FrieslandCampina Ingredients

The influence of process parameters on soy hydrolysate chemical composition

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Introduction

Proyield® Soy SE50MAF-UF is an animal component-free protein hydrolysate manufactured by FrieslandCampina Ingredients. It is widely used in mammalian cell culture processes, such as Chinese hamster ovary (CHO) cell systems, as part of basal or feed media formulation. It has been shown to complement commercially available chemically defined (CD) media to enhance cell culture performance. The manufacturing of Proyield® Soy SE50MAF-UF involves multiple steps including protein hydrolysis, multiple filtration steps and drying of the end product to powder. In this poster, FrieslandCampina Ingredients presents the work from our Quality by Design (QbD) project to demonstrate the use of production data and statistical analysis. The goal is to elucidate how the chemical composition of the hydrolysate can be controlled via process.

Quality by Design (QbD)

QbD is a systematic approach to development with emphasis on the product, process understanding and its control. The European Medicines Agency (EMA) stated "One of the goals of quality by design is to ensure that all sources of variability affecting a process are identified, explained and managed by appropriate measures"[1]. FrieslandCampina Ingredients has implemented this systematic approach to our study of Proyield® Soy SE50MAF-UF manufacturing process. Figure 1 illustrates the approach during this project. It was divided into three stages: data mining, statistical analyses and Design of Experiment (DOE) studies.

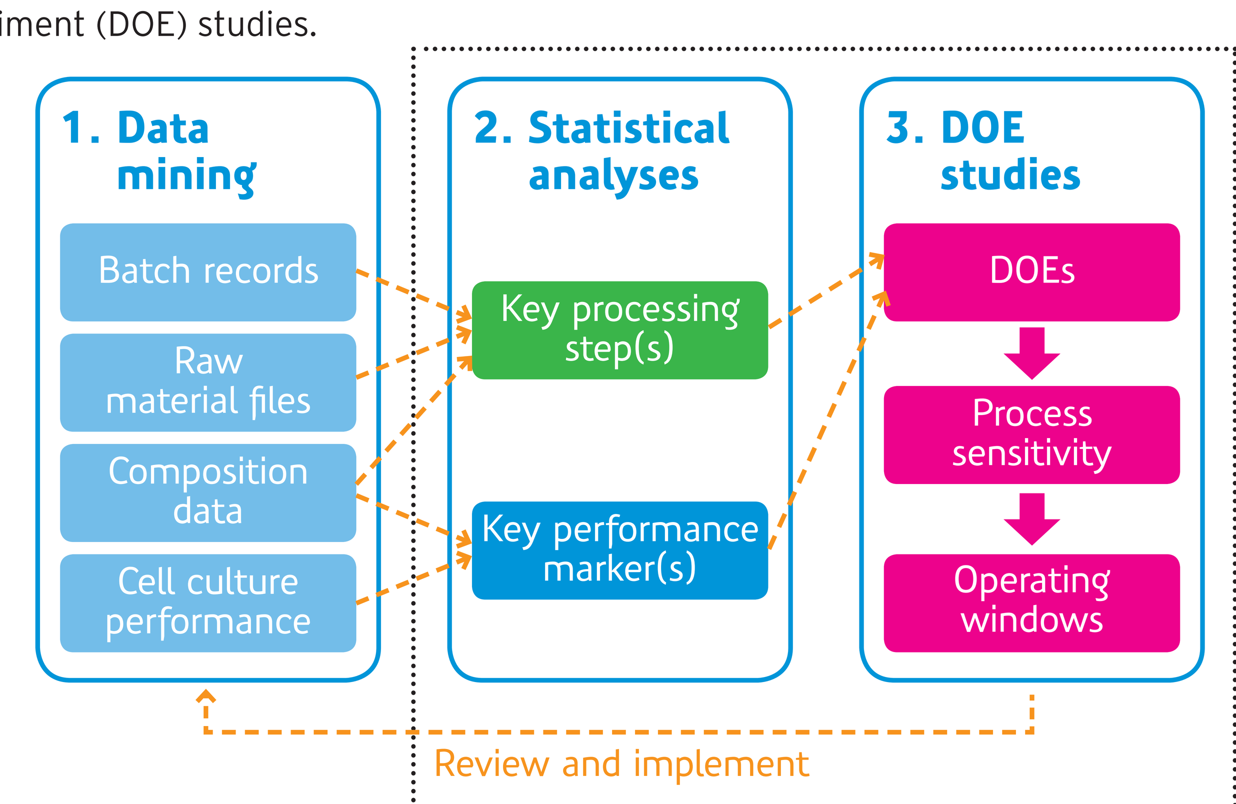

Figure 1: A schematic showing FrieslandCampina Ingredients approach in our QbD project. The dotted black section shows our study on the effect of our process on the composition of Proyield® Soy SE50MAF-UF. (*DOE were carried out at pilot scale).

Results and discussion

Data analyses were conducted, correlating performance from multiple cell culture systems against composition data of Proyield® Soy SE50MAF-UF. 20 cell culture performance indicators shared across multiple cell culture systems were identified by FrieslandCampina Ingredients (Figure 2). This list of indicators was generated by correlating against each chemical component (700+, study not shown here) in Proyield® Soy SE50MAF-UF.

5 amino acid derivatives	4 for product titre and cell growth	+ve
	1 for product titre	-ve
5 unique derivatives	4 for product titre and cell growth	+ve
	1 for cell growth	+ve
3 Lipid derivatives	1 for product titre and cell growth	+ve
	2 for product titre	+ve
		-ve
2 carbohydrate derivatives	2 for product titre and cell growth	-ve
2 nucleotide derivatives	1 for product titre	+ve
	1 for cell growth	-ve
2 peptides	1 for product titre and cell growth	+ve
	1 for cell growth	+ve
1 xenobiotic from plant	1 for product titre	-ve

Figure 2: Shared cell culture performance indicators identified in Proyield® Soy SE50MAF-UF hydrolysate using multiple cell culture systems. +ve indicates positive impact on cell culture whereas -ve indicates negative impact on cell culture study.

By maximizing the 14 positive indicators and minimizing the 6 negative indicators, a favorable hydrolysate composition can be achieved to yield high performing batches. Therefore, it is critical to identify factor(s) which can influence the levels of those indicators in the end hydrolysate.

References 1. www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000162.jsp

FrieslandCampina Ingredients recorded detailed manufacturing data for each production batch. The production data was used to study any statistically significant correlation between each process variable against each of the 20 shared performance indicators (Figure 2). A "heat map" was produced (Figure 3) to identify correlating quality control parameters (QCP's) within the manufacturing design space. This study shows there is a key manufacturing step which can significantly influence half of the identified shared performance indicators. Further study has shown that the process response for the key manufacturing step correlates strongly with the identified shared indicators. This provides FrieslandCampina Ingredients with the opportunity to improve the hydrolysate performance within the current manufacturing design space.

Process step	Process parameters	Amino acid derivative	Carbohydrate derivative	Amino acid derivative	Carbohydrate derivative	Lipid derivative	Amino acid derivative	Amino acid derivative	Nucleotide derivative	Xenobiotic (from plant)	Nucleotide derivative	Lipid derivative	Peptide	Peptide	Lipid derivative	Unique component	Unique component	Unique component	Unique component	Unique component	Amino acid derivative
A	A1	−0,33	0,38	0,55	−0,31	0,00	−0,45	0,26	0,42	−0,71	0,38	−0,12	0,40	−0,45	0,71	−0,31	0,26	0,86	−0,43	0,69	−0,07
B	B1	0,21	−0,38	−0,31	−0,29	0,05	0,39	0,31	−0,25	−0,14	−0,38	0,12	0,15	0,01	0,06	0,35	0,35	0,18	0,35	0,57	0,04
B	B2	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
B	B3	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
C	C1	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
C	C2	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
D	D1	0,20	0,15	0,50	−0,11	0,63	0,15	0,13	0,26	−0,30	0,35	−0,06	0,21	0,37	0,20	0,07	0,24	0,14	0,15	−0,09	0,46
D	D2	0,20	0,15	0,50	−0,11	0,63	0,15	0,13	0,26	−0,30	0,35	−0,06	0,21	0,37	0,20	0,07	0,24	0,14	0,15	−0,09	0,46
E	E1	−0,23	0,34	−0,40	0,59	0,07	−0,48	−0,61	0,19	0,01	−0,14	−0,35	−0,48	−0,37	−0,12	−0,44	−0,31	−0,22	−0,33	−0,33	−0,22
E	E2	−0,09	0,24	−0,29	0,44	−0,03	−0,29	−0,57	0,05	−0,06	−0,18	−0,21	−0,35	−0,23	−0,11	−0,27	−0,19	−0,19	−0,20	−0,19	−0,07
E	E3	0,22	−0,11	0,32	−0,18	0,13	0,43	0,07	−0,03	0,14	−0,07	0,32	0,17	0,66	−0,32	0,27	0,18	0,05	0,33	0,07	0,48
E	E4	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
E	E5	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
E	E6	0,12	−0,03	−0,31	0,05	−0,15	0,07	0,04	−0,13	0,06	−0,53	−0,20	−0,02	−0,13	−0,21	0,04	−0,06	−0,25	−0,07	−0,08	−0,06
F	F1	−0,67	0,79	−0,30	0,81	−0,06	−0,83	−0,76	0,37	0,41	−0,14	−0,42	−0,78	−0,41	−0,03	−0,91	−0,84	−0,47	−0,81	−0,56	−0,29
F	F2	0,59	−0,74	0,25	−0,68	0,15	0,73	0,65	−0,34	−0,40	0,23	0,50	0,76	0,34	0,09	0,87	0,87	0,62	0,86	0,61	0,24
G	G1	0,48	−0,68	0,03	−0,36	0,16	0,48	0,43	−0,23	−0,32	0,00	0,03	0,43	0,32	−0,25	0,60	0,57	0,26	0,59	0,15	0,20
G	G2	0,48	−0,68	0,03	−0,36	0,16	0,48	0,43	−0,23	−0,32	0,00	0,03	0,43	0,32	−0,25	0,60	0,57	0,26	0,59	0,15	0,20
G	G3	0,47	−0,62	0,00	−0,30	0,10	0,41	0,44	−0,27	−0,25	−0,03	−0,05	0,34	0,18	−0,21	0,53	0,44	0,15	0,47	0,01	0,07
H	H1	−0,57	0,03	−0,48	0,16	0,00	−0,45	−0,20	0,18	−0,10	0,20	−0,23	0,02	−0,43	0,02	−0,22	−0,07	0,30	−0,10	0,25	−0,46
I	I1	−0,54	0,77	0,37	0,31	0,54	−0,54	−0,54	0,20	0,26	0,03	−0,31	−0,03	0,14	−0,14	−0,66	−0,37	−0,20	−0,43	−0,26	0,37
J	J1	−0,32	0,40	−0,29	0,46	−0,07	−0,37	−0,58	0,11	0,24	−0,36	−0,08	−0,44	−0,42	−0,04	−0,38	−0,36	−0,02	−0,38	0,12	−0,24
J	J2	−0,02	−0,24	−0,09	−0,17	0,24	0,18	0,09	−0,06	−0,16	−0,09	−0,04	0,17	−0,10	0,05	0,24	0,38	0,40	0,24	0,68	−0,01
J	J3	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
J	J4	0,24	0,13	0,51	−0,13	0,62	0,19	0,16	0,21	−0,30	0,33	−0,02	0,23	0,38	0,17	0,11	0,27	0,15	0,19	−0,06	0,48
J	J5	−0,17	−0,05	−0,11	−0,02	0,30	0,13	−0,12	0,23	0,52	0,04	−0,15	0,04	0,04	−0,55	0,13	−0,08	−0,13	0,02	−0,07	−0,10
K	K1	0,20	0,15	0,50	−0,11	0,63	0,15	0,13	0,26	−0,30	0,35	−0,06	0,21	0,37	0,20	0,07	0,24	0,14	0,15	−0,09	0,46
K	K2	0,20	0,15	0,50	−0,11	0,63	0,15	0,13	0,26	−0,30	0,35	−0,06	0,21	0,37	0,20	0,07	0,24	0,14	0,15	−0,09	0,46

Figure 3: A correlation heat map between hydrolysate manufacture data against each shared cell culture performance indicator (Figure 2). The values showed the Spearman's ranked correlation coefficients between each process step and each shared marker. The orange section highlights the process parameters which have the most significant impact on the levels of shared performance markers. The heat map was generated from 60 lots of Proyield® Soy SE50MAF-UF (n = 60, P< 0.01).

The statistical analysis indicates a favorable mixture of the shared performance indicators (Figure 2) in Proyield® Soy SE50MAF-UF can be achieved by careful control of the QCP's (Figure 3). With this information, FrieslandCampina Ingredients conducted a DOE to identify a robust normal operating range (NOR) to improve the performance of Proyield® Soy SE50MAF-UF.

The DOE results confirm a tighter control of QCP's can yield a desired process response. The resultant contour plots were constructed (Figure 4) to see where the NOR is located within the design space. The orange region in both contour plots provide a robust NOR and yielded a desired process response which is in agreement to our initial correlation studies using production records.

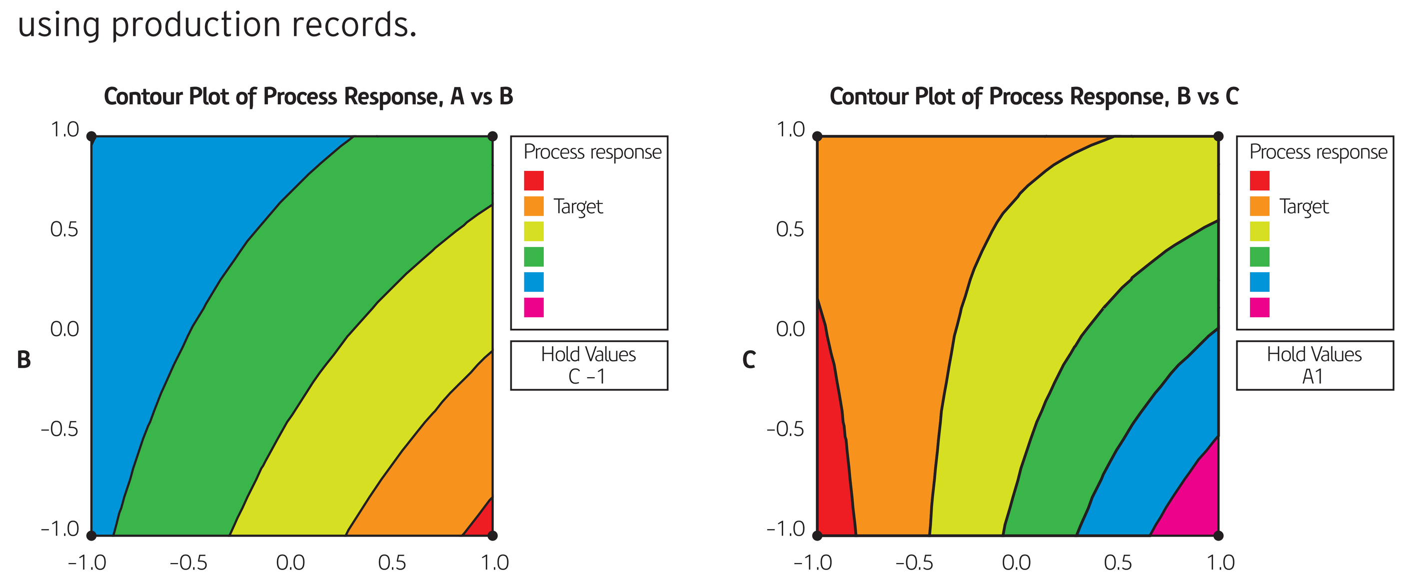

Figure 4: Contour plots of process response F1, using DOE experiment results at pilot scale. Three process parameters (A and B on the left, B and C on the right) were varied during the experiment (normalized range, -1 to +1) based on a two level full factorial design.

Conclusion

FrieslandCampina Ingredients has shown that production data can yield valuable insight into process control and yield hydrolysate with higher cell culture performance and improved consistency. By combining this knowledge with DOE experimentation, FrieslandCampina Ingredients has located a robust NOR within the current manufacturing design space.