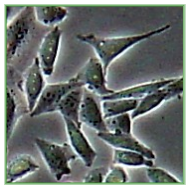

Addressing Hydrolysate Variability Through the Application of Novel Production Process Technology.

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Introduction

Supplementation of culture media with protein hydrolysates can provide a number of benefits to cell culture systems. Cell viability, cell proliferation and target protein production all may be improved. For this reason, hydrolysates have found widespread application in eukaryotic biopharmaceutical production systems, including mammalian and insect cell culture. However, inter-lot variability has been known to compromise the effectiveness of protein hydrolysates in certain commercial systems, thus diminishing their overall value as performance enhancing media supplements.

It has commonly been suggested this inter-lot variability may be attributed to the inherent variability within the primary raw materials. While, to a certain extent this may be true, data have shown that discrete lots of a given hydrolysate, manufactured from a single lot of raw material, yield disparate performance in a common production system. This suggests that a significant source of inter-lot variability may lie in the manner in which these supplements are produced.

Protein hydrolysates have traditionally been produced by the proteolytic digestion of a given raw material, followed by iterative filtration and evaporation steps, ultimately yielding a spray-dried powder. Due to the crude nature of the digests produced in these traditional processes, filtration of this material can often be problematic and inefficient. Much of the original raw material is not fully utilized, large amounts of undigested material may be discarded, and the full nutritional potential of the given raw material may not be fully realized.
In order to ameliorate the various negative effects of these filtration issues, a number of processing aids and adjuncts may be employed. However, many of these substances are not fully removed during subsequent purification steps, and may have detrimental effects on the performance of the final product in eukaryotic cell systems. All of these factors may also contribute to variability among different lots of a given hydrolysate.

Recent research, involving an extensive characterization of inter-lot variability in a traditionally manufactured hydrolysate, coupled with an intensive investigation into the potential root causes of this variability, yielded a number of valuable insights. The resulting knowledge was then applied to the design and production of a new generation of hydrolysates for use in cell culture media. The first of these, UltraPep™ Soy, was introduced to the market in October 2007.

At the core of this new technology is a novel, highly optimized digestion step, which takes into account specific characteristics of the raw material. The result is an extremely tractable digest, amenable to an efficient filter stream, precluding the need for processing adjuncts or additives. This streamlined process reduces or eliminates many of the root causes that may lead to inter-lot variability of the finished product and subsequent inconsistency in end-use performance.

Data are presented which demonstrate that hydrolysates manufactured using this novel approach support elevated cell densities, enhance target protein production, and prolong cell viability when compared with non-supplemented media controls. In addition, we demonstrate the vastly improved inter-lot consistency of end use performance found among lots of UltraPep™ Soy.

Results

Figure 1: CHO-K1 performance in a basal medium supplemented with three different lots of UltraPep™ Soy at 5 g/l.

Figure 2: CHO-K1 cell viability over seven days in a basal medium supplemented with an UltraPep™ Soy at 5 and 10 g/l as compared with an unsupplemented medium control.

Figure 3: CHO-K1 performance variability in media supplemented with a traditionally manufactured hydrolysate, as compared with media supplemented with UltraPep™ Soy.

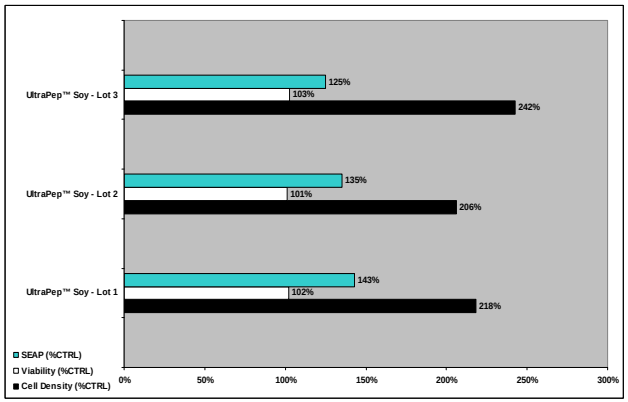


Figure 1. These results demonstrate the enhanced performance achieved with CHO-K1 cells in a basal medium supplemented with three different lots of UltraPep™ Soy at a rate of 5 g/l. Cell density, viability and SEAP production are all improved as compared with the unsupplemented basal medium control. In addition, a high degree of consistency among the three lots can be observed.

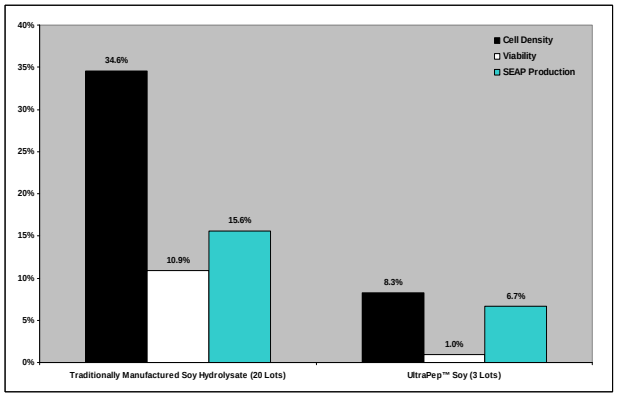
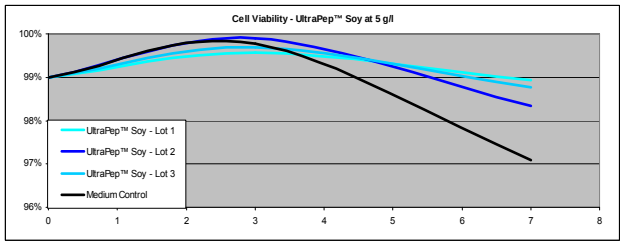


Figure 3. These data compare the inter-lot variability for twenty lots of a traditionally manufactured soy hydrolysate produce over a four year period, and the first three production lots of UltraPep™ Soy. A significant reduction in variability among lots of UltraPep Soy™ can be observed, as measured by the percent relative standard deviation of the data for each performance parameter. These data indicate that the novel digestion and streamlined processing of the UltraPep™ platform has led to a four-fold reduction in variability for cell density at 5 days, a ten-fold reduction for viability, and a more than two-fold reduction for SEAP production as compared to the traditionally manufactured hydrolysate..



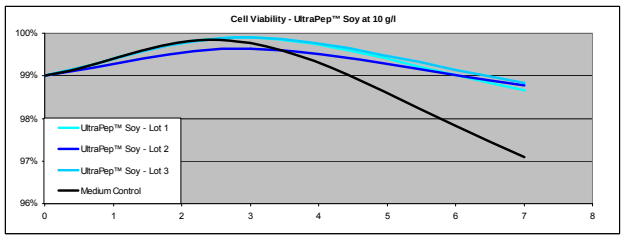


Figure 2. Seven-day viability curves for CHO-K1 cells cultivated in basal medium supplemented with three different lots of UltraPep™ Soy at dosages of 5 and 10 g/l. These data illustrate that UltraPep™ Soy consistently prolongs cell viability as compared with cells cultivated in the unsupplemented medium.

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. Monolayer cultures were grown in six-well microplates containing a final medium volume of 3 ml/well. The basal medium consisted of 50% chemically defined medium (CDM) and 50% Ham's F12-K, 1 mg/ml G-418, supplemented with 5% FBS. UltraPep™Soy was added at either 5 or 10g/l . Triplicate cultures were seeded at 2 x10^5 cells/well, and incubated at 37°C in 5% CO_2. In our standard five day test (Figure 1), 200 µl of the culture supernatants were removed for SEAP analysis. Cell monolayers were rinsed, trypsinized and neutralized for counting.

In the seven day test (Figure 2), three sets of triplicate cultures were seeded at 2 x10^5 cells/well, and incubated at 37°C in 5% CO_2. One set of cultures was harvested at 3, 5, and 7 days, and data collected as in the 5 day test.

Levels of functional SEAP in the supernatants were measured using an absorbance-based activity assay. Cell monolayers were rinsed, trypsinized and neutralized for counting. Cells were counted using a NucleoCounter fluorescence-based automated cell counter.