

Abstract

Today's biopharmaceutical and biotech industries require comprehensive knowledge of raw materials to achieve consistent culture process performance and product quality attributes. In response, SAFC launched a raw material characterization program to evaluate raw materials used in media preparations, employing biological and analytical methods. Soy hydrolysate was a key target in the program because it is a complex, undefined medium component and a recognized source of variability in animal cell culture processes.

SAFC has investigated variability in soy hydrolysates using biological assays to characterize growth, production and product quality using three industrially relevant cell lines. The study included four vendors and at least twenty-five lots of soy hydrolysate at a range of concentrations. The individual cell line responses to soy in the assay ranged from limited to highly dependent. While variability in cell growth and productivity was observed among soy lots from the same vendor, differences were more pronounced comparing soy among different vendors. Analytical methods were also employed to study product glycosylation resulting from different soy concentrations in the medium among all the lots. The lots demonstrated a soy concentration-dependent shift in glycosylation patterns.

Soy hydrolysate is just one of many components that SAFC continues to evaluate using various biological and analytical methods to characterize variability, impurities and identify markers for performance in complex raw materials.

Introduction

Biological assays are an important tool for screening raw materials (complete media or individual components) for culture performance. While these assays are subject to variability, suitable analytical methods for raw material characterization are not available for all raw materials. This is especially true for complex, undefined raw materials such as soy hydrolysate where growth, productivity and product quality have not been correlated with specific, analytically quantifiable performance markers.

SAFC utilizes a bioassay procedure based on a dose response model to evaluate equivalency of individual raw material lots originating from the same and different vendors. Three test cell lines are grown in a range of concentrations for each raw material lot in TPP tubes over a seven day batch culture assay. Growth, IgG titres and glycosylation patterns are considered over the dose response range to assess lot comparability. The following study describes the results of an evaluation of 25 soy hydrolysate lots, most from SAFC's qualified supplier of this raw material (Vendor A).

Materials and Methods

Cell Lines and Media

	Cell Line	Product	Assay Medium
Test Line 1	CHO	IgG	Sigma-Aldrich CD CHO Fusion (modified) P/N 82787C
Test Line 2	CHO	IgG	Sigma-Aldrich CD CHO Fusion (modified) P/N 82787C
Test Line 3	Avian	Vaccines	Sigma-Aldrich GRO I (modified) P/N 82788C

Cell Culture and Growth Assay

Cells are maintained and expanded in VWR vent cap shake flasks, 125 mL -1 L at 37 °C/ 5% CO₂ at 110-120 rpm. On the first day of the assay, expanded seed cells are washed and inoculated into replicate TPP tubes in test medium (30 mL working volume) with a range of soy concentrations (final concentrations of 0-15 g/L). A control using the same test medium lot was included in all assays for normalization of results. The cultures are grown in either a Kühner or Multitron shaking incubator at 37 °C/ 5% CO₂ at 200 rpm for 7 days.

Analytical Methods

Cultures are counted on the Cedex (Roche Innovatis, Germany) on days 0 and 3-7. Cultures are harvested on day 7. CHO Test Line 1 and 2 samples are assayed for IgG quantitation on the Octet QK using bio-layer interferometry (ForteBio, Inc, CA, USA). Samples for IgG glycosylation analysis were purified using a Protein-A batch method in 96-well plates (Protein A Agarose FastFlow, PALL Acroprep 96-Well Filter Plate, 0.45 µm and Waters 96-Well 350 µL Acuity collection plate). Glycoforms were analyzed by SEC-MS (Waters Acuity UPLC/Masslynx 4.1 software and TSK Gel SW3000XL, 300 x 2.0 mm, 4 µm).

All data was analyzed using SigmaPlot statistical software (Systat Software, Inc. SigmaPlot for Windows, Version 11.0.).

Results

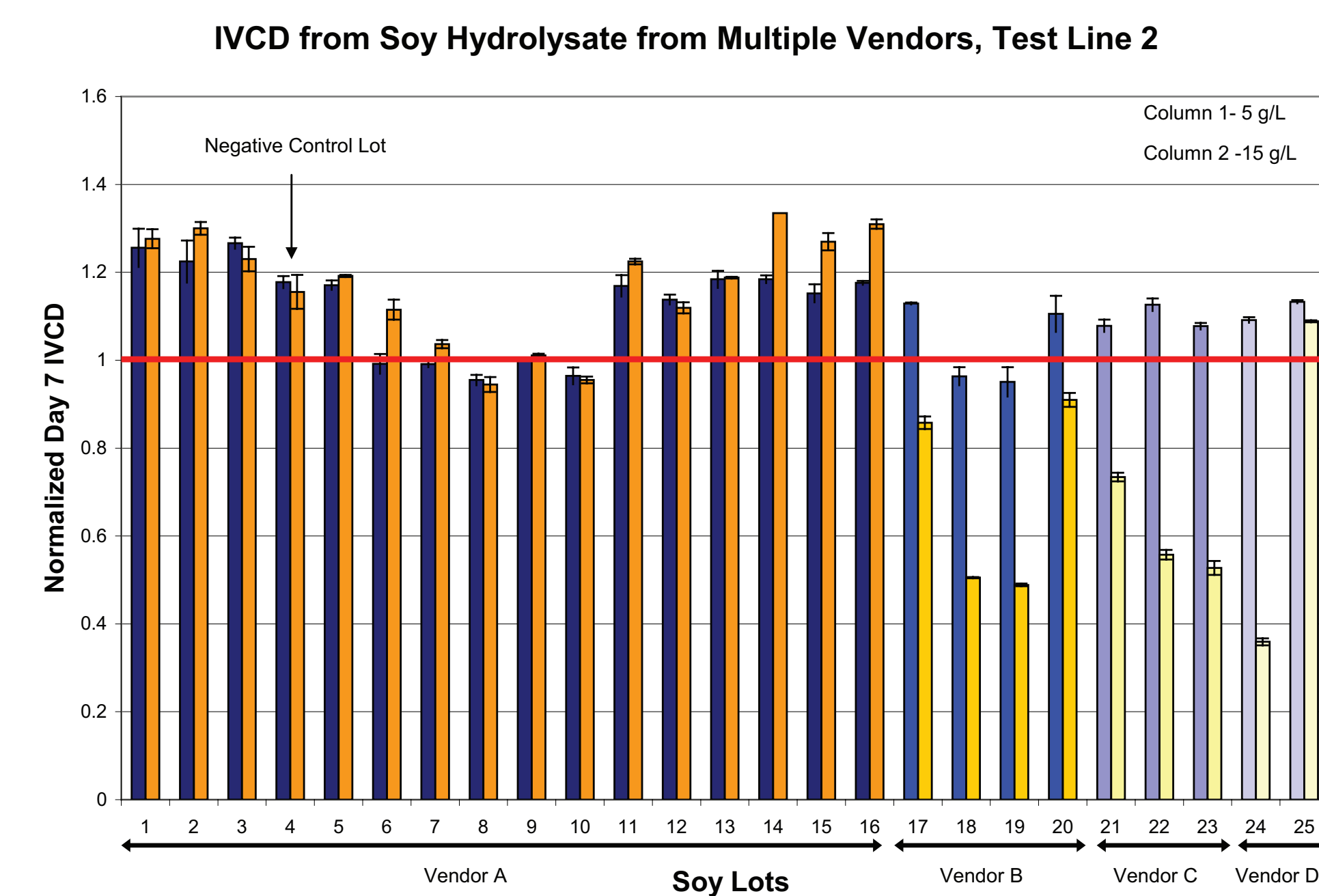


Figure 1: Normalized Integrated Viable Cell Density (IVCD) for 7 day cultures of Test Line 2 grown in 25 soy lots from 4 vendors. The IVCD was normalized to the assay control (red line). Test Line 2 is not dependent on soy hydrolysate and is therefore less sensitive to lot variations. At 5 g/L, significant inter-vendor lot differences were observed but there were few intra-vendor lot differences (except within vendor B lots). Soy at 15 g/L showed higher inter- and intra-vendor lot variability in growth. The negative control lot (lot 4) contains an inhibitory impurity but did not affect growth of this cell line.

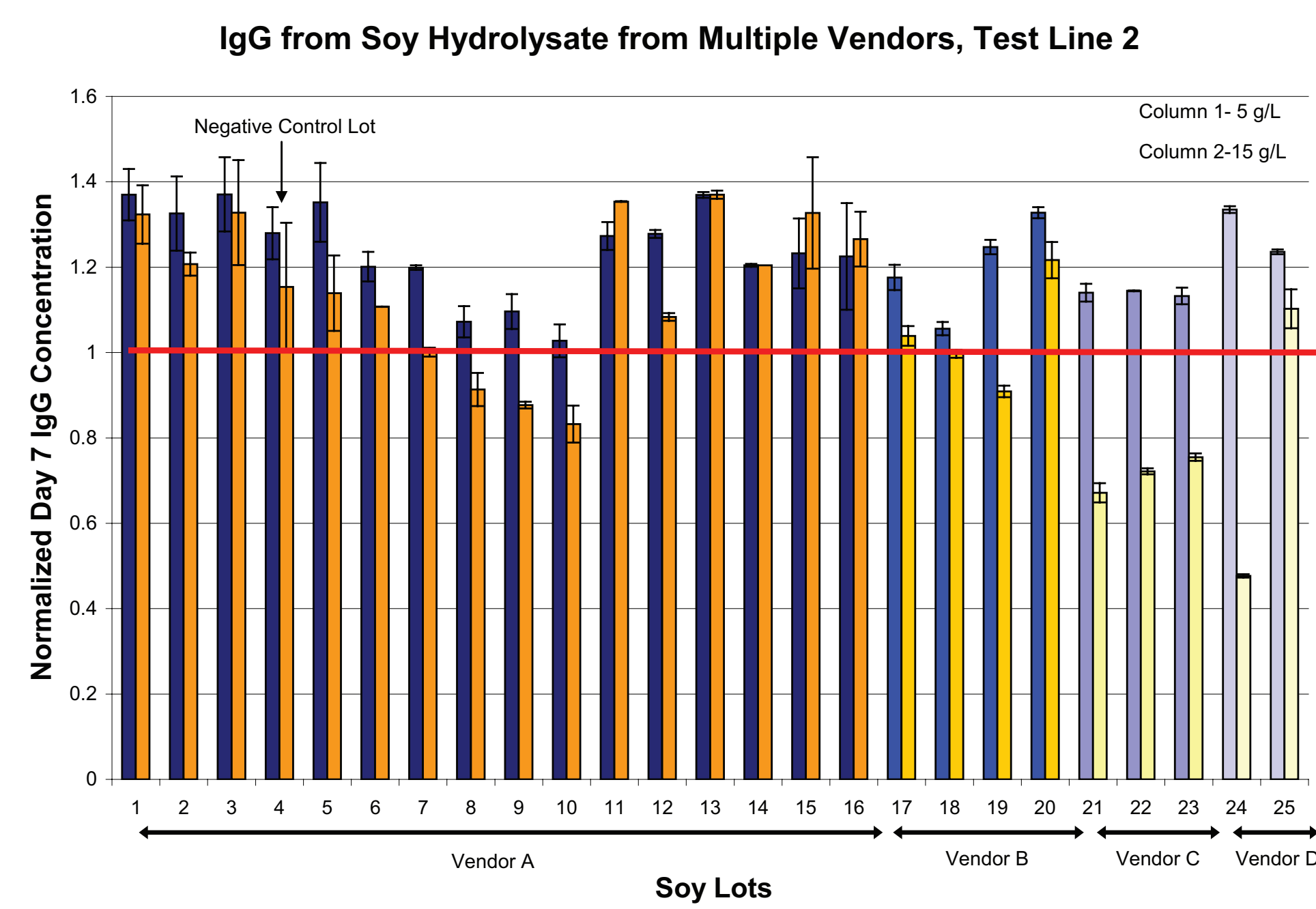


Figure 2: Normalized IgG concentration from day 7 for cultures of Test Line 2 grown in 25 soy lots from 4 vendors. The day 7 IgG titres were normalized to the assay control (red-line). At 5 g/L, there were significant inter-vendor and intra-vendor lot differences, but these differences were again more apparent at the 15 g/L soy concentration.

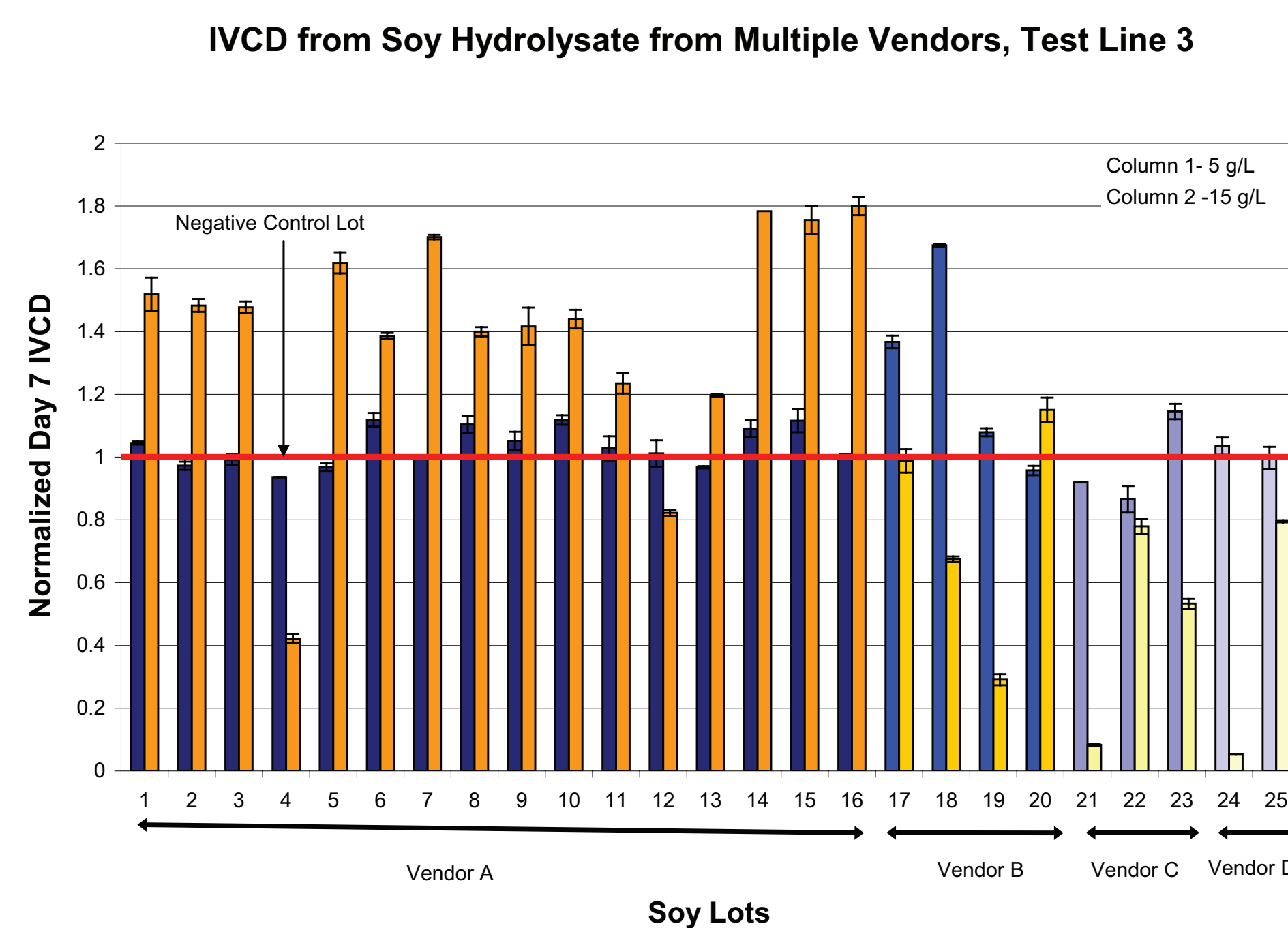


Figure 3: Normalized Day 7 IVCD for 7 day cultures of Test Line 3 grown in 25 soy lots from 4 vendors. The IVCD was normalized to the assay control (red line). Test Line 3 is dependent on soy for growth and is therefore the most sensitive to soy differences compared to the CHO test lines. There were significant inter-vendor lot differences at 5 g/L and 15 g/L. Within vendor A, growth was not significantly different at 5 g/L but intra-vendor lot differences were significant at 15 g/L.

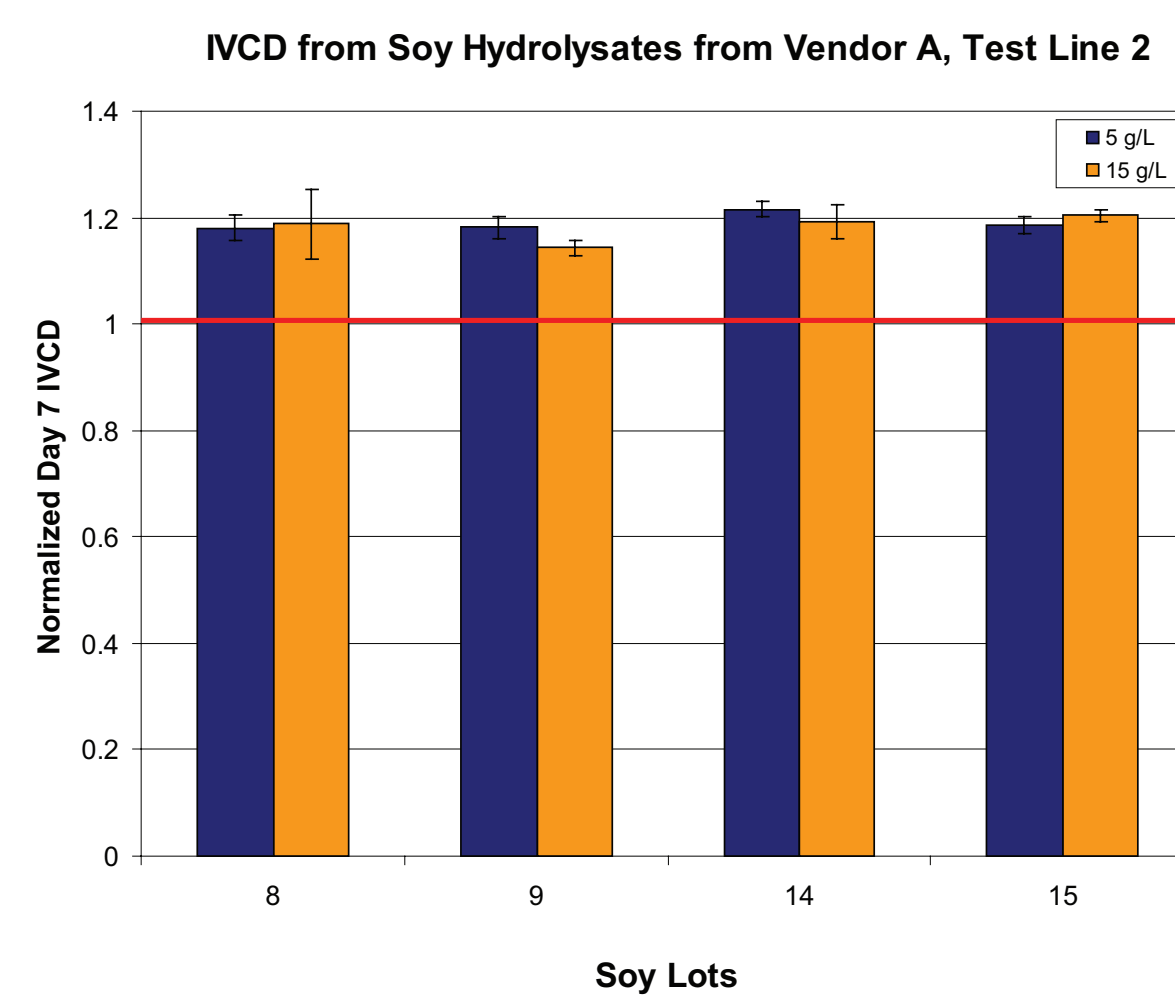


Figure 4: Normalized Integrated Viable Cell Density (IVCD) for Test Line 2 from selected Vendor A lots. Four lots (8, 9, 14 and 15) from Vendor A were chosen from the first 25 lot screen to be run for further evaluation of lot differences and eliminate inter-assay variability. This experiment used triplicate cultures from two operators (n=6) for each of the soy lots at 4 different concentrations (5 g/L and 15 g/L shown).

A) IVCD - 5 g/L		B) IVCD - 15 g/L		
LOT #	8	9	14	15
8	N	Y	N	N
9		N	Y	N
14			N	Y
15				N

Table 1: ANOVA analysis for Test Line 2 IVCD grown with 5 g/L and 15 g/L of 4 selected soy hydrolysate lots (refer to Figure 4). Significant differences are indicated by "Y" and insignificant differences by "N" ($\alpha = 0.05$). (A) There is a significant difference for growth at 5 g/L for lot 14 compared to other lots. However, the graph shows this to be a minor difference. (B) There is no significant difference for the growth between any of the lots at 15 g/L based on ANOVA analysis. This confirms that Test Line 2 has a limited ability to distinguish soy lots based solely on growth. Apparent differences between these lots were greater in the initial screen (Figure 1) due to inter-assay variability.

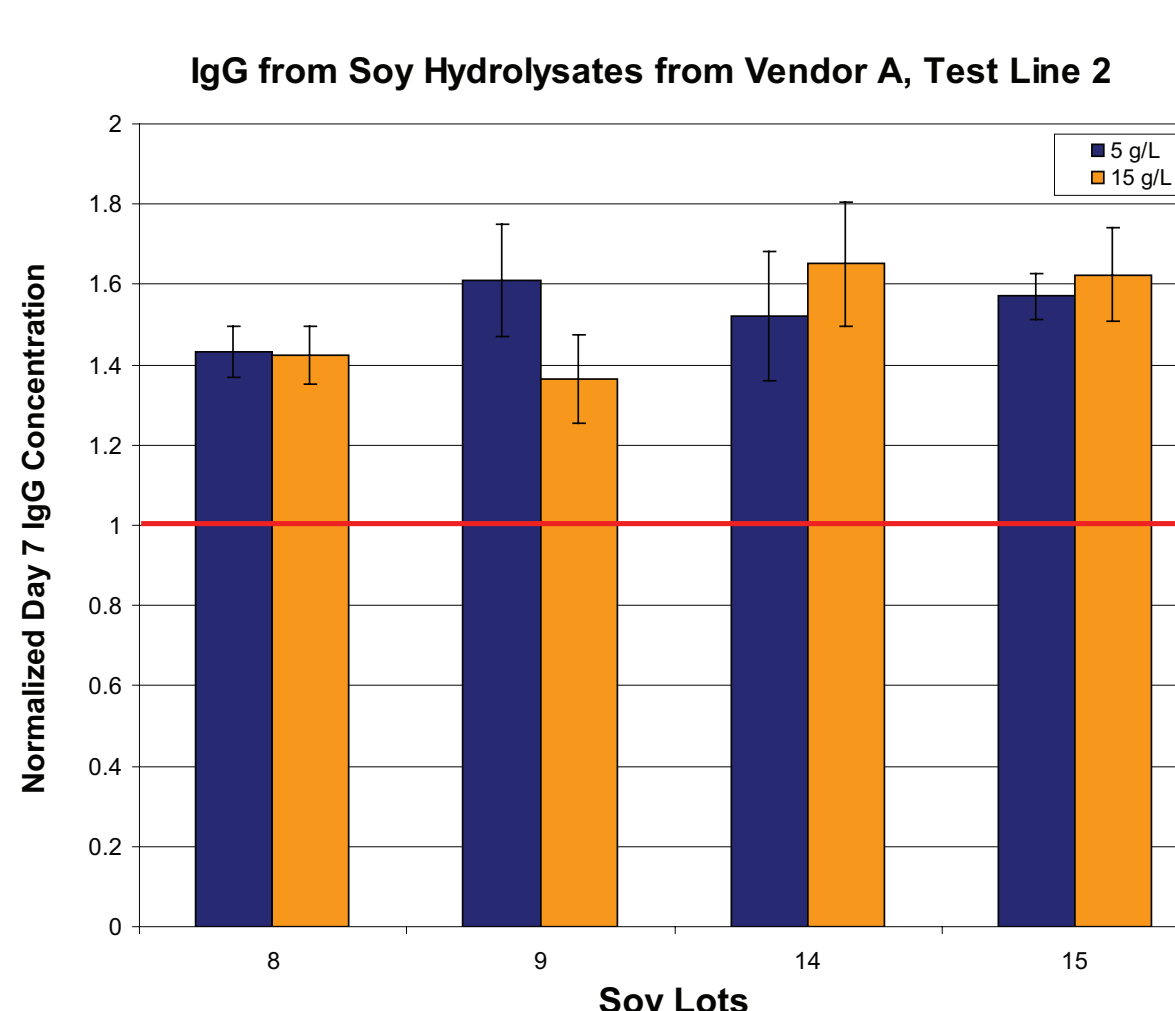


Figure 5: Normalized IgG titres for Test Line 2 from selected vendor A lots assayed in Figure 4. This graph depicts the IgG titres on Day 7 from cultures with soy lots 8, 9, 14 and 15 for confirmation of IgG production.

A) IgG - 5 g/L		B) IgG - 15 g/L		
LOT #	8	9	14	15
8	N	N	N	N
9		N	N	N
14			N	N
15				N

Table 2: ANOVA analysis for Test Line 2 IgG titres for cultures with 5 g/L and 15 g/L of 4 selected soy hydrolysate lots (refer to Figure 5). (A) There is no significant difference (N) for productivity at 5 g/L between any of the lots. (B) At 15 g/L there is no significant difference between lots 8 and 9 or between 14 and 15. However, there is a significant difference (Y) for productivity between lots 8, 14 and 15 as well as 9, 14 and 15 ($\alpha = 0.05$). This confirms that differences in the productivity are more likely at a higher concentration of soy hydrolysate.

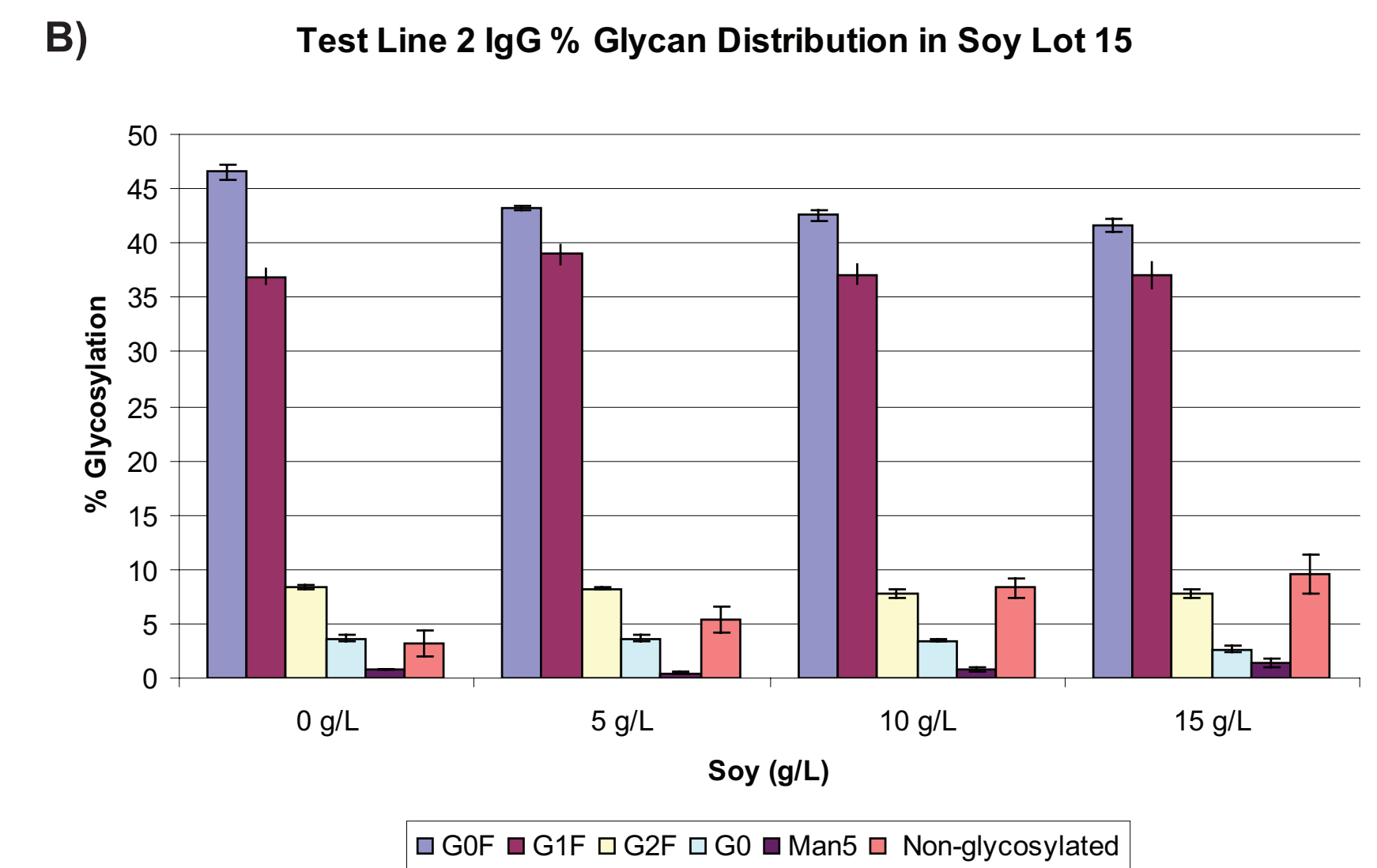
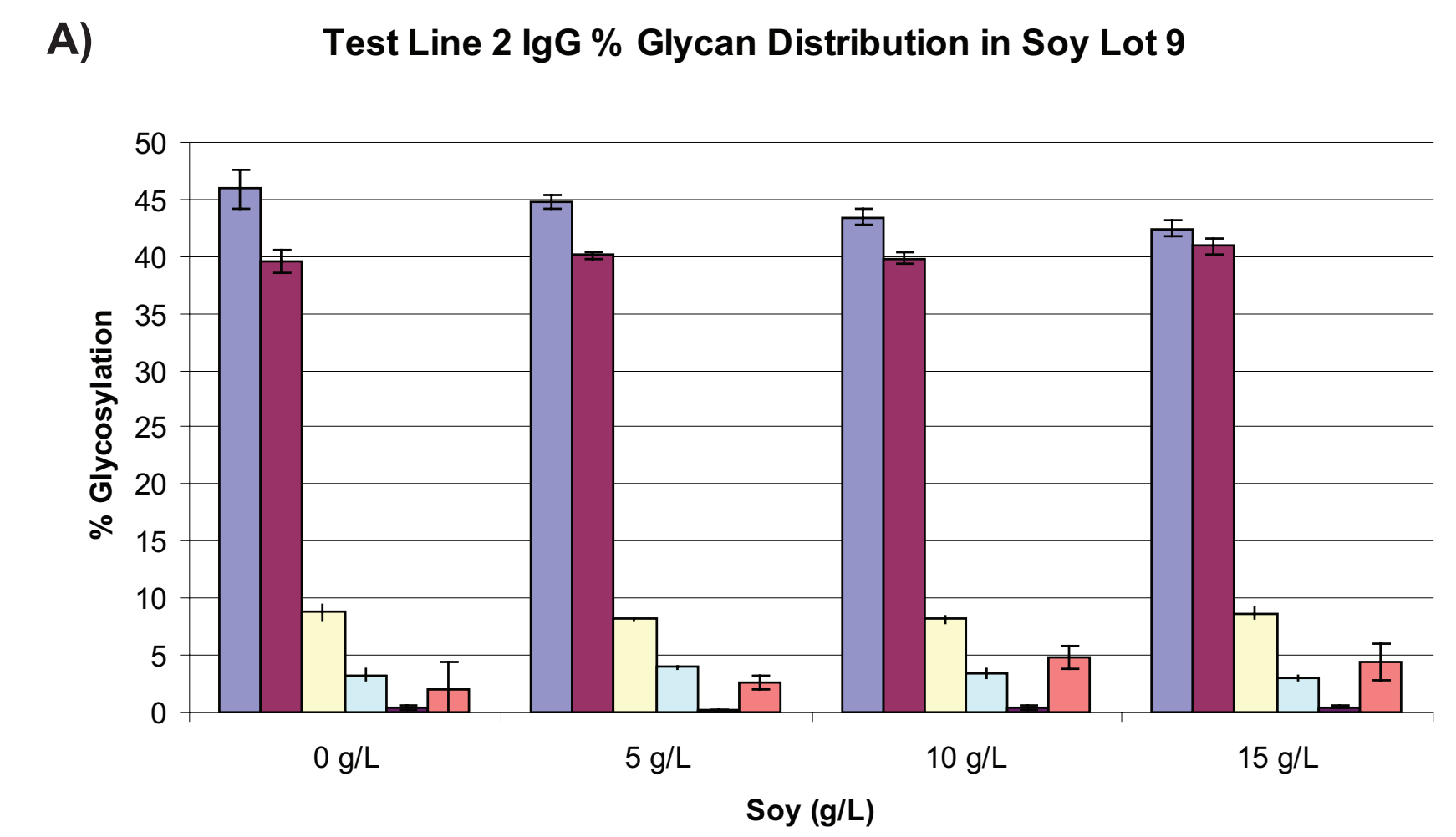


Figure 6: IgG N-linked glycan distribution for Test Line 2 (high IgG producer) was determined for four selected soy lots (refer to Figure 5). Test Line 2 was run in replicate TPP cultures (n=6) for the four soy lots at 0, 5, 10 and 15 g/L. The % glycan distribution is shown for (A) soy lot 9 and (B) soy lot 15. Increasing soy additions resulted in a trend of increased non-glycosylated moiety for soy lot 15 but this pattern was less apparent in soy lot 9. IgG glycan distribution was similar when comparing soy lots 8 to 9 and also 14 to 15.

	Soy Concentrate (g/L)			
	0 g/L	5 g/L	10 g/L	15 g/L
G0F	N	Y	Y	Y
G1F	Y	N	Y	Y
G2F	N	Y	Y	Y
G0	N	N	N	Y
Man5	N	N	Y	Y
non-glycosylated	N	Y	Y	Y

Table 3: ANOVA analysis of IgG % glycan distribution for Test Line 2 grown with four soy lots (8, 9, 14, 15) at 0, 5, 10 and 15 g/L. ANOVA was performed on replicate % glycan values (n=6) to compare each glycan moiety at a specific soy concentration. A significant difference among each glycan species is designated by "Y" and insignificant as "N" ($\alpha = 0.05$). For example, % G0F replicate values at 5 g/L for each soy lot were compared in the ANOVA analysis. Increasing soy concentration resulted in differences among more of the glycan species. Differences in glycan profiles among lots were therefore most apparent at the highest soy concentration tested (15 g/L).

Discussion

The test cell lines used for SAFC's RMC bioassay screening were selected based on sensitivity to a range of raw materials. Test Lines 1 and 2 are rCHO lines that have a limited growth and production response to this raw material. Test Line 3 was established as dependent on hydrolysate for growth. The effect of soy lot and concentration on product quality are also a key part of the RMC program. Therefore, results for Test Line 2, which is a higher IgG producer, was a focus for this study.

Figures 1 and 3 indicate that both Test Lines 2 and 3 are relatively insensitive to soy lot differences in terms of growth at lower supplement concentrations (5 g/L), even when comparing different vendors (inter-vendor). However, when the soy concentration is increased to 15 g/L, lot differences can clearly be detected in lots, even within the same vendor (intra-vendor) for Test Line 3. Productivity differences were also most apparent at the high soy concentration (Figure 2). Since these lots were screened over several assays, albeit with a reference control in each assay, inter-assay variability could account for some of the observed growth and/or productivity differences. Four lots were therefore selected among these and tested with additional replicates for Test Line 2. Growth differences were minor, even at 15 g/L (Figure 4). Productivity was generally higher in lots 14 and 15 compared to 8 and 9 at 15 g/L hydrolysate (Figure 5). This was consistent with the initial 25 lot screen but were less distinct in the second screen with the four lots performed together in the same assay. This suggests that inter-assay variability accounts for some of the observed lot differences.

Glycosylation analysis of the four selected soy lots 8, 9, 14 and 15 confirmed soy lots from the same vendor induced different glycan distributions. These differences are shown in Figure 6, where lot 9 has higher G1F and lower non-glycosylated IgG at most soy concentrations compared to lot 15. These differences were most apparent at the highest Soy concentration (15 g/L). The overall glycosylation patterns were also more similar when comparing lots 8 to 9 and 14 to 15 (data not shown). Table 3 also confirmed with ANOVA analysis that higher soy concentrations resulted in more glycosylation differences among lots. Although these differences represent relatively small changes in individual glycosylation moieties in this assay, these lots may induce greater differences in fed-batch bioreactor cultures or with other cell lines more sensitive to hydrolysate. Subtle glycosylation differences, particularly in fucosylated forms, may also have a significant impact on biological activity. Together these results confirm a soy concentration and lot-dependent effect on IgG product quality.

Conclusion

- Variation in culture performance resulting from soy hydrolysate supplements in animal cell culture media can be evaluated using TPP tube batch culture assays.
- Cell growth, product yield and product quality differences occur within the same hydrolysate vendor (intra-vendor).
- Higher soy concentrations are likely to emphasize lot-induced variation.
- Standardized bioassays may be an effective tool for soy hydrolysate performance screening or lot matching.

Acknowledgements

The authors would like to thank Analytical Research and Development (ARD) (St. Louis, MO) for performing glycosylation analysis of test samples.