Wüst A., Schmidt A., Klausing S., Heinrich C., Northoff S.

Xell AG, Bielefeld, Germany | www.xell.ag

presenting author | anica.schmidt@xell.ag

Define the undefined - Towards real chemically defined media in cell culture

Nowadays stable and reproducable bioprocesses are needed to guarantee biopharma productions with low lot-to-lot variations. The medium formulation as an important influence factor have to be controlled to achieve high medium consistencies. The specific influences of impurities are foremost process and cell line dependend, therefore the specific response has to be checked individually. The usage of controlled high quality products for medium formulations can exclude cost intensive screenings for the impact of single components on product qualities and process stabilities.

We observe concentration variations of trace elements in proteinogenic amino acids from different supplier and quality grades. Substantial impurity profils were measured for cystine and tyrosine, whereas all other components show significant lower values of trace elements, indepedend of the tested quality grade.

RESULTS

A. Trace element impurities of amino acids

The most popular impurities of components used in cell cultivation media with an impact on process performances are trace elements. Here, an analytical screening of different proteinogenic amino acids is shown to evaluate trace element contaminations for 12 different suppliers (amino acid-dependent) and quality grades. The resultes illustrate amino acid-dependent impurity profiles. How important these factors are for process performance is extremly cell line- as well as product- dependent and have to be evaluated seperatly, and an examplary screening would not be meanigful. Additional impurities (e.g. organic compounds) are not investigated here, but they also have to be taken into account as possible impurities.

Amino acids	
L-Alanin	L-Isoleucine
L-Arginine	L-Leucine
L-Asparagine	L-Lysine x HCl
L-Asparagine x H_2O	L-Methionine
L-Aspartic acid	L-Phenylalanine
L-Cysteine	L-Proline
L-Cystine	L-Serine
L-Cystine x 2 Na x H_2O	L-Threonine
L-Cystine x 2 HCl	L-Tryptophan
L-Glutamic acid	L-Tyrosine
L-Glutamic acid x Na	L-Tyrosine x 2 Na
L-Glutamine	L-Tyrosine x 2 Na x 2 H_2O
Glycin	L-Valine
L-Histidine x HCl x H_2O	

FIG. 1: Tested amino acids that are regularly used in cell cultivation media with different quality grades (min. 97 %) where tested for 10 trace element contaminations. All over, 12 different supplier where investigated, but not every supplier deliever each amino acid shown.

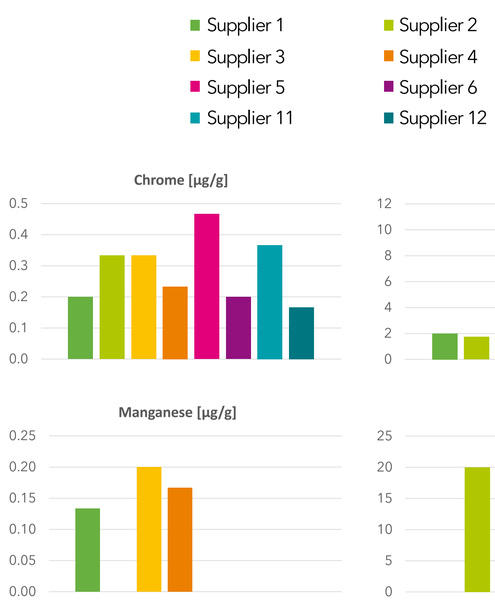

FIG. 2 : Highest trace element contaminations for **glutamic acid batches** of 8 different suppliers were detected for chrome, copper, manganes and iron.

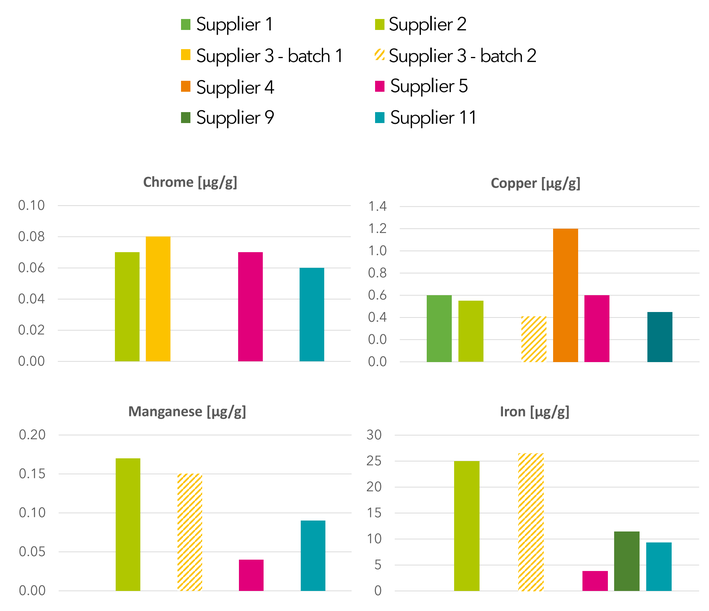

FIG. 3: Highest trace element variations for 7 different suppliers of **asparagine batches** were detected for chrome, copper, manganese and iron.

B. Cystine and Tyrosine impurities

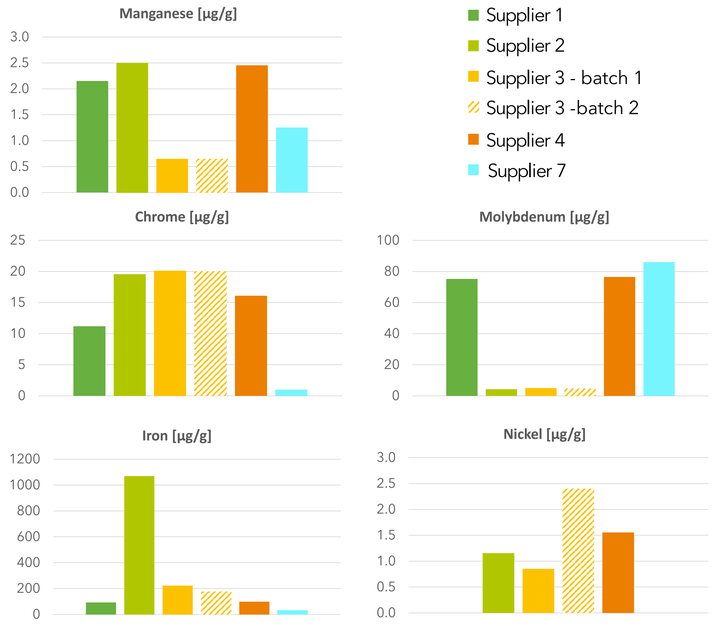

FIG. 4 : Detection of concentration variations of Cr, Mn, Fe, Ni and Mo in **cystine batches** of 5 different suppliers and different quality grades.

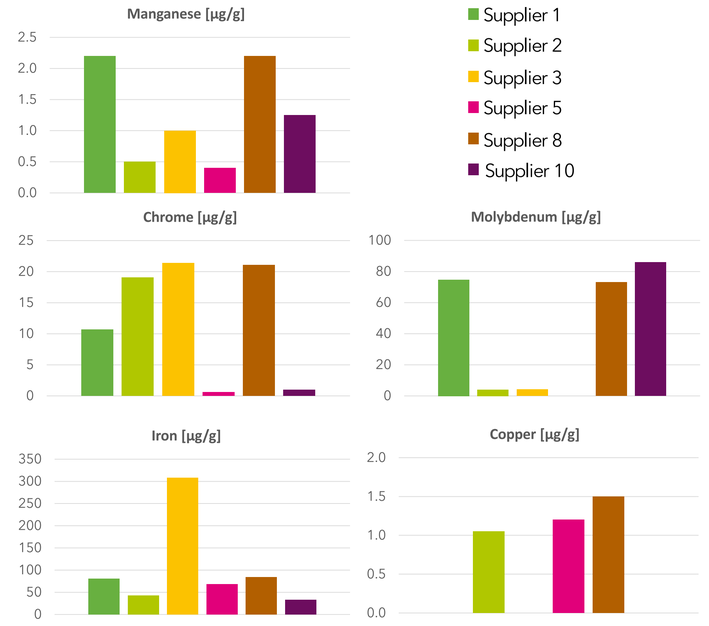

FIG. 5 : Analysis of trace element impurities (Cr, Mn, Fe, Mo, Cu) in **tyrosine batches** of 6 different suppliers, with different quality grades.

METHODS

Trace element Analytics

The chemicals were solubilized at different concentrations (solubility-dependent) and analysed via ICP/MS.

LOQ values for measured trace elements

Trace element	LOQ [µg/L]
Cadmium	0.2
Chrom	0.5
Cobalt	0.2
Copper	2
Iron	10
Manganese	0.2
Molybdenum	0.5
Nickel	0.5
Selenium	2
Zinc	50

CONCLUSIONS

- Amino acids are one of the major components in media formulations used in cell cultivation, therefore the quality of the raw material have to be evaluated to identify possible contaminations that can influence the cultivation performance.
- Known contaminations that can have an impact on cell cultivation processes at low concentration levels are trace elements like iron, zinc, copper and manganese, less investigated are nickel, chrome and molydbenum, which are also detectable as impurities.
- Most of the tested amino acids are without detectable impurities for tested elements, for glutamic acid weak concentrations of chrome (0.47 µg/g for 99% quality grade) and copper (11 µg/L for pure quality grade) were measured. In addition, a high iron concentration in one asparagine batch up to 26.5 µg/L (98% quality grade) could be detected.
- The qualitiy grades varied between pur. (purum; 97%) up to p.a (pro analysis) and amino acids signed as cell culture reagent. The data indicate no concrete trend in terms of higher trace element contaminations for lower quality grades, therefore the values have to be checked individually.
- As the effect of trace elements is higly component, cell line and product specific, also lower concentration ranges (shown for TYR, CYS-CYS, GLU and ASN) have to be taken into account for a precise chemical definition of a medium.
- The trace element impurity amount was highest for the proteinogenic amino acids tyrosine and cystine, whereas all other tested component show significant lower impurities (data not shown).
- Detailed analytics show highest concentration values for iron (up to 1070 µg/g) and molybdenum (up to 85 µg/g) impurities in cystine of different suppliers.
- High contamination values of tyrosine and tyrosine sodium salt batches are detected for iron (309 µg/g), molybdenum (74.7 µg/g) and chrome (19 µg/g).

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