systems. 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. Today, many high-performing, richly formulated chemically defined media have become available as stand-alone substrates for biopharmaceutical production. 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.

Part I of this report, presented at ESACT 2009 in Dublin, data were shown from a series of experiments conducted comparing the performance of CHO cell cultures cultivated in full-strength chemically defined media, full-strength chemically defined media supplemented with plant-derived hydrolysates, and diluted chemically defined media re-enriched with plant-derived hydrolysates. It was established that even at full strength, the performance of cells cultivated in these rich media was enhanced by the addition of hydrolysates. In addition, it was shown that in some cases, it is possible to replace a significant portion of the active ingredients of these chemically defined media with plant-derived hydrolysates, yielding CHO cell performance that equals or surpasses that of the un-supplemented full-strength formulation. In this report, using data from these same experiments, we demonstrate that CHO cells undergo a beneficial shift in metabolism when cultivated in the presence of plant-derived hydrolysates, as compared with those grown in un-supplemented chemically defined media.

Materials and Methods

SheffCell™ 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 3x10⁵ 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.

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. Glucose, lactate, glutamine and glutamate data were collected using a YSI 2700 Biochemical Analyzer.

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 80% CDM. Each dilution/dosage scheme provided positive, although dissimilar results. For the purpose of clarity, only the data from the dilution scheme yielding the best overall results for a given chemically defined medium are included.

Growth Curves

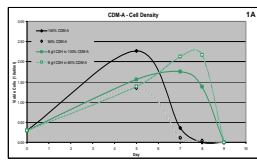


Figure 1A:

Dilution of CDM-A resulted in a significant reduction in both cell density and SEAP production (Figure 2A). Addition of hydrolysate to the full strength medium resulted in a pronounced growth curve and the maximum cell density fell short of the 100% medium control. However, addition of the hydrolysate resulted in a 2.5-fold increase in SEAP production. Addition of the hydrolysate to the diluted medium also resulted in a pronounced growth curve, and a maximum cell density was achieved that was equivalent to the 100% medium control. Addition of the hydrolysate to the diluted medium yielded a 2.3-fold increase in total SEAP produced.

Total SEAP

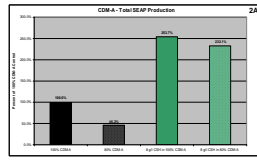


Figure 2A:

Dilution of CDM-A resulted in a marked 10% reduction in total SEAP produced in the un-supplemented culture. Addition of hydrolysate to the full strength medium resulted in a significant increase in total SEAP production. In the 80% medium supplemented with hydrolysate, the loss in SEAP production was the smallest, un-supplemented sample was recovered, and the culture yield more than twice the total SEAP than the 100% medium control.

Glucose

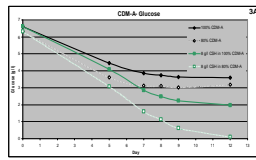


Figure 3A:

CDM-A contained the most glucose of the three media tested. As expected, dilution of the medium resulted in a decrease in initial glucose content of the media. Addition of hydrolysate did not appear to increase the initial glucose levels in the media. However, the hydrolysate supplemented samples appeared to consume glucose at a slower rate than both the 80% and 100% medium controls. Glucose levels in the control cultures remained essentially constant after the majority of cells had died. The hydrolysate supplemented cultures, with their extended growth curves, continued to consume glucose until the end of the experimental run. The diluted medium, supplemented with hydrolysate effectively consumed all available glucose from the medium.

Lactate

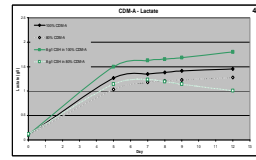


Figure 4A:

In CDM-A, the hydrolysate supplemented samples produced lactate at a higher rate than the medium controls, as would be expected based on the glucose consumption rates. Lactate production in all cultures began to level off between Days 4 and 7, regardless of the time that each culture achieved its maximum cell density. After Day 7, lactate production in the cultures grown in hydrolysate medium began to drop at Day 8. By Day 9, the glucose level in this culture had fallen considerably more than in any of the other treatments, reaching only about 1 g/l. The culture did not achieve its maximum cell density until Day 8 of the experiment.

Glutamine

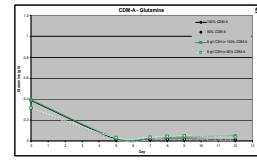


Figure 5A:

CDM-A medium contained the greatest amount of initial glutamine of the three media tested. Glutamine consumption rates for the 100% medium control and the 10% medium control supplemented with hydrolysate were slightly higher than for the cultures grown in the 80% medium, with and without hydrolysate. In all cases, glutamine was effectively exhausted by Day 5 of the experiment, regardless of the point at which the cultures reached their respective maximum cell densities.

Glutamate

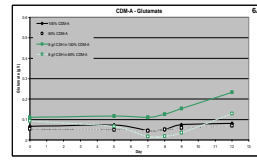


Figure 6A:

In CDM-A, hydrolysate supplementation appeared to slightly increase the initial glutamate concentration. Initial glutamate levels were, on average, higher in CDM-A than in the other media. The rate of increase was nearly identical for all the cultures through Day 2 of the experiment. After Day 2, glutamate levels in the hydrolysate supplemented cultures began to rise only marginally higher than the initial concentrations. In the hydrolysate supplemented cultures, glutamate concentrations began to rise at Day 5, and continued to increase through the end of the experiment.

Growth Curves

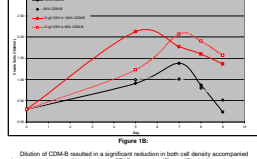


Figure 1B:

Dilution of CDM-B resulted in a significant reduction in both cell density accompanied by an approximately 15% reduction in SEAP production (Figure 2B). Addition of hydrolysate to the full strength medium resulted in an abbreviated growth curve, however the maximum cell density was significantly higher than for the 100% medium control. Addition of the hydrolysate to the diluted medium resulted in growth curves similar to the 100% medium control, but yielded a significantly higher maximum cell density. Addition of the hydrolysate to the diluted medium yielded a nearly 1.5-fold increase in total SEAP produced.

Total SEAP

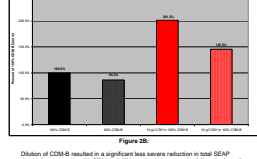


Figure 2B:

Dilution of CDM-B resulted in a significant less severe reduction in total SEAP production than was seen with CDM-A. Addition of hydrolysate to the full strength medium resulted in a 2-fold increase in SEAP production. Addition of the hydrolysate to the diluted medium yielded a nearly 1.5-fold increase in total SEAP produced. The reduction of SEAP production in the 80% medium supplemented with hydrolysate as compared with the 100% medium supplemented with the same amount of hydrolysate was more pronounced than the difference in SEAP production between the 80% and 100% un-supplemented media.

Glucose

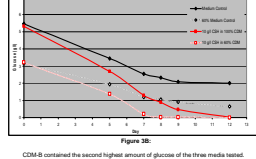


Figure 3B:

CDM-B contained the second highest amount of glucose of the three media tested. Again, dilution of the medium resulted in a decrease in initial glucose content of the media, and addition of hydrolysate did not appear to increase the initial glucose levels in the media. As with CDM-A, the hydrolysate supplemented samples appeared to consume glucose at a slightly slower rate than both the 80% and 100% medium controls. The 100% medium supplemented with hydrolysate effectively consumed all available glucose from the medium within a day of reaching the maximum cell density. The 100% un-supplemented culture continued to consume glucose until it was exhausted at the end of the experiment.

Lactate

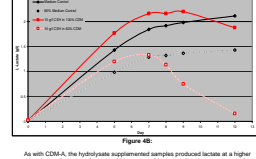


Figure 4B:

As with CDM-A, the hydrolysate supplemented samples produced lactate at a higher rate than the medium controls, as would be expected based on the glucose consumption rates. Lactate production in the medium control cultures began to level off by Day 7, rising slowly from that point until the end of the experiment. After Day 7, lactate production in the cultures grown in hydrolysate medium supplemented with hydrolysate began to drop rapidly. This corresponds with the exhaustion of glucose in that culture, as well as the medium cell density. The 100% medium supplemented with hydrolysate, the lactate level began to drop at Day 5.

Glutamine

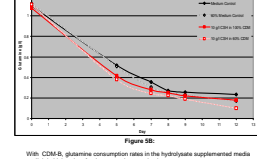


Figure 5B:

With CDM-B, glutamine consumption rates in the hydrolysate supplemented media were slightly higher than for the un-supplemented cultures. In all cases, glutamine consumption rates began to slow at Day 6. The greatest amount of glutamine was consumed by cells grown in 100% medium supplemented with hydrolysate. Glutamine was not fully consumed in any of the cultures.

Glutamate

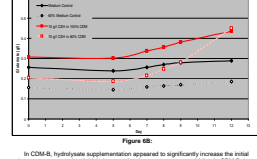


Figure 6B:

In CDM-B, hydrolysate supplementation appeared to significantly increase the initial glutamate concentration. Initial glutamate levels were, on average, higher in CDM-B than in the other media. The rate of increase was nearly identical for all the cultures through Day 5 of the experiment. After Day 5, glutamate levels in the hydrolysate supplemented cultures began to rise only marginally higher than the initial concentrations. In the hydrolysate supplemented cultures, glutamate concentrations began to rise at Day 7, and continued to increase through the end of the experiment. The rate of increase was highest in the 80% medium supplemented with hydrolysate, and resulted in the highest final glutamate concentration of all the cultures.

Growth Curves

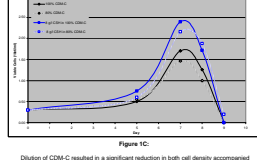


Figure 1C:

Dilution of CDM-C resulted in a significant reduction in both cell density accompanied by an approximately 15% reduction in SEAP production (Figure 2C). Addition of hydrolysate to the full strength medium resulted in an abbreviated growth curve, however the maximum cell density was significantly higher than for the 100% medium control. Addition of the hydrolysate to the diluted medium resulted in growth curves similar to the 100% medium control, but yielded a significantly higher maximum cell density. Addition of the hydrolysate to the diluted medium yielded a nearly 1.5-fold increase in total SEAP produced.

Total SEAP

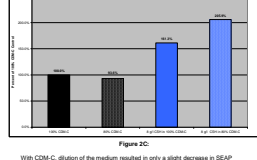


Figure 2C:

With CDM-C, dilution of the medium resulted in only a slight decrease in SEAP production. Addition of hydrolysate to the full strength medium resulted in more than 1.5-fold increase in SEAP production. Addition of the hydrolysate to the diluted medium resulted in a more than 2-fold increase in total SEAP produced.

Glucose

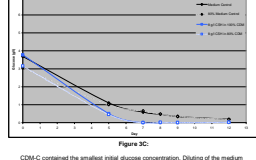


Figure 3C:

CDM-C contained the smallest initial glucose concentration. Diluting of the medium resulted in a decrease in initial glucose content of the media, and addition of hydrolysate appeared to slightly increase the initial glucose levels in the media. As with CDM-A and CDM-B, the hydrolysate supplemented samples appeared to consume glucose at a slightly slower rate than both the 80% and 100% medium controls. The hydrolysate supplemented cultures also consumed all available glucose until they had reached their maximum cell density. The 100% medium supplemented with hydrolysate, the glucose was not completely consumed in these cultures. Although, at a much slower rate after the cultures had achieved their maximum cell density. The glucose was not completely consumed in these cultures.

Lactate

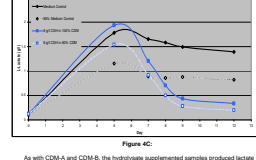


Figure 4C:

As with CDM-A and CDM-B, the hydrolysate supplemented samples produced lactate at a higher rate than the medium controls, as would be expected based on the glucose consumption rates. Lactate production in the medium control cultures began to level off between Days 5 and 7, the medium control cultures began to drop rapidly until Day 5, while in the hydrolysate supplemented cultures lactate levels continued to drop rapidly until Day 5, when they began to level off. The rate of drop for all cultures corresponds with the point at which the cultures reached their maximum cell densities.

Glutamine

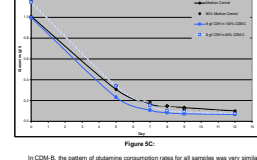


Figure 5C:

In CDM-C, the pattern of glutamine consumption rates for all samples was very similar. The rate of glutamine consumption in 100% medium control was slightly slower than for all other samples. In all cases, lactate levels began to fall between Days 5 and 7, the medium control cultures began to drop slowly at Day 5, while in the hydrolysate supplemented cultures lactate levels continued to drop rapidly until Day 5, when they began to level off. The rate of drop for all cultures corresponds with the point at which the cultures reached their maximum cell densities.

Glutamate

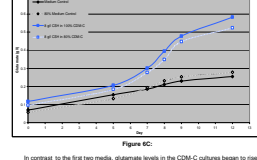


Figure 6C:

In contrast to the first two media, glutamate levels in the CDM-C cultures began to rise from the outset. The rate of increase was nearly identical for all the cultures through Day 2 of the experiment. After Day 2, glutamate levels in the hydrolysate supplemented cultures began to increase more rapidly than in the un-supplemented cultures. Rates of increase slowed again at all cultures at Day 5, with the hydrolysate supplemented cultures showing the slowest rates of increase. The hydrolysate supplemented samples continue to rise more quickly than in both the 80% and 100% medium controls.

Summary

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. 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 hydrolysate supplementation of diluted chemically defined media can achieve equivalent or enhanced cell culture performance as undiluted media alone, providing a cost-effective alternative to full-strength chemically defined media. Furthermore, these data suggest that in certain medium formulations, addition of a cottonseed hydrolysate facilitates a shift in cellular metabolism, allowing for the utilization of lactate as an energy source when more abundant carbon sources such as glucose and glutamine have fallen below certain critical levels, or are entirely depleted in the culture media. This may, in part, explain why in most cases hydrolysate supplemented cultures exhibit extended growth curves and sustained cell viabilities as compared with un-supplemented cultures. This extension of the productive life of the cultures in turn translates into higher target protein titers in hydrolysate-supplemented cultures as compared with cultures grown in hydrolysate-free media.