

# Soy Hydrolysate Optimization for Cell Culture Applications

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## Abstract

Soy hydrolysates often provide significant performance enhancements to the serum-free cultivation of mammalian cells and are frequently used in the biomanufacturing industry. However, historically there have been concerns with variability in performance which could directly impact cell culture protein titers. Given the critical nature of soy hydrolysates in the production of biotherapeutic proteins, there is value in understanding what parameters in the manufacture of soy hydrolysates have significant impact on cell culture performance. By modifying and/or better controlling these critical hydrolysate manufacturing parameters, it may be possible to significantly reduce variability, as well as optimize performance.

SAFC Biosciences (Lenexa, KS USA) and DMV International (Delhi, NY USA) have collaborated on a project in which the current process for the manufacture of soy hydrolysates was assessed. A multivariate statistical design approach was taken to evaluate a number of steps in the manufacturing process of soy hydrolysate. Hydrolysates were manufactured using modified processes and then evaluated in the culture of Chinese Hamster Ovary (CHO) cells as well as by analytical methods. More than 75 test hydrolysates were manufactured at bench scale for the purpose of performing these tests. Statistical analyses revealed that the manufacturing process had a significant impact on CHO cell culture performance and analytical test results. Some manufacturing steps were observed to have a positive impact on performance, while others had a negative impact. Additionally, one step in particular was determined to influence product variability. Test hydrolysates produced using modified processes yielded a range in performance of 50% – 200% of the control hydrolysate, demonstrating that optimization of the manufacturing process can yield a higher performing hydrolysate as compared to the existing product.

## Introduction

Soy hydrolysates have been used extensively for the serum-free cultivation of many mammalian cell lines for the purposes of improving viable cell densities, recombinant protein titers, increased culture longevity, reduced apoptosis and general robustness in performance. However, soy hydrolysates are largely undefined complex raw materials comprised of enzymatically digested soy and have been used historically in microbial applications with less challenging cell lines prior to being employed in the cultivation of mammalian cell lines. Given that soy hydrolysates were not specifically developed for all of their current applications including those with mammalian cell lines and the reliance of the biomanufacturing industry on their use with these lines, there is significant importance in investigating the existing manufacturing process. It may be possible to elucidate what parts of the process affect cell culture performance in order to better control the process, improve product consistency and potentially develop and manufacture a higher performing soy hydrolysate.

## Materials and Methods

### Hydrolysate manufacturing

Ten soy hydrolysate manufacturing parameters were investigated using a multivariate design of experiment (DoE) approach (Figures 1, 2) and tested in a cell culture application to determine the effects when the test soy hydrolysates were used in an animal-component free, protein-free medium. A total of 77 test hydrolysates were manufactured and tested using analytical methods by DMV International and cell culture tested for performance relative to the existing soy hydrolysate product by SAFC Biosciences.

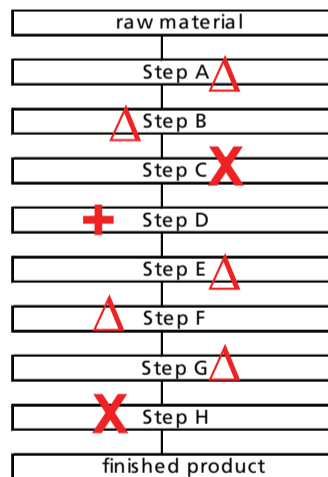


Figure 1: Conceptual outline of soy hydrolysate manufacturing process modifications

Run Order	Parameter 1	Parameter 2	Parameter 3	Parameter 4	Parameter x
1	1	1	1	1	1
2	1	1	1	-1	1
3	-1	-1	-1	-1	1
4	-1	-1	1	-1	-1
5	1	-1	-1	-1	-1
6	1	1	1	-1	-1
7	-1	1	-1	1	1
8	-1	-1	-1	1	1
9	-1	1	-1	1	-1
10	1	1	-1	-1	1
11	1	1	-1	1	-1
12	-1	-1	1	1	1
13	-1	1	-1	-1	1
14	1	-1	-1	-1	-1
15	-1	-1	-1	1	-1
16	-1	1	1	1	1
17	-1	-1	1	1	-1
y	1	-1	1	1	-1

Figure 2: Generalized DoE outline used to investigate manufacturing affects on performance

## Cell culture

A suspension CHO DUKX-IgG producing cell line, CRL-11397, (ATCC, Manassas, VA USA), was used throughout hydrolysate development. Stock cell cultures were maintained in erlenmeyer flasks at 37 °C in a 5% CO<sub>2</sub> environment in soy hydrolysate containing EX-CELL™ 325 (Item No. 14340C) supplemented with 4 mM L-glutamine. EX-CELL™ 325 dry powder deficient of hydrolysate was manufactured by imMEDIATE ADVANTAGE™, which then was supplemented with each of the test lots of hydrolysate to the same concentration as in EX-CELL™ 325. The control hydrolysate used was SE50MAF-UF (DMV International). pH (7.0–7.2) and osmolarity (340 – 360 mOsm) were adjusted and the media were filter sterilized. All media were supplemented with 4 mM L-glutamine prior to use.

Prior to screening test hydrolysates, stock cells were passaged into EX-CELL™ 325 without hydrolysate for a brief culture interval. Cells were then “recovered” by passaging into complete media containing any of the test hydrolysates or a control hydrolysate at a seeding density of 2e5/mL (Figure 3). Additionally, an untreated control culture was maintained in parallel in order to assess how well the “recovered control” culture performed. Cell growth and viability were assessed using ViaCount reagent (Guava Technologies, Hayward, CA USA) and measured using a Guava PCA-96 (Guava Technologies). IgG titers were measured using an ELISA kit (Zeptomatrix, Buffalo, NY USA).

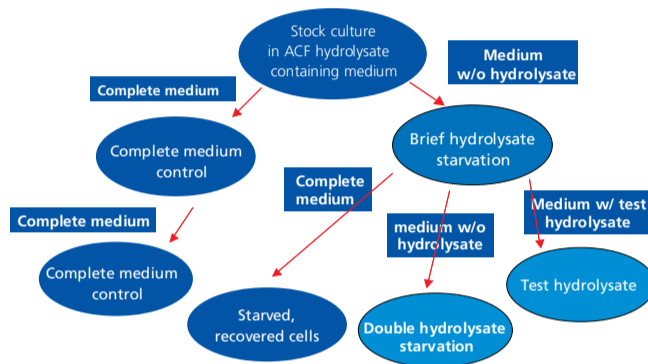


Figure 3: Cell culture screening process for test hydrolysates

## Chemical Composition

All test hydrolysates had the following analytical tests performed on them by DMV International: molecular weight profile, free and total amino acid analysis and elemental analysis.

## Results and Discussion

### Cell Culture

The goal for this development project included identification of key manufacturing parameters which affect cell culture performance in order to (1) improve consistency in performance as can be affected by manufacturing and (2) improve overall recombinant protein productivity. In our baseline analyses to assess performance in hydrolysate lots using the existing manufacturing process, six lots were screened for both cell growth and productivity using the outlined process (Figure 3). The performance amongst these initial six lots (“complete medium controls”, also known as untreated controls) demonstrated approximately a 1.5 – 2-fold range between lowest and highest for both growth and productivity (70–130% and 80–120%, respectively) (Figures 4, 5). This established the baseline lot-to-lot variability in hydrolysate performance for this cell culture application. Cell cultures exposed to a brief hydrolysate starvation (“starved-recovered”) yielded comparable results for growth (100 – 130%) when compared to untreated controls (70 – 130%) and slightly lower productivity (55 – 90%) than untreated controls (80 – 120%). Furthermore, cells cultured in the absence of hydrolysate (“double starvation”) yielded substantially lower growth and productivity (60 – 70% and 20 – 40% respectively) when compared to untreated controls and “starved recovered” cultures (100 – 130% and 55 – 90% respectively). The presence or absence of hydrolysate had a significant impact on both cell growth and productivity, allowing us to exploit this methodology for testing. The cells were capable of recovering comparably to that of the untreated controls when passaged into supportive hydrolysate, enabling us to identify which test hydrolysates performed positively. Cells cultured in the absence of hydrolysate maintained viability but did not grow or produce substantially, enabling us to identify hydrolysates which were potentially inhibitory or cytotoxic.

The initial 44 test hydrolysates tested in this manner (Figures 4, 5) indicated that some of the hydrolysates were as supportive as the control hydrolysate, some performed much better (by 220% for growth and 180% for productivity) and some yielded results worse than the hydrolysate-free condition, suggesting either inhibition or cytotoxicity caused by the test hydrolysate.

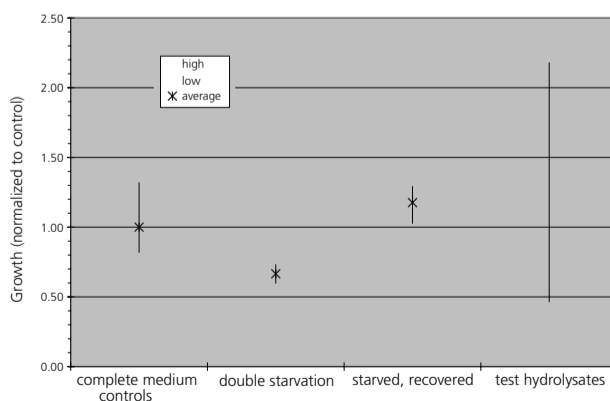


Figure 4: Cell growth, as reported by cell mass (integral viable cell density between days 0 and 7) and normalized to control

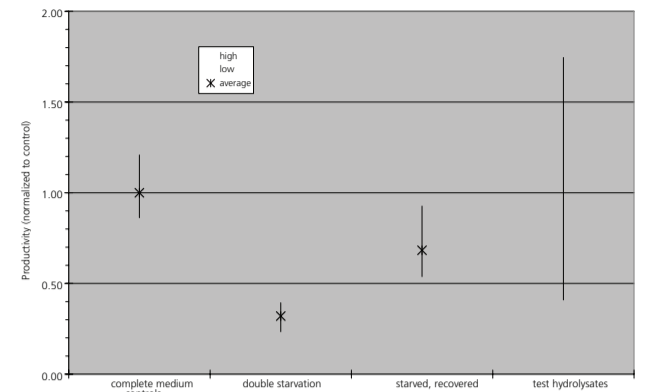


Figure 5: Productivity (day 7, normalized to control) in control cultures and those in test hydrolysates

The DoE analyses revealed that seven of the ten investigated manufacturing parameters significantly affected cell culture performance. One was found to have a negative effect; one had a significant effect on performance variability; one had an inverse relationship on growth and productivity; and four had positive impacts on cell culture performance. Additionally, analytical results indicated that manufacturing processes had significant effects on chemical composition (Figure 6). The range in measured component concentrations was typically between 2 and 5-fold, but sometimes higher (data not shown). By combining a collection of data from chemical composition analyses and cell culture using the test hydrolysates, it was possible to trend performance with respect to hydrolysate component concentration. Figure 7 demonstrates that modifications made to the standard manufacturing process resulted in productivity levels of up to 200% as compared to the starting process. This was accomplished by modifying the process in such ways as to affect component concentrations in final hydrolysate. These data demonstrate that improvement of existing soy hydrolysate is achievable through modifications to the manufacturing process and that by correlating chemical composition with cell culture data it may be possible to predict cell culture results based on specific analytical criteria.

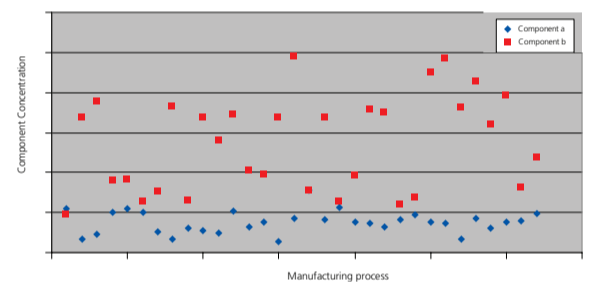


Figure 6: The effect of hydrolysate manufacturing process on component concentrations

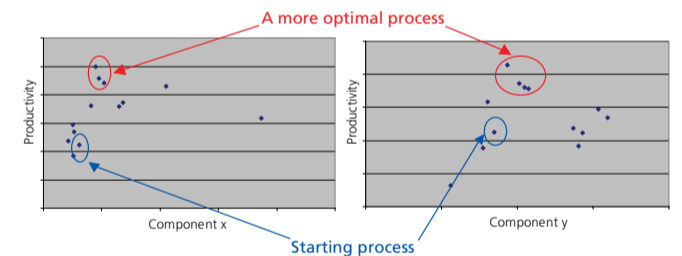


Figure 7: Analytical test results trend with cell culture performance

## Conclusions

These studies have demonstrated that the manufacturing processes used for soy hydrolysate have significant effects on both chemical composition as well as cell culture performance. Key manufacturing parameters were identified which had substantial impact, either positively or negatively, on performance in a cell culture application. Additionally, there were observed trends between chemical composition and cell culture performance, suggesting that analytical testing may be a valuable tool in assessing hydrolysate performance in cell culture applications.

Furthermore, these studies indicate that modifications to the existing process can result in a higher performing hydrolysate as indicated by the significantly improved productivity observed in some test hydrolysates.

## Acknowledgements

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