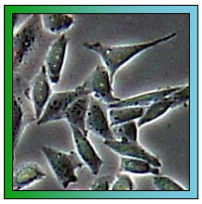

Cottonseed Hydrolysate Facilitates the Consumption of Lactate in CHO Cell Cultures Resulting in an Extended Growth Profile and a Significant Increase in Target Protein Yield

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Introduction

Not all protein hydrolysates perform equally in different biopharmaceutical production systems. The interaction among components of the basal medium and those of the hydrolysate supplement can have a significant impact on overall system performance. While both culture media and protein hydrolysates share a number of common components, the unique composition of various hydrolysates may elicit distinct beneficial responses in cultured cells.

In recent investigations, we discovered that in certain media, CHO cell cultures supplemented with a cottonseed hydrolysate begin to metabolize lactate once the majority of available stores of glucose and glutamine are depleted. Prior to this shift in metabolism, both glucose and glutamine are consumed at a more rapid rate than in unsupplemented cultures. This metabolic shift is accompanied by extended growth profiles and significant increases in target protein yield.

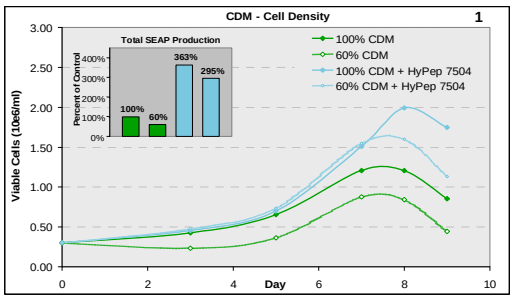
This metabolic shift was also seen in cultures supplemented with (FBS). Interestingly, this same behavior was not observed when cultures were grown in a commercially available chemically defined medium. This suggests that both the FBS and cottonseed hydrolysate share a functional component not found in the chemically defined medium.

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, 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 $3x10^5$ cells/ml, and incubated at 37°C in 5% CO_2 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.

Optimum hydrolysate supplementation rates for the cottonseed hydrolysate (HyPep™ 7504) in the full strength and diluted media were determined by dose-response experiments. The commercially available chemically defined medium was dosed at 10 g/l hydrolysate, and the F-12K + 1% FBS medium was dosed at 8 g/l.

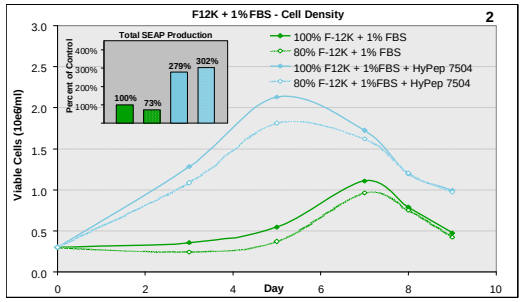
Cell counts and metabolite analyses were performed using a Nova BioProfile® Flex automated cell culture analyzer. Levels of SEAP were determined using anion exchange chromatography.

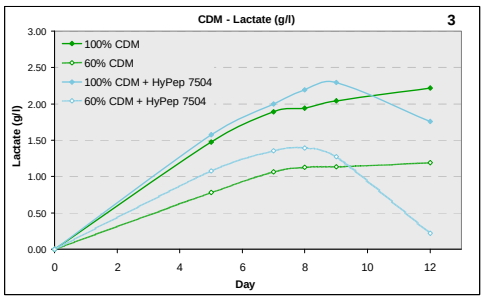


Figures 1 & 2:

Dilution of both the chemically defined medium and the F-12K + 1% FBS medium resulted in a significant reduction in maximum cell density, accompanied by a reduction in SEAP production (Figures 1 & 2).

Hydrolysate supplementation of both media at full strength significantly enhanced both growth and SEAP production. When the dilute versions of the media were supplemented with HyPep 7504, similar performance enhancement was seen, and the re-enriched media out performed the un-supplemented, full strength media.

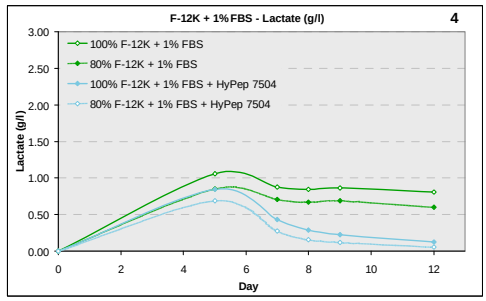


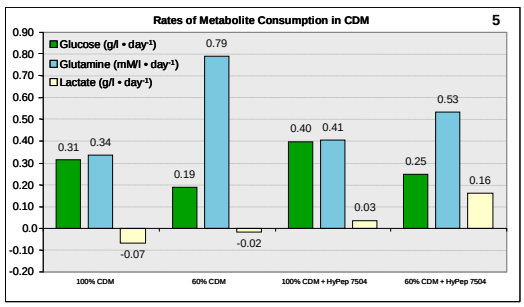


Figures 3 & 4:

These figures illustrate the shift in metabolism to lactate consumption seen in the media supplemented with cottonseed hydrolysate. In the chemically defined medium supplemented with HyPep 7504, this shift occurs at Day 9, while in the F-12K + 1% FBS the shift occurs at Day 5. While the shift was not seen in the un-supplemented CDM, it was seen to a lesser extent in un-supplemented treatments containing FBS. This suggests the cottonseed hydrolysate and the FBS share a common component not found in the CDM, which facilitates the change in metabolism.

These inflection points coincide with the total depletion of glucose in all but one of the supplemented media. The 100% CDM supplemented with hydrolysate still had approximately 25% of the original glucose remaining when the shift occurred. Glutamine was also totally depleted by Day 5 in the supplemented F-12K treatments. Less than 15% of the total glutamine was still available in the supplemented CDM treatments (Data not shown).



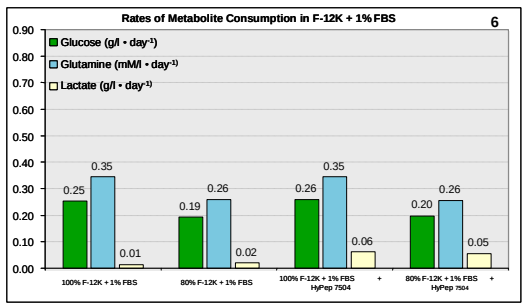


Figures 5 & 6:

In all cases, the rates of glucose and glutamine consumption were higher in those media supplemented with HyPep 7504 than in the un-supplemented media. Interestingly, dilution of the CDM resulted in a significantly higher increase in the rate of glutamine consumption, which was not seen in the diluted F-12K + 1% FBS.

In the chemically defined medium, the un-supplemented cultures continued to produce lactate after the supplemented cultures had shifted to lactate consumption. The rate of lactate utilization was highest in the diluted CDM supplemented with HyPep 7504.

Only those cultures supplemented with either HyPep 7504 and/or FBS demonstrated the shift from lactate production to lactate consumption.



Summary

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.

It has been 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 has been 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. Furthermore, it has been demonstrated that supplementation of chemically defined media with a cottonseed hydrolysate (HyPep 7504) facilitates a metabolic shift, allowing for the cells grown in such media to utilize lactate as an energy source once the majority of glucose and glutamine are depleted from the media.

The same has now proven to be true in a classical medium containing 1% fetal bovine serum (FBS). However, the metabolic shift to lactate consumption has also been demonstrated to occur to a lesser extent in a medium containing only FBS as a supplement. This suggests that both the FBS and cottonseed hydrolysate share a functional component not found in the chemically defined medium.