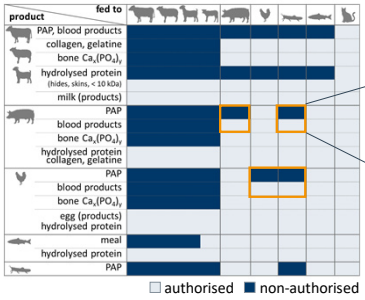
German Federal Institute for Risk Assessment

Distinguishing animal-derived feed ingredients based on their processing status

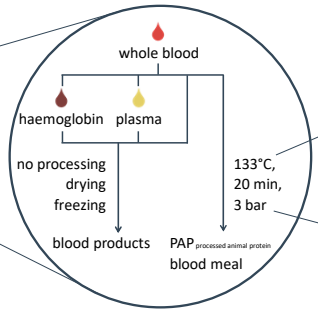
Tobias Stobernack, Paula Mühlpforte, Uta M. Herfurth
National Reference Laboratory for Animal Protein in Feed, Department Food Safety, German Federal Institute for Risk Assessment (BfR)

Introduction

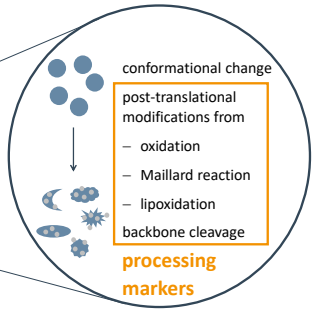
Feeding Rules: Regulation 999/2001



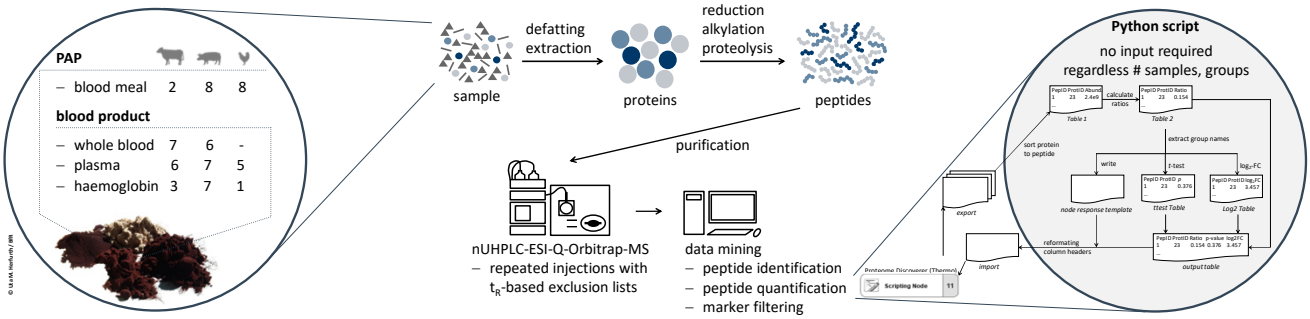
Processing Animal-derived Feed Ingredients: Regulation 142/2011



Processing Effects on Proteins



Experimental



peptide normalisation to its corresponding proteins' abundance

Results

Dataset overview

	identified peptides		
non-modified[a]	6,710	6,654	7,613
modified[a]	12,958	10,437	3,832
semi-tryptic	2,079	2,421	4,238
Σ	21,747	19,512	11,851

[a] Tryptic peptides including those containing Cys carbamidomethylation (non-modified) or at least one modification other than the latter (modified).

Filtering

group differentiation	– $\lvert log_2\text{-FC} \rvert$	≥ 4	≥ 4	≥ 2
	– p	-	-	≤ 0.05
peptide identification	– Xcorr	pass[a]	pass[a]	pass[a]
	– # protein	1	-	1
reliability	– found in … BM	≥ 1/2	≥ 3/8	≥ 5/8
manual check	– reference peptide ratio	pass	pass	pass

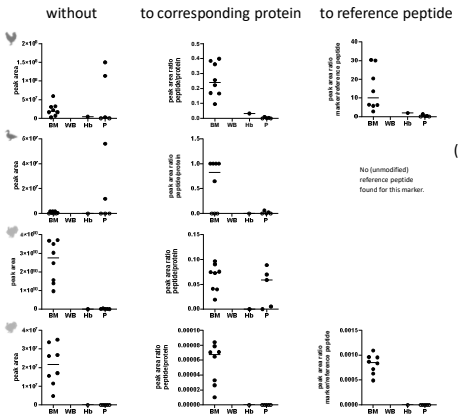
[a] XCorr ≥ 2.20 (2+), 3.75 (3+), 4.5 (4+), 4.45 (≥ 5+); adapted from Wolters et al. *Anal. Chem.* **2001**, *73*, 5683-5690

Processing markers

	processing markers[b]		
non-modified[a]	18 (2)	3 (-)	8 (6)
modified[a]	23 (5)	11 (8)	7 (3)
semi-tryptic	18 (1)	4 (1)	5 (5)
Σ	59 (8)	18 (9)	20 (14)

[a] Tryptic peptides including those containing Cys carbamidomethylation (non-modified) or at least one modification other than the latter (modified).
[b] Peptides filtered for their potential to differentiate differently processed blood products and PAPs. Only part of these potential markers were manually checked for suitable reference peptides (ratios) and those successfully passing this step will be further validated with targeted LC-MS/MS methods (and are summed here in parentheses).

Impact of peptide normalisation



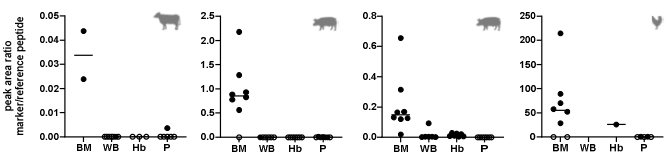
Samples for which peak area ratios could not be calculated due to either the marker or reference peptide not being detected are depicted here with empty circles at a peak area (ratio) of 0 for better visibility.
BM – blood meal, WB – whole blood, Hb – haemoglobin / blood cells, P – plasma.

✓ Uncover previously missed true positives

(✓)

✗ Ruling out false positives, i.e. change due to protein concentration not processing degree

✓ Retain true positives



Samples for which peak area ratios could not be calculated due to either the marker or reference peptide not being detected are depicted here with empty circles at a ratio of 0 for better visibility.
BM – blood meal, WB – whole blood, Hb – haemoglobin / blood cells, P – plasma.

conclusions

- Most markers contain processing-induced change, non-modified markers may origin from extractability and/or proteolytic accessibility improved by processing
- Site-specific detection of processing-induced changes allows to differentiate between medium and mild processing conditions
- Marker normalisation to reference peptide (representing the corresponding protein) is required for accurate results

German Federal Institute for Risk Assessment
bfr@bfr.bund.de | bfr.bund.de/en