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ABSTRACT

Since the beginning of the feed ban regarding the use of processed animal protein (PAP) in feedstuffs, one of the main challenges has been to develop methods to detect and identify the type and origin of PAP or animal products. By now, in the context of a controlled relaxation of the ban, complementary methods are sought to characterize the product when positive responses are delivered by current official methods, with special attention on feed materials (whether or not authorized) originating from ruminants. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics was identified as the most promising method to meet this challenge. This study presents the first inter-laboratory test constructed to evaluate and compare the LC-MS/MS methods currently developed in six different laboratories from four countries. Nine samples consisting of blank feed matrix or feed adulterated with bovine processed animal proteins and/or milk products were analyzed using in-house protocols and the results obtained were discussed. This study confirms the potential of LC-MS/MS-based proteomics to resolve the current analytical gaps in the detection and differentiation of PAP and highlights the technical challenges that need to be addressed in future.

1. Introduction

PAP is defined by the European Commission (2011) as animal protein derived entirely from Category 3 material, which has been treated to render them suitable for direct use as feed material, organic fertilizers or soil improvers. This definition includes both blood meal and fishmeal but excludes blood products, milk products, colostrum products, gelatin, collagen, egg products, hydrolyzed proteins, dicalcium phosphate and tricalcium phosphate. In practice, PAPs look like meal and are produced by heat treatment under high temperature and pressure in a kind of "carcass soup" made up of, among other things, bone, cartilage, muscle, blood and fat.

Official control of the use of PAP in feedstuffs is based on two analytical methods, light microscopy (LM) and polymerase chain reaction (PCR), which are combined according to the feed destination (European Commission, 2009). With the relaxing of the feed ban, complementary analytical methods must be developed to support the forthcoming lifting of the feed ban. In a previous publication, Lecrenier et al. (2020) highlighted the analytical gaps that exist, the possible scenarios for future operational schemes, and the new expected challenges that should be associated. Complementary methods need to bridge these gaps by making an accurate distinction between authorized feed materials and non-authorized ones when current official methods deliver positive results. In particular, the joint discrimination of the type of tissues (e.g. dairy products *vs* PAP) and the species or taxonomic origin (e.g. ruminant *vs* porcine) is required.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics is a promising approach to fill the analytical gaps

Based on the results obtained for the targeted peptides and individual evaluation criteria, laboratories had to make a conclusion on the presence or absence of prohibited ruminant by-products in the blind samples.

4. Methods

Six laboratories analyzed the samples using their own in-house LC-MS/MS-based proteomic approach. The applied methods used were based on recent publications (Belghit et al., 2019; Lecrenier et al., 2016, 2018; Marchis et al., 2017; Niedzwiecka et al., 2018; Steinhilber et al., 2019). Major differences between methods are summarized in Tables 2–5.

4.1. Sample preparation

Table 2 summarizes the major differences observed in the sample preparation protocols used by the participating laboratories. Test portion size ranged from 15 mg up to 1 g of sample. All the laboratories performed the analyses in technical duplicates, except for Lab 5 that analyzed only one test portion of each sample. Labs 2 and 5 did not pre-treat the samples before extraction, whereas Labs 1 and 3 included an additional grinding step. Lab 1 ground a larger subsample before weighing the test portions in order to ensure a good homogenization. Labs 4 and 6 primarily defatted the samples by using a buffer containing trichloroacetic acid (TCA) and acetone. Each lab used a different extraction buffer, but the reduction and alkylation agents were similar, except for Lab 1 that used another reduction agent. The tryptic diges-

Table 2
Comparison of the sample preparation steps used by the participating labs.

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
Test portion size	15 mg	100 mg	50 mg	1 g	1 g	50 mg
No of replicates	2	2	2	2	1	2
Pre-treatment	Grinding	/	Grinding	Defatting step with TCA 10%/2-ME in acetone	/	Precipitation/defatting step with TCA 10%/acetone washes.
Extraction buffer	TEA-HCl (50 mM), 0.5% (w/v) nOG	Laemmli Buffer 4x (4% SDS, 20% glycerol, 10% 2-ME, 0.004% bromophenol blue, Tris.HCl (125 mM)	0.1 M Tris HCl, SDS 4%	Urea (7 M), Thiourea (2 M), 0.03% (w/v) CHAPS, 1.25 mg/L alpha-Amylase	Tris.HCl (200 mM), Urea (2 M)	Urea (7 M), Thiourea (2 M), Tris (30 mM), 4% (w/v) CHAPS
Reduction agent	TCEP	DTT	DTT	DTT	DTT	DTT
Alkylation agent	IAA	IAA	IAA	IAA	IAA	IAA
Digestion enzyme	Trypsin, HPD	Trypsin	Trypsin	Trypsin	Trypsin	Trypsin
Digestion time	2 h	Overnight	16 h	>12 h	1 h	Overnight
Purification method	Immuno-precipitation [b]	SDS-page [a]	C18 spin columns [b]	Immuno-affinity enrichment [a]; C18 spin columns [b]	C18 SPE cartridges [b]	FASP digestion

Legend: 2-ME, 2-mercaptoehtanol; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; DTT, dithiothreitol; IAA, iodoacetamide; FASP, filter aided sample prep; HCl, hydrogen chloride; HPD, heterogeneous phase digestion; No, number; nOg, n-octylglucoside; SDS, sodium dodecyl sulfate; SPE, solid-phase extraction; TCA, trichloroacetic acid; TCEP, tris (2-carboxyethyl) phosphine; TEA, triethanolamine.
[a] Applied prior to digestion.
[b] Applied after digestion.

Table 3
Comparison of the liquid chromatography (LC) and Mass spectrometer (MS) system used by the participating labs and the main parameters.

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
LC system	Ultimate [TM] 3000 nano RSLC (Thermo Fisher Scientific)	ExionLC [TM] system (SCIEX)	Vanquish [TM] Horizon binary UHPLC (Thermo Fisher Scientific)	nanoHPLC (Agilent Technologies)	UPLC [TM] Acquity system (Waters)	NanoElute ® (Bruker)
Gradient time (min)	10	15	15	74	16	40
MS system	Q-Exactive [TM] Plus (Thermo Fisher Scientific)	QTRAP [TM] 5500 System (SCIEX)	Q-Exactive [TM] Orbitrap (Thermo Fisher Scientific)	maXis [TM] (Bruker)	Xevo ® TQ-S micro (Waters)	Tims TOF [TM] Pro (Bruker)
Acquisition mode	PRM	MRM	PRM	DDA with inclusion list	MRM	DDA
Ionization mode	ESI positive	ESI positive	ESI positive	ESI positive	ESI positive	ESI positive

Legend: DDA, Data-dependent acquisition mode; ESI, electrospray ionization; LC, liquid chromatography; MRM, multiple reaction monitoring; MS, mass spectrometry; PRM, parallel reaction monitoring.

Table 4
List of the peptide sequences used by the participating labs and the targeted tissue/product.

Protein	Peptide	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
Alpha-2-Macroglobulin	GSGGTAEHPFTVEEFVLPK	Blood					
Alpha-2-Antiplasmin	LPPLSLLK	Blood					
Protein HP-25 homolog 2	FGFDIELFQHAVK	Blood					
Complement Component 9	YTPVEAIEK	Blood					
Myosin-7	MLSSLFANYAGFDTPIEK	Muscle					
Osteopontin	YPDAVATWLKPDPSQK	Bone/Milk					
Matrilin-1	AGGIELFAIGVGR	Cartilage					
Desmin	TSGGAGGLGALR		Muscle				
Vimentin	TLYTSSPGGVYATR		Muscle				
Myoglobin	YLEFISDAIIHVLHAK		Muscle				
Hemoglobin alpha-chain	VGGHAAEYGAEALER			Blood	Blood	Blood	Blood
Hemoglobin beta-chain	AAVTAFWGK			Blood	Blood	Blood	Blood
	EFTPVLQADFQK			Blood	Blood	Blood	Blood
	VVAGVANALAHR				Blood	Blood	Blood
Collagen alpha-2 (I)	GEPGPAGPAGAVGPGAGAVGPR						Bone/Tendon
Casein alpha-S1	FFVAPFPEVFGK			Milk		Milk	
	HQGLPQEVLNENLLR			Milk		Milk	
	YLGYLEQLLR					Milk	
Casein alpha-S2	NAVPITPTLNR			Milk		Milk	Milk
Beta-lactoglobulin	LSFNPTQLEEQCHI					Milk	
	VLVLDTDYK			Milk		Milk	Milk
	VYVEELKPTPEGDLEILLQK			Milk		Milk	

Table 5
Comparison of the evaluation criteria applied by the participating labs.

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
To detect and identify the peptide	RT identical to IS (± 1 s) Qualifier ion > LLOQ (CV = $\pm 20\%$, CV = $\pm 25\%$ at LLOQ) Specificity of antibody Ion ratios similar to IS ≥ 3 product ions detected	RT similar to ref sample [a] S/N > 3 for all product ions Ion ratios similar to ref sample [a]	RT similar to IS (± 0.07 min) Product ions < 3 ppm mass error S/N > 3 for all product ions ≥ 3 product ions detected ≥ 1 product ion is a y-ion type	≥ 1 peptide identified Peptide Mascot score ≥ 39.0	RRT similar to ref sample [a] S/N > 10 for quantifier ion Ion ratios similar to ref sample [a]	RT and IM closed to QC sample Product ions < 5 ppm error Peptide Mascot score $\geq$ identity score Peptide threshold 1% FDR
To conclude on the presence of the targeted by-product	CV of the ratio between quantifier ion and IS intensity $\leq 20\%$	≥ 2 process IS identified IS identified ≥ 1 peptide identified	≥ 1 peptide identified	≥ 1 peptide identified in both replicates. If results differ, bovine Hb considered to be present, if detected in a 3rd replicate	≥ 2 peptides identified (including AAVTAFWGK for bovine Hb, and FFVAPFPEVFGK for Milk or LSFNPTQLEEQCHI if casein is not main milk source	≥ 1 peptide identified

Legend: CV, coefficient of variation; FDR, false discovery rate; Hb, hemoglobin; IM, ion mobility; IS, internal standard; LLOQ, lower limit of quantification; ppm, part per million; QC, quality control; ref sample, reference sample; RRT, relative retention time; RT, retention time; S/N, signal to noise ratio.
[a] RT or ion ratio tolerances described by European Commission (2002).

tion time varied between the laboratories: Labs 1 and 5 had a rapid digestion step ranging from 1 to 2 h, while the other labs performed the digestion for more than 12 h. A last important distinction among the methods relates to purification and enrichment. Lab 1 used an immuno-affinity enrichment technique (peptide immunoprecipitation) and Labs 3 and 5 used C18 solid phase extraction (SPE) cartridges. Lab 4 combined an immuno-affinity enrichment technique (protein immuno-affinity) and a C18 SPE using spin columns. Lab 2 performed SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Lab 6 carried out the reduction, alkylation, and digestion in ultrafiltration filters by a filter-aided sample preparation (FASP) (Distler, Kuharev, Navarro, & Tenzer, 2016).

4.2. LC-MS/MS

The LC-MS/MS systems applied by the participants are presented in Table 3. All laboratories worked with ultra-high-pressure liquid chromatography (UHPLC), except for Lab 4 that used high-pressure liquid chromatography (HPLC). Labs 2, 3 and 5 used conventional internal diameter columns of 2.1 mm whereas Labs 1, 4 and 6 worked with nano

columns. Labs 1, 2, 3 and 5 applied a short gradient time (≤16 min), while Labs 4 and 6 had a longer gradient time ≥ 40 min.

Two classes of MS instruments were used by the laboratories: (i) high-resolution mass spectrometers (HRMS) with quadrupole-orbitrap (Q-Orbitrap) or quadrupole-time-of-flight (Q-TOF) used by Labs 1, 3, 4, and 6 and (ii) low-resolution mass spectrometer (LRMS) with triple quadrupole (triple-Q) used by Labs 2 and 5. Differences can also be observed in the acquisition mode: Parallel reaction monitoring (PRM) for Labs 1 and 3, multiple reaction monitoring (MRM) for Labs 2 and 5 and data-dependent acquisition (DDA) for Labs 4 and 6. In summary, the laboratories can be separated in three groups based on their MS approach: LRMS with MRM mode (Labs 2 and 5), HRMS with PRM mode (Labs 1 and 3) and HRMS with DDA mode (Labs 4 and 6).

Table 4 lists the peptide sequences (and the protein from which they origin) used in this study. The targeted bovine tissues or products (i.e., blood, muscle, bone, cartilage, tendon and milk) were identified for each peptide. Lab 1 designed its set with plasma, muscle, bone and cartilage peptides. The selected bone peptide is also present in milk products. Lab 2 has focused its method on muscle peptides. The four other labs (Labs 3, 4, 5 and 6) have chosen comparable sets of hemoglobin peptides, supplemented by milk peptides for Labs 3, 5 and 6 and a bone/tendon marker for Lab 6.

4.3. Criteria for interpretation of the results

The last major parameter of variation concerns the interpretation of the results. Table 5 summarizes the criteria used by each lab to evaluate the MS data and to deliver a conclusion on the presence or absence of the targeted by-product. Regarding the identification and detection of the peptides, criteria were similar for a same cluster in acquisition mode (MRM/PRM or DDA). However, an important difference was the number of identified peptides requested to conclude on the presence of the related by-product. Some laboratories concluded on the identification of by-products with at least one protein marker whereas Lab 5 sets its criteria to a minimum of two peptides.

5. Results and discussion

Individual results are summarized in Supplementary Table 1–6. Table 6 summarizes each participating laboratory's conclusion on their findings. Erroneous results are marked with an asterisk.

The overall results, expressed in terms of global accuracy confirm the suitability of the mass spectrometric approaches for the detection of bovine PAP. The percentage of total error only accounted for 16.5% of the total responses. There are no false positive results for the detection of prohibited ruminant by-products. However, bovine PAPs, when present in the samples, were not always detected. Labs 1, 5 and 6 had no false negative results. Labs 2 and 3 showed one false negative result (sample #8) and Lab 4 had three false negative results (samples #5, #6

Table 6
Summary of the labs conclusion on the presence of prohibited ruminant by-products in blind samples.

Sample #	Adulteration	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
5	**1% PAP-1**	+	+	+	-*	+	+
6	**1% PAP-2**	+	+	+	-*	+	+
7	**5% Milk**	-	-	-	-	-	-
8	**1% PAP-1 + 1% Milk**	+	-*	-*	-*	+	+
9	**Blank**	-	-	-	-	-	-

Legend: PAP, processed animal protein; +, the sample contains prohibited ruminant by-products; -, the performed analyses does not allow a conclusion on the presence of prohibited ruminant by-products. False results are marked with an asterisk. Concentrations are expressed as weight-weight (w/w) percentage (%).

and #8). The first conclusion is that, except for sample #8, five out of six participating laboratories correctly detected bovine PAPs at the level of 1% (w/w).

To explain the results obtained by Lab 4 for samples #5 and #6, the method employed in Lab 4 was compared to those of Labs 3, 5, and 6. Even if the methods used by the laboratories listed above differed in sample preparation protocols, MS instrument and the acquisition mode, all four laboratories targeted hemoglobin chains with a comparable set of peptides. Labs 5 and 6 used the same set of peptides as Lab 4, while Lab 3 used only three out of this set of four. Whereas Lab 4 did not detect any of the target peptides, Lab 3 detected one of them (VGGHAAEYGAEALER) in one of the two replicates of samples #5 and #6. Lab 5 detected three hemoglobin peptides (VGGHAAEYGAEALER, AAVTAFWGK and VVAGVANALAHR) in sample #5 and four hemoglobin peptides (VGGHAAEYGAEALER, AAVTAFWGK, EFTPVLQADFQK and VVAGVANALAHR) in sample #6. Lab 6 also detected three hemoglobin peptides (VGGHAAEYGAEALER, EFTPVLQADFQK and VVAGVANALAHR) in sample #5 and four hemoglobin peptides (VGGHAAEYGAEALER, AAVTAFWGK, EFTPVLQADFQK and VVAGVANALAHR) in sample #6. Lab 4 further investigated the results obtained by analyzing pure bovine PAP-1 (data not shown). This sample was found to be positive for bovine material based on the detection of two out of the set of four peptides (VGGHAAEYGAEALER and VVAGVANALAHR). Repeated analysis of the QC sample (containing PAP-1 at a much lower level) by Lab 4, once again yielded negative results for all targeted peptides. This inter-laboratory comparison confirms the presence of bovine hemoglobin in PAP-1 and PAP-2 and suggests that the false negative results obtained by Lab 4 in the study is probably due to a lack of sensitivity of the method on the PAPs used. After investigation, it appeared that the lack of sensitivity compared to prior tests with the method may result from a lowered LC resolution.

The results obtained by all participants on sample #5 (1% PAP-1) and sample #6 (1% PAP-2) were also compared. Labs 5 and 6 detected 100% (4/4) and 75% (3/4) of their hemoglobin markers in sample #6 and in sample #5, respectively. Similar results were also observed by Lab 1 for their blood marker peptides. Lab 3 detected only one blood peptide (VGGHAAEYGAEALER) in both samples (samples #5 and #6). Comparison of the signal intensities or peptide counts for the peptides detected in both samples revealed that signals for the blood peptides were generally higher in sample #6 than in sample #5. Muscle markers (myosin-7 and myoglobin) were detected in both samples by Labs 1 and 2. The signal intensity of myosin-7 (MLSSLFANYAGFDTPIEK) and of myoglobin (YLEFISDAIIHVLHAK) was also higher in sample #6 than in sample #5. In contrast, bone markers (osteopontin and collagen alpha-2 (I)) used by Labs 1 and 6 were detected in sample #5 and not in sample #6. In order to see whether these differences originate from the PAP's composition, these observations were compared to the results obtained by LM. Using tetramethylbenzidine-hydrogen peroxide staining (European Union Reference Laboratory for Animal Proteins in feedingstuffs, 2013), PAP-2 gave a positive reaction for blood (immediate blue-green coloring and release of O_2 bubbles), while PAP-1 did not react positively to this staining. Therefore, the blood concentration in PAP-1 is probably lower than in PAP-2. The comparison of sediment percentage indicates a higher bone content in PAP-1 than in PAP-2. LM observations thus confirms the higher blood content of PAP-2 and the higher bone content of PAP-1 that were suggested by the MS data.

Sample #8, containing 1% PAP-1 and 1% milk powder, appears to be the most complex sample as three out of six laboratories (Labs 2, 3 and 4) failed to detect PAP in it. Lab 3 detected two product ions of the peptide VGGHAAEYGAEALER in sample #8 but declared it as negative because their criteria for peptide identification (≥3 product ions detected) were not met. Lab 2 did not detect any product ion in this sample. When the same PAP material (PAP-1) was used at the same concentration in sample #5, Labs 2 and 3 could correctly detect it. Labs 1, 5 and 6 detected the presence of prohibited ruminant by-products but the comparison of the data obtained for sample #8 and sample #5

shows some differences. Peak intensities observed by Lab 5 for the hemoglobin peptides VGGHAAEYGAEALER, AAVTAFWGK and VVAGVANALAHR were lower in sample #8 than in sample #5. Peptide counts detected by Lab 6 for VGGHAAEYGAEALER and VVAGVANALAHR were also lower in sample #8 than in sample #5. EFTPVLQADFQK (hemoglobin) and GEPGPAGAVGPAGAVGPR (collagen) had the same peptide counts. For Lab 1, the comparison was limited to muscle and cartilage peptides as the other ones may appear in both adulterants of sample #8, PAP and milk. In this case, the signal intensities of MLSSLFANYAGFDTPIEK (myosin-7) and AGGIELFAIGVGR (matrilin-1) were higher in sample #8 than in sample #5. These results revealed that the presence of milk can have different impacts on the detection of PAP depending on the method and the peptides used. This phenomenon is probably linked to a cause of disturbance well described in ESI-LC-MS: matrix effects. These effects are often due to the alteration of the ionization efficiency of the target analytes due to the co-elution of other compounds. Matrix effects can affect analytical performance in the form of a decrease (ion suppression) or an increase (ion enhancement) in signal intensity (Zhou, Yang, & Wang, 2017). The fact that the presence of milk in sample #8 has a different impact on the results of Lab 1 vs other labs can therefore be explained: the specific purification (immunoaffinity enrichment) used by Lab 1 minimizes the matrix effect by removing more co-eluting molecules.

Some participants also included the detection of milk powder (Labs 1, 3, 5 and 6) in their results. Correct detection of milk proteins was achieved by four laboratories (more details in the Supplementary Tables 1–6), meaning that no false negative result was reported. However, milk powder induced many false positive results and all laboratories found milk peptides at least in one sample that did not contain milk powder. As PCR analyses cannot distinguish the origin of detected ruminant DNA, QC and blind samples (samples #4, #5, #6, #7, #8 and #9) were analyzed by enzyme-linked immunosorbent assays (ELISAs) in order to evaluate if the unexpected results were not due to an unknown milk contamination during sample preparation. Analyses were performed by the CER Groupe using ELISAs developed for allergen detection in food (Dumont et al., 2010). These methods are very sensitive for the detection of beta-lactoglobulin and casein, with a limit of quantification of 0.25 ppm and 0.5 ppm, respectively. No milk protein was detected in samples #5, #6 and #9 and both beta-lactoglobulin and casein were detected in samples #4, #7 and #8.

Lab 1 erroneously classified sample #5 as positive with regard to milk content. The classification was based on the presence of high osteopontin content in the sample. YPDAVATWLKPDPSQK (osteopontin) is used by Lab 1 both to detect the presence of milk and of bone-containing PAP. The identification of the tissue of origin is based on the protein ratio of osteopontin *vs* the blood proteins. The high bone content of PAP-1, used for sample #5 adulteration, probably caused this misclassification. This study underlines the difficulty of the approach to identify the tissue origin of a protein based on its ratio to other proteins, especially when analyzing mixtures with variable tissue ratios. Hence, a broader data knowledge of PAP composition ranges will be necessary for more reliable milk detection based on the ratio between osteopontin and other tissue markers. The reasons for false milk detection of Labs 3, 5, and 6 are less obvious though based on the same peptides. Lab 3 found milk peptides in one of the two replicates for sample #5 and sample #6. Lab 5 detected one casein peptide in sample #6 but did not declare the sample as positive for milk according to their evaluation criteria, which requires the detection of two peptides to confirm the presence of the targeted by-product. Lab 6 also detected only one milk marker in sample #6 and declared the sample as positive for milk, according to their acceptance criterion. As described by Ramachandran, Yang, and Downs (2020), some milk peptides are known to be sticky leading to increase carryover effects. This phenomenon may explain the false positive results observed. Carry-over is due to non-specific adsorption of peptides that can occur at every part of the LC-MS

system (Maes, Smolders, Michotte, & Van Eeckhaut, 2014). The potential risk is increased consecutively to the injection of high-concentration samples. In order to overcome this problem, it is essential to understand the origin of carry-over and to develop efficient strategies to reduce it. If, despite everything, it is not possible to manage it, the exclusion of troublesome peptides from the target list is another option.

6. Conclusion

The versatility and discriminative power of LC-MS/MS-based proteomics methods to identify both species and tissue origin allows to efficiently and unambiguously determine an array of prohibited and authorized animal by-products in feed samples. The present study shows that LC-MS/MS is able to successfully identify the presence of various proteins of bovine origin in feed at an adulteration level of 1% (w/w). Both LRMS and HRMS exhibited very similar performances in term of accuracy. The results also revealed that there are still some pitfalls to solve. For example, as similarly observed when using other analytical approaches for PAP detection and differentiation, the heterogeneity of the composition of feed materials can interfere with the correct detection of specific peptide in certain matrices. This inter-laboratory study thus provides important data required to build robust and reproducible LC-MS/MS methods for future routine PAP analyses for the detection of prohibited animal by-products. In addition to the very specific application of MS-based proteomics for PAP detection, the results obtained in the present study also provide evidence that protein markers may be a very useful tool for more general questions of feed and food authentication issues, especially in cases of highly processed products.

bank (FKZ 28RZ6IP002) with regard to the work of Uta M. Herfurth, Oliver Poetz and Andreas Steinhilber.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2021.107944.

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