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Detecting fraudulent additions in skimmed milk powder using a portable, hyphenated, optical multi-sensor approach in combination with one-class classification

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ABSTRACT

The detection of fraudulent additions to milk powder is an ongoing research subject for governmental agencies, industry and academia. Current developments steer towards the application of so-called fingerprint approaches, describing authentic, reference samples with spectroscopy and using one-class classification (OCC) to identify "out-of-class", or adulterated samples. Within this article we describe the application of a novel, portable device hyphenating ultraviolet–visible, fluorescence and near-infrared spectroscopy in combination with OCC modelling to discriminate authentic skimmed milk powders from adulterated ones. As adulterated samples we analyzed skimmed milk powder with the addition of plant protein powder, whey powder, starch, lactose, glucose, fructose as well as non-protein nitrogen like ammonium chloride, ammonium nitrate, melamine and urea in different concentrations. After fusion of the classification results from the three spectral techniques and several models two scenarios are presented. 100% (scenario 1) or 80% (scenario 2) of the authentic skimmed milk powders were correctly identified as "in-class", whereas respectively 64% or 86% of the adulterated samples were correctly classified as "out-of-class". In brief, this article provides insights in the application of novel, portable devices that may be applied in a non-invasive manner and gives an outlook on data handling and a new data fusion strategy.

1. Introduction

In food authenticity and food safety testing, targeted analysis of hazardous compounds is increasingly replaced with fingerprinting analysis (Gao et al., 2019; Riedl, Esslinger, & Fauhl-Hassek, 2015). Data from authentic or reference samples are collected and are described as one group or class via multivariate statistics. By choice, all samples that show abnormal fingerprints are identified and may be flagged for further in-depth analyses (Callao & Ruisánchez, 2018; Rodionova & Pomerantsev, 2020). In addition to the shift of analytical methodology in food fraud detection, measurements are preferred to be performed on-site and in a non-invasive and fast manner. This drives the development of portable devices that carry miniaturized optical spectrometers (Crocombe, 2018; Ellis, Muhamadali, Haughey, Elliott, & Goodacre, 2015; McGrath et al., 2018; Yeong, Jern, Yao, Hannan, & Hoon, 2019). The approach to measure fraudulent additions to food with benchtop optical spectroscopy has been investigated thoroughly and is currently in place in multiple governmental agencies, food industry and respective academia. In particular milk powders' authenticity and safety have been investigated using near-infrared (NIR) (Cattaneo & Holroyd, 2013; Pasquini, 2018) or mid-infrared (MID) spectroscopy (Romero Gonzalez, Cobuccio, & Delatour, 2019) as well as Raman spectroscopy (Karunathilaka, Farris, Mossoba, Moore, & Yakes, 2017). Behkami, Zain, Gholami, and Khir (2019) even used the combination of ultraviolet–visible and NIR radiation with a benchtop, hyphenated, three-sensor device to classify the origin of spray-dried cow milk. So-called hyphenated devices may combine multiple sensors and technologies such as spectrometers covering multiple wavelengths or Raman within one device (Crocombe, 2018). The combination of the data, i.e. fusion of spectra or statistical output is then believed to give a more accurate fingerprint of the sample (Callao & Ruisánchez, 2018). Besides hyphenated sensors, the application of miniaturized sensors in portable devices is of current interest. For example, Karunathilaka, Yakes, He, Brückner, and Mossoba (2018) examined the performance of two portable Raman spectrometers identifying melamine addition in milk powders. Nevertheless, literature on the application of miniaturized devices and the usage of hyphenated devices with different sensors is lacking. For fast spectroscopic applications in food sensing, data needs to

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be assessed by multivariate statistics. Targeted multivariate classification and regression models are the common method of choice (e.g. linear Partial Least Square Discriminant Analysis or Regression or non-linear Support Vector Machine Classification or Regression) in spectroscopy. In targeted analysis, both classes of products (authentic versus adulterated) or a range of the interested analyte, e.g., adulterant or food component in the authentic food commodity, are required as input for these type of models. However, when dealing with food integrity issues of an unknown origin, such supervised approaches tend to fail or overlook emerging threats. By means of multivariate one-class classification (OCC) this problem can be tackled, as only the 'authentic' product is considered in the multivariate model and defined with an appropriate uncertainty limit. The latter may be used to reduce the number of false positive or false negative classifications in accordance with the needs. Using OCC, any abnormalities may be flagged, provided that they have any contribution to the response of the optical sensor(s).

In this work we present the application of a portable, hyphenated, optical device developed and built during the EU-H2020 project 'PhasmaFood' for the detection of skimmed milk powder (SMP) fraud. The prototype records a NIR, visible (VIS) and fluorescence (FLUO) spectrum and captures a RGB-camera picture in addition. The data acquired from the different approaches originate from the same sample at the same time and the same spot. The data were processed using OCC strategies. Optimized approaches for multivariate data fusion are presented in detail. The accuracy of fraud detection using the OCC approach was tested on multiple adulterants that are of concern to governmental agencies, industry and academia. Different scenarios are presented to illustrate the flexible application of different class limits for the authentic class.

2. Materials & methods

2.1. Materials and sample collection

In January and March 2019, 14 skimmed milk powders (SMPs), and in November 2017, 6 SMPs were purchased directly from producers within the Netherlands, Belgium and Germany or were acquired from the routine national SMP intervention program of the Netherlands Food and Consumer Product Safety Authority (NVWA). All were stored at −18 °C in the dark and were shielded from hygroscopic conditions to the best of our abilities. The SMPs from 2017, further named 'old SMPs', were used in blends with the ones from 2019 in this report. This is not a common case of food fraud but may be an issue of food integrity, as SMPs stored for a period of more than three months show a change in phospholipids which is accompanied by a sensory change (Romeu-Nadal, Chávez-Servín, Castellote, Rivero, & López-Sabater, 2007). Various plant protein powders (total 16 unique samples) originating from soy (4), pumpkin, hemp (2), rice, pea (5), a pea/rice/hemp plant protein mix as well as whey protein (5) were purchased from Pursana NV (Haarlem, the Netherlands), Pulsin (Gloucester, UK), Mattisson Healthcare BV (De Meern, the Netherlands), Biotona (Oostkamp, Belgium), Myprotein (Manchester, UK), Lucovitaal (Uden, the Netherlands) and Bulkpowders (Colchester, UK). Starch from Merck KGaA (Darmstadt, Germany) and fructose, glucose and lactose (sugars) from Sigma Aldrich (St Louis, MO, USA) were used as fillers. Ammonium chloride (99.8% *w/w*), ammonium nitrate (>95% *w/w*) and urea (99.5% *w/w*) from Merck and melamine (99% *w/w*) from Sigma Aldrich were used as non-protein nitrogen to adulterate the SMPs.

To obtain the SMP reference values following chemicals were used: boric acid (for analysis), sodium hydroxide solution (30%), sulphuric acid (95–97% *w/w*, nitrogen-free), ammonia solution (25%), congo red from Merck, diethyl ether (>99.5% *w/w*) petroleum ether (boiling point 30–60 °C, PEC grade) from Actu-All Chemicals (Oss, the Netherlands), hydrochloric acid (0.1 mol/L) from Boom B.V. (Meppel, the Netherlands), L-Tryptophan and sucrose from Duchefa Biochemie B.V. (Haarlem, the Netherlands), Special Kjeltabs No. 4, AB04 from Thompson & Capper Ltd. (Runcorn, UK), and ethanol (96 ± 2% (V/V)) from Klinipath, (Amsterdam, the Netherlands).

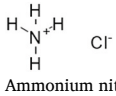
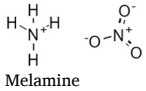
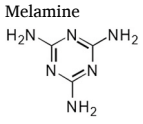
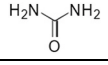
2.2. Reference values for SMPs

To approve if the SMPs meet the EU composition specifications, they were analyzed using the reference methods in the European legislation (Regulation (EU) 2018/150). Relevant provisions from this regulation for this research include protein, moisture and fat content. The protein content was analyzed in accordance with ISO 8968-1, using nitrogen determination by the Kjeldahl principle and conversion to crude protein by means of calculation. The fat content (ISO 1736) and moisture content (ISO 5537) of the SMPs were analyzed gravimetrically according to standard procedures.

2.3. Preparation of adulterated samples

All adulterated samples were prepared by dry-blending as detailed in the overview in Table 1. To ensure homogeneous samples, the containers with the mixtures were manually shaken 1 min, checked visually and if necessary shaken again. To SMP from the year 2019, old SMPs were added in a ratio of 10, 25 and 50% (*w/w*). Whey and plant protein, sugars and starch were mixed in a ratio of 10, 25 and 50% (*w/w*) with 2019 SMPs. These fillers have been fraudulently added to SMPs in the past, to increase the volume at low costs (Amaral, Mafra, Pissard, Pierna, & Baeten, 2018; Nascimento, Santos, Pereira-Filho, & Rocha, 2017). Samples with the addition of non-protein nitrogen were blends in 1, 2 and 5% (*w/w*) ratio, only ammonium chloride was blended in a ratio of 0.1, 1, and 2% (*w/w*). This way, the apparent protein content in the adulterated samples increased in comparison to the authentic SMPs (see Table 1). According to Nascimento et al. (2017), this common type of adulteration is used because the non-protein nitrogen cannot be distinguished by the legal reference methods like Kjeldahl and Dumas used for determining total protein content in skimmed milk powders. It is acknowledged that the dry-blending method employed in this study commonly overestimates NIR detection capabilities in wet-blended

Table 1
Overview of prepared adulterated samples.

	Diluent/Adulterant	Concentration of diluent/adulterant in % (w/w) of the prepared sample	Increase in apparent protein content in %
Filler	Old SMPs (purchased in 2017)	10, 25, 50	none
	Plant protein powders	10, 25, 50	n.d.
	Starch and sugars	10, 25, 50	n.d.
	Whey protein powder	10, 25, 50	n.d.
Adulterant (non-protein nitrogen)	Ammonium chloride 	0.1	0.13
		1	1.33
		2	2.65
	Ammonium nitrate 	1	1.89
		2	3.77
		5	9.44
	Melamine 	1	3.91
		2	7.81
		5	19.53
	Urea 	1	2.63
		2	5.26
		5	13.15

n.d. not determined.

samples as a result of matrix effects (Scholl, 2017). Each adulterated sample was prepared 3 times, each with a different randomly chosen 2019 SMP. This way the genuine variety of skimmed milk powders, i.e., protein, moisture and fat content, were covered. In addition to the 14 authentic samples this resulted in 154 adulterated SMP samples. The latter was divided in 3 classes, namely: old SMP and mixes (1, 23 samples), fillers and mixes (2, 87 samples), non-protein nitrogen mixes (3, 33 samples).

2.4. Optical multi-sensor measurements using the 'PhasmaFood' sensor

Optical multi-sensor measurement data was acquired using the prototype portable, hyphenated, optical sensor 'PhasmaFood' (type I, Fraunhofer IPMS, Dresden, Germany and WINGS ICT solutions, Athens, Greece). The hyphenated sensor was equipped with a miniaturized commercial UV-VIS spectrometer (range 320–889 nm, 288 individual wavelengths recorded, C12880, Hamamatsu, Japan), a prototype in-house developed miniaturized NIR sensor (range 939–1833 nm, 895 individual wavelengths recorded, MEMS-type, Fraunhofer IPMS, Patent no. WO 2003069289 A1, Pügner, Knobbe, and Grüger (2016)), and a miniaturized RGB-camera (MU9PC-MH, CMOS, Ximea, Münster, Germany). The UV-VIS spectrometer was used for both fluorescence and diffuse reflectance VIS spectroscopy. The NIR and UV-VIS sensing front-ends together with their respective light sources are positioned in a circular integrated setup as displayed in Fig. 1. The RGB-sensor is positioned as the central sensor and was in this study solely used to check the positioning of the sample and the focus of the illumination. The 'PhasmaFood' sensor was operated by a custom-build 'PhasmaFood' Android application (developed by VizLore Labs Foundation, Novi Sad, Serbia). Data was sent to an online cloud repository associated with this application. The settings of the individual sensors were optimized for SMP powders targeting short acquisition times with an appropriate signal-to-noise ratio (SNR) and no saturation of the signal. Illumination currents of the VIS and FLU lamps were 1 mA and 8 mA respectively and integration time 55 μs for both sensors. The NIR microlamps illumination current was set at 800–900 mA, being warmed up for at least 5 s before conducting the measurement (integration time 5 s). As integration times of the NIR were not changeable, the number of measurements was increased and illumination conditions were changed to increase the SNR. During one measurement cycle of around 1 min 10 VIS spectra, and 255 NIR spectra were acquired in diffuse reflectance mode and 10 fluorescence spectra under UV illumination (365 nm). The 'PhasmaFood' sensors were calibrated prior to SMP measurement by conducting a 99% diffuse reflectance and dark acquisition measurement of a white standard material (Spectralex White Diffuser WDF-030-95, Lake Photonics, Uhldingen-Muehlhofen, Germany). Spectral measurements were corrected as follows:

- VIS and NIR: (raw sample data – dark acquisition sample data)/(99% diffuse reflectance white standard data – dark acquisition white standard data)
- FLUO: raw sample data – dark acquisition sample data

Samples were transferred to 5 cm diameter plastic petri dishes and homogenized using a spoon (Fig. 1). The layer thickness of 1 cm was chosen in order to prevent acquiring spectral data which did not originate from the sample but for example the underlying laboratory table. The 'PhasmaFood' sensor node was positioned on top of the Petri dish by means of a customized spacer (Fig. 1) allowing approximately 3 cm between the sample surface and the sensor and light modules.

All 168 samples (14 SMP and 154 adulterated samples) were measured in triplicate on three different days, yielding 9 measurements per sample and are summed up 1512 sample spectra per sensor. Between the three days, several days of storage were included to cover a total storage period of 3 months for every sample. In the sensor extreme wavelengths for VIS and NIR the sensor response was irregular or noisy, and these regions of the spectra were discarded. VIS wavelengths from 400 to 740, fluorescence signals from 340 to 780 nm, and NIR wavelengths from 1020 to 1833 nm were found suitable for further data analysis.

2.5. Multivariate data analysis

VIS, FLUO and NIR data was downloaded from the 'PhasmaFood' cloud repository. All data analysis was conducted using R 3.6.1 (R Core Team, 2018). Spectral outliers within each set of 9 measurements per sample and per sensor were identified using Euclidian distance of each scan to the sample mean. Visually divergent distances were dubbed outliers, resulting in 3, 7 and 3 spectra to be discarded for VIS, FLUO, and NIR, respectively. As no outliers were exclusively found for one sample, no sample was excluded from further analysis. The dataset available for multivariate analysis, therefore, consisted of 1509 spectra of 129 spectral points (VIS), plus 1505 spectra of 200 spectral points (FLUO) and 1509 spectra of 814 spectral points (NIR). On this dataset, one-class modelling was performed. That is, the spectral properties of only the SMP samples were modelled, yielding class distances for all spectra being predicted. A threshold was set to determine whether a sample (spectrum) fits into the class of SMP or not.

A systematic screening of data preprocessing and one-class classification algorithms was performed (Fig. 2A and B), separately for the data for each sensor. Each combination of preprocessing and classification algorithm was evaluated using a 40 times repeated random cross validation (80% split) on the target class (authentic SMP), where sample replicates were kept together in test/training sets. Performance was evaluated calculating 'area under the receiver operating characteristic' (AUROC) of the target class SMP against each of the classes mentioned in section 2.3 (Fig. 2C). Ten models, detailed in Table 2, jointly covering the highest obtained AUROCs for each of the classes were selected manually and used in the decision scheme (as described below). Throughout model screening, preprocessing steps were executed in the order from left to right as in Table 2. A high-level approach was chosen to fuse the data from different sensors (and multiple models) together,

Fig. 1. Pictures and schematic diagram of the 'PhasmaFood' device: left the sensing node, middle top the handling of the prototype, middle bottom the customized spacer for measurements of solid samples, right the schematic diagram.

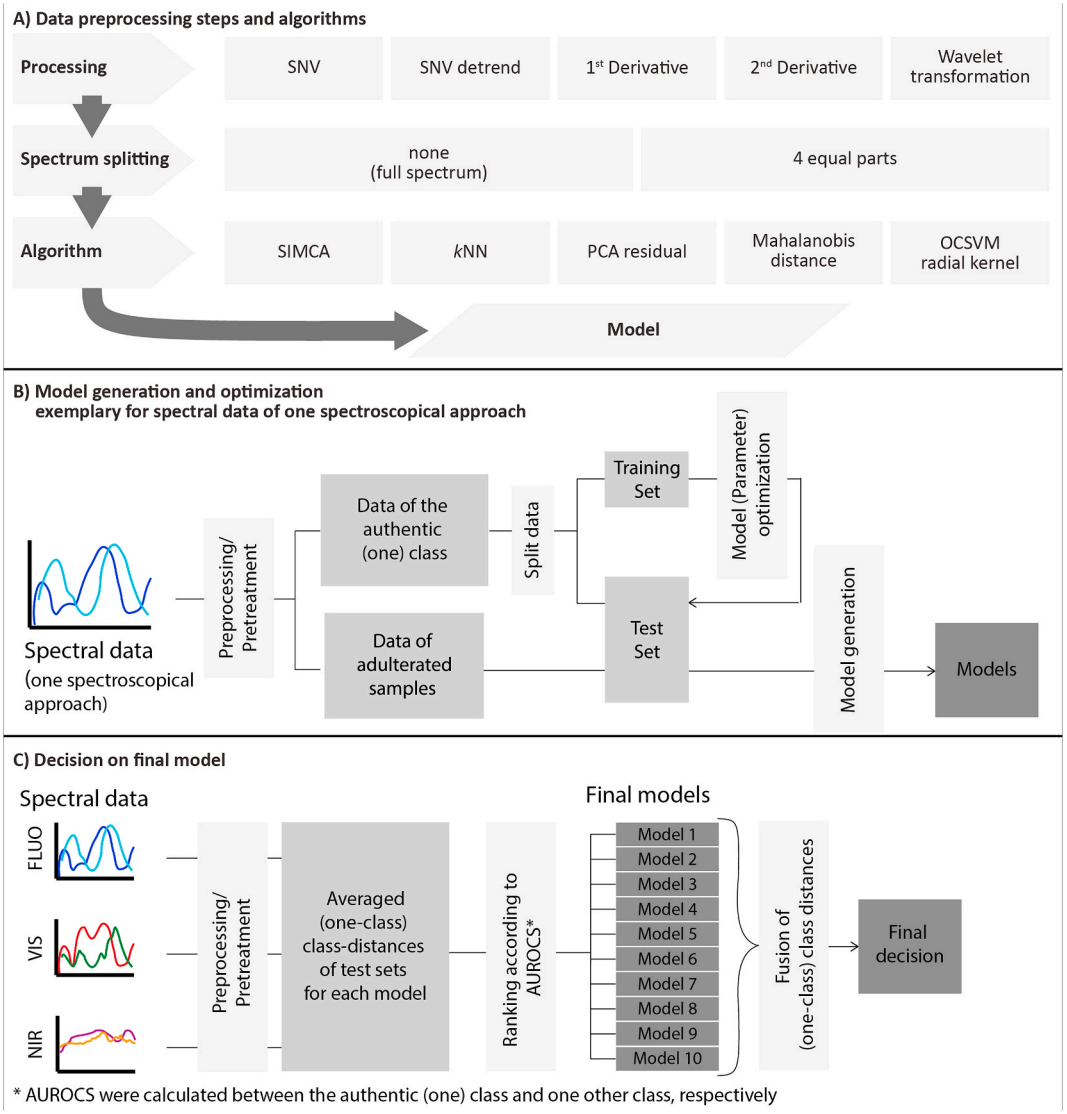


Fig. 2. Detailed multivariate statistics processes (A), exemplary spectral data processing, model generation and optimization for one spectroscopic approach (B), evaluation of performance results, decision on final models and data fusion (C).

which means that the model results were combined, rather than the raw spectral data (low-level) or extracted spectral features (mid-level data fusion). As reported by Callao and Ruisánchez (2018), high-level data fusion is recommended when dealing with a large amount of data and differing database sizes. The final classification was based upon a simple decision tree (scheme), i.e., if two or more out of the 10 models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for further calculations.

3. Results and discussion

3.1. SMP reference samples

All SMPs were investigated in accordance with EU regulations. SMPs included in the study had protein contents between 31.7 and 38.2% (Average 34.6% ± 1.8) and fat contents in a range of 0.37–0.76% (Average 0.56% ± 0.16). Both fat and protein content were all within the respective legal limits for SPMs. The moisture contents were between 3.58 and 5.10% (Average 4.07 ± 0.41). Whereas most SMPs from 2019 met the criteria set in the Regulation (EU) 2016/1238 for public storage of SMPs, the ones that were stored for a longer period (old SMPs) had all moisture contents above 4.00%. The 14 genuine SMP samples do not fully account for the natural diversity in SMP available on the market, as only limited geographic location and production years have been included. Therefore, the application range of this specific sample set is limited to the geographical origin and production years as described in section 2.1 (USPC, 2019).

3.2. Raw spectral dataset

The spectral data (white and dark corrected) prior to processing are displayed in Fig. 3 for the three types of measurements resulting from the 'PhasmaFood' sensor. The FLUO reflectance spectra immediately showed high variability throughout the spectra amongst the different classes of samples (SMPs (black), fillers including old SMPs and mixes (green) and non-protein nitrogen mixes (red)). Striking to observe was the sensitivity towards porphyrin-based structures, i.e. chlorophylls and their derivatives, that are present in most plant protein powders (Tetenkin, 2003). Furthermore, differences in FLUO emittance between SMPs and other white protein powders (soy, whey) and chemical nitrogen enhancers was observable. The VIS diffuse reflectance data provided complementary data to the FLUO sensors by means of subtle changes in the yellow hue of the powders and thus provided a more

Table 2
Overview of the picked models with the calculated AUROCs.

Sensor	Pre-processing				Algorithm	AUROC			
	SNV	*Derivative*	*Subset*	*DWT*		SMP vs class 1	SMP vs class 2	SMP vs class 3	SMP vs all
VIS	–	1st	3rd	–	SIMCA *(3PCs)*	0.53	0.86	0.54	0.69
VIS	DT	–	4th	–	PCAresid *(3PCs)*	0.63	0.86	0.55	0.72
FLUO	–	1st	4th	–	Mahalanobis	0.56	0.58	0.55	0.57
FLUO	–	2nd	4th	–	kNN *(2neighbors)*	0.52	0.54	0.57	0.55
NIR	SNV	–	(full)	la8 (3–5)	SIMCA *(3PCs)*	0.64	0.92	0.60	0.76
NIR	–	1st	4th	–	kNN *(2neighbors)*	0.65	0.89	0.61	0.76
NIR	–	–	(full)	d2 (5–7)	PCAresid *(3PCs)*	0.62	0.89	0.59	0.79
NIR	DT	–	4th	–	SIMCA *(3PCs)*	0.64	0.89	0.60	0.75
NIR	–	–	(full)	la8 (3–5)	PCAresid *(3PCs)*	0.63	0.91	0.62	0.76
NIR	–	1st	4th	–	OCSVM	0.63	0.89	0.61	0.75

Abbreviations and details: **SNV**: Standard Normal Variate (SNV), R-package 'prospectr' (Stevens & Ramirez-Lopez, 2013). SNV-**DT**: Detrend, SNV followed by baseline correction (Stevens & Ramirez-Lopez, 2013). Derivative: 1st or 2nd derivative (Savitzky-Golay) with an 11-point filter length using R-package 'signal' (Signal developers, 2013).**Subset**: Each spectrum was modelled in **full**, and as 4 quarter sections with equal lengths. Number indicates the quarter (**3**rd or **4**th) section being modelled (sorted by increasing wavelength).**DWT**: Discrete wavelet transformation. The spectrum (section) was spline-interpolated to 128 points (DWT requires a power of 2). Then, either a **d2** (Daubechies, filter length 2) or a **la8** (Least Asymmetric, filter length 8) transformation was applied, and the indicated wavelet coefficients were returned, using R-package 'wavelets' (Aldrich, 2019).**SIMCA:** Soft Independent Modelling of Class Analogies (SIMCA), using R-package 'mdatools' (Kucheryavskiy, 2020). The number of components is selected using a 5-fold (inner loop) cross validation.**PCAresid:** Principal Components Analysis (PCA) residual, calculating the sample residuals (Q residuals). The number of components is selected using a 5-fold (inner loop) cross validation.**Mahalanobis:** The Mahalanobis distance was calculated using means and covariance of the training set.**kNN:** distance to the k-Nearest Neighbor (kNN), using R-package 'kknn' (Schliep & Hechenbichler, 2016). The number of neighbors is selected using a 5-fold (inner loop) cross validation.**OCSVM:** One Class Support Vector Machine (OCSVM) with radial basis kernel and automatic parameter estimation, using R-package 'kernlab' (Karatzoglou, Smola, & Hornik, 2004).

quantitative insight in the types of powders present. In comparison to the first two spectroscopic approaches, the NIR data majorly represents the general macro-composition of the powders (protein, fat, moisture, carbohydrates). Hence, it may give specific information on chemical bonds present non-protein nitrogen enhancers. Differences between the sensors' abilities may be observed by comparing the median line of the respective raw spectra (thick line) in Fig. 3. Both the VIS and FLUO sensors are able to detect most differences between authentic SMPs and ones with fillers. However, only the NIR is clearly able to detect differences between the authentic SMPs and the fillers and old SMPs and mixes thereof with SMP (class 1 and 2) and non-protein nitrogen mixes with SMP (class 3).

3.3. OCC of skimmed milk powders – Threshold decision

All chosen OCC models yielded class distances for each spectrum predicted. To turn these class distances into one OCC model, a decision threshold was applied. It was possible to choose thresholds such that all (authentic) SMPs were classified correctly (scenario 1, Table 3), or such that there is an optimal balance between SMPs being predicted "in-class" and adulterated or other samples being predicted as "out-of-class" (scenario 2, Table 4). In the first scenario, thresholds were such that no false negative classifications appear, that means all SMPs were correctly identified. On the other hand, that led to more false positive classifications, when an adulterated sample was wrongly identified as SMP. For both the mixes of old and fresh milk powders as well as the powders with added non-protein nitrogen this leads to unacceptable low classification rates of 19% and 12%, respectively. From the powders with filler additions, 89% were identified correctly as adulterated. To improve the correct classification rate and decrease the number of false positive classifications, in the second scenario the number of correctly identified authentic SMPs was set via the thresholds to approximately 80%. This way, one out of five SMPs were identified wrongly as adulterated. We believe that this may be more in the interest of an industrial player as 65%, 60%, and 99%, of mixes with old SMP, non-protein nitrogen or fillers were correctly flagged as "adulterated". This could make this technique an effective screening tool, requiring additional methods for confirmation of all flagged samples. It is worth mentioning that in both scenarios the fusion of classifications from the three different sensing approaches led to a better overall detection rate. However, this food commodity leans heavily on the performance of NIR technology, which is in accordance with literature results on applying solely NIR in SMP fraud detection. For other food commodities the combination of models may differ. In section 3.4 and 3.5, scenario 2 will be detailed further.

3.4. Detection of filler additions to SMPs in scenario 2

In Fig. 4, the fused classifications are presented as function of concentration of filler added. All results were normalized using the thresholds, so every classification below 1 is classified as "in-class" whereas every classification above 1 is "out-of- class". In some cases, fresh SMP might be mixed with one that has been in storage for a longer period. Only 65% of the skimmed milk powders adulterated with old SMPs, ones that have been stored for over a year, were detected, evenly distributed over the different concentrations. Hence, no clear separation was observed. In order to simulate volume increments at low costs, SMPs were adulterated with plant protein powders, whey powders, sugars or starch that are cheaper than fresh skimmed milk powder. The 'PhasmaFood' device flagged 99% of these adulterations correctly as "adulterated". Within this group, 99% of SMPs with added plant proteins, and 100% of SMPs with added whey, sugars and starch were classified correctly, respectively. The samples containing plant proteins which were wrongly classified as authentic SMP, were mixtures of 10% plant protein addition. As visualized in Fig. 4, the overall detection of 10% fillers mixed in SMPs scored lower fused class distances than the addition in higher concentrations. Protein powders from plants may contain traces of secondary metabolites (i.e. (degradation products of) porphyrin structures), that result in a fluorescent or VIS signal deviating from the one of a pure SMP as outlined in the raw data section. Therefore, the detection of a possible adulteration may be based on all three sensors. When whey, starch or sugar are added the mixtures did not differ visually from SMP. Hence, their detection relied on the NIR sensor to detect differences in protein composition (Table 4). Interestingly, in the case of lactose addition, the increased levels of lactose were detected by the sensor-OCC model combination. Results on the detection accuracy of starch and sugar addition are in agreement with previous reports using near-infrared approaches (Capuano, Boerrigter-Eenling, Koot, & van Ruth, 2015). It is worth mentioning that in the present experiment both the visible and the fluorescence approach added some information and increased the correct classification rate for this specific type of adulteration after the data fusion step (Table 4).

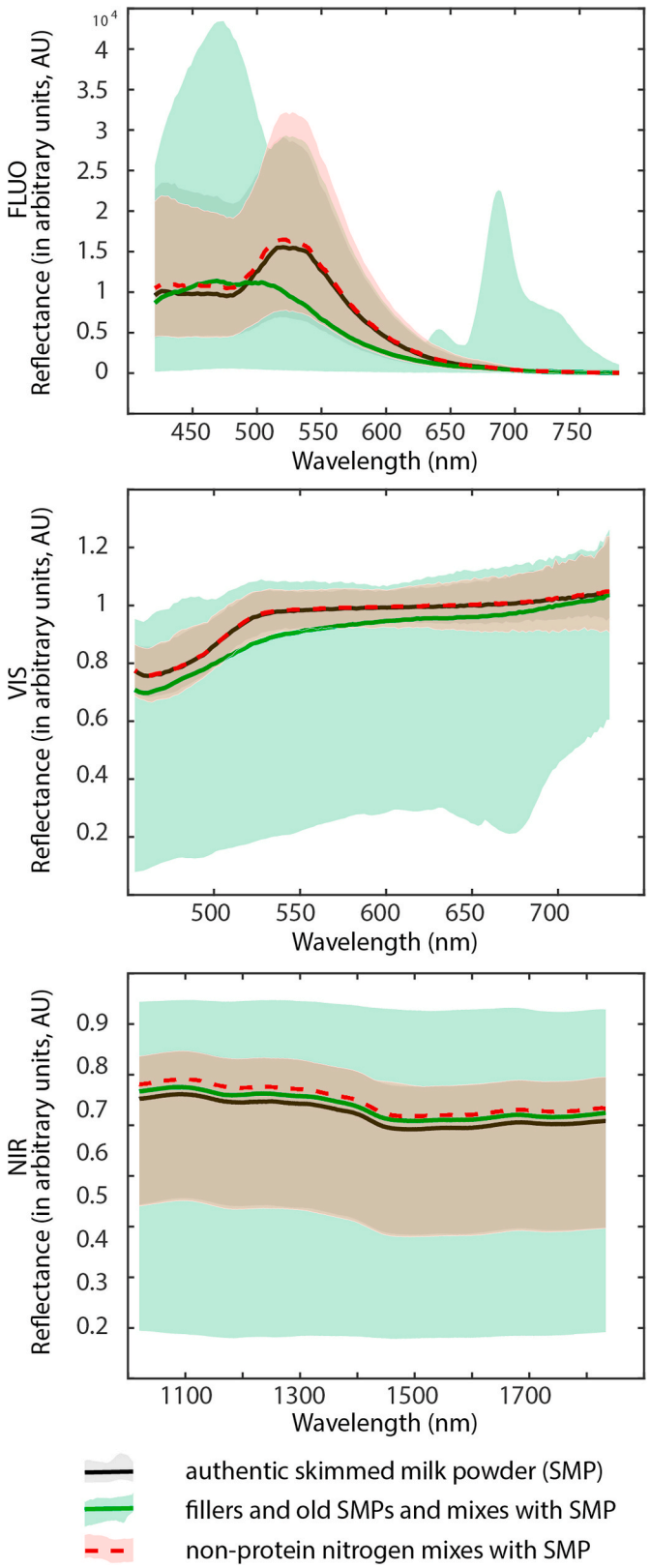


Fig. 3. Raw data of the three respective spectroscopic approaches (FLUO, VIS and NIR) before processing plotted in sample classes: authentic SMPs in black, fillers including old SMPs and mixes in green and non-protein nitrogen mixes in red (dashed). The thick lines represent the median of the respective spectra, while the range depicts all spectra between the respective maximum and minimum spectrum. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Correctly identified samples in %, when applying the thresholds that lead to 100% correct identification of the authentic class (SMP) as described for scenario 1.

	Combination (Decision tree[a])	Only NIR[b]	Only FLUO[c]	Only VIS[d]
SMP	100%	100%	100%	100%
Old SMP (10,25,50, 100%)	19%	6%	4%	3%
Adulterated with fillers (10,25,50, 100%)	89%	58%	5%	80%
Adulterated with non-protein nitrogen (0.1,1,2,5%)	12%	4%	5%	2%
All old SMPs, adulterants (fillers and non-protein nitrogen), adulterated samples	64%	43%	5%	51%

[a] If two or more out of the 10 models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.
[b] If two or more out of the 6 NIR models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.
[c] If two out of the 2 FLUO models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.
[d] If two out of the 2 VIS models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.

Table 4
Correctly identified samples in %, when applying the thresholds that lead to around 80% correct identification of the authentic class (SMP) as described for scenario 2.

	Combination (Decision tree[a])	Only NIR[b]	Only FLUO[c]	Only VIS[d]
SMP	80%	80%	80%	80%
Old SMP (10,25,50, 100%)	65%	54%	22%	36%
Adulterated with fillers (10,25,50, 100%)	99%	92%	25%	93%
Adulterated with non-protein nitrogen (0.1,1,2,5%)	60%	41%	24%	27%
All old SMPs, adulterants (fillers and non-protein nitrogen), adulterated samples	86%	77%	25%	70%

[a] If two or more out of the 10 models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.
[b] If two or more out of the 6 NIR models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.
[c] If two out of the 2 FLUO models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.
[d] If two out of the 2 VIS models (Table 2) classified a sample as 'out-of-class' it was flagged as 'not within the SMP class'. The median class distances of sample triplicates were used for the calculations.

3.5. Detection of non-protein nitrogen addition to SMPs in scenario 2

In contrast to the fillers, non-protein nitrogen was added in lower concentrations to simulate an increased apparent protein content of up to 20% (Table 1). 42%, 75%, 67% and 56% of ammonium chloride, ammonium nitrate, melamine and urea additions were classified correctly, respectively. Fig. 5 shows that more classifications were correct when the concentrations increased. However, it is obvious that the detection of these non-protein nitrogen additions remains challenging. The addition of non-protein nitrogen to milk powder and other milk products has been investigated with multiple approaches (Cattaneo &

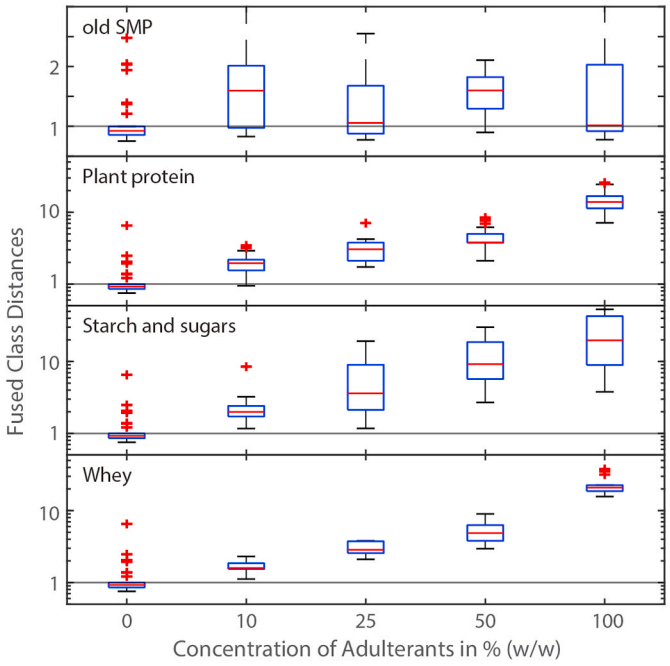


Fig. 4. Normalized with thresholds, fused class distances for skimmed milk powders adulterated with fillers. All classifications above 1 are classified as "out-of-class".

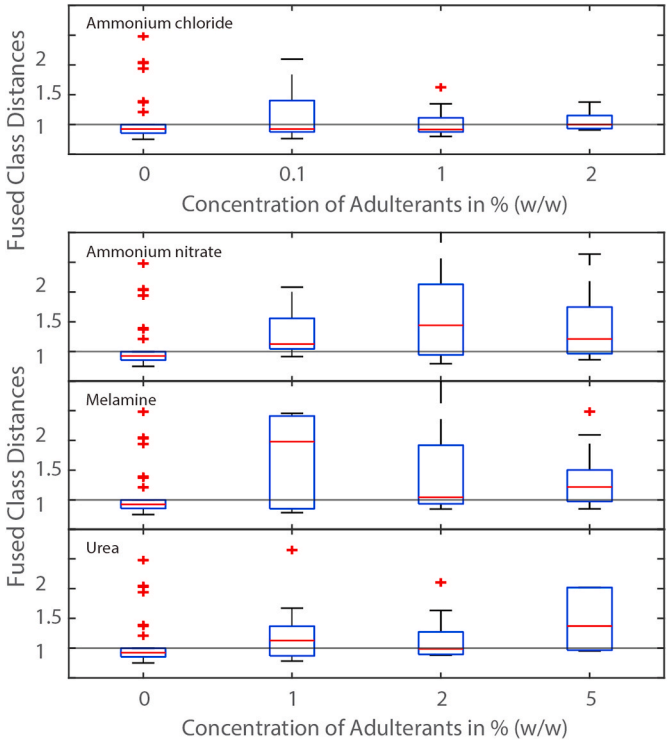


Fig. 5. Normalized with thresholds, fused class distances for skimmed milk powders adulterated with non-protein nitrogen. All classifications above 1 are classified as "out-of-class".

Holroyd, 2013; Poonia et al., 2017). For example, Karunathilaka, Yakes, He, Chung, and Mossoba (2018) examined melamine, dicyandiamide, aminotriazole, biuret, and cyanuric acid and observed good classification results (non-targeted SIMCA approach) when applying two bench-top devices. Their application of a hand-held infrared instrument led to lower correct classification results, due to the devices' lower resolution and limited spectral range. Further, is it important to keep in mind that most reports on very good results with spectroscopic approaches might be misleading due to not-randomized experimental design as outlined recently by Pasquini (2018). To overcome this issue, in this study different SMPs (with deviating moisture content) were adulterated with non-protein nitrogen. This might be the reason for the lower detection rate than in previous reports on the detection of adulterated milk powders with spectroscopic approaches. We believe, however, that there is great potential in the usage of hyphenated sensors and fusing several classification results as may be seen in Table 4.

4. Conclusion

For the first time, we report the usage of a novel, portable, hyphenated sensor in the detection of food fraud that generates information from different spectroscopic approaches at the same time from the same sample and spot. By combining three miniaturized spectroscopic approaches, VIS, fluorescence and NIR, detection of adulterations in skimmed milk powder was possible with an overall accuracy of 86%. The fusion of the classification results from ten OCC models for three different optical approaches improved the overall classification accuracy. The multivariate statistics approach used enables a tailored application of different thresholds to balance the false negative or false positive classifications targeting the needs of the respective operators such as governmental agencies, industry and academia. For example, when the correct classification rate for skimmed milk powder was set at about 80%, 99% of SMP samples with added plant protein, whey, sugars and starch to SMP were identified correctly. However, correct classification of skimmed milk powders with low concentrations (<5% w/w) of non-protein nitrogen remained challenging as only 60% of these adulterated samples were identified correctly. We believe that this report on a novel, portable, hyphenated device and data fusion attempt will support the fight against food fraud. Further research will be conducted using the 'PhasmaFood' device on other food commodities or developing further hyphenated devices.

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CRediT authorship contribution statement

Judith Müller-Maatsch: Conceptualization, Data curation, Methodology, Formal analysis, Project administration, Supervision, Writing - original draft, Visualization, Writing - review & editing. **Martin Alewijn:** Data curation, Methodology, Formal analysis, Software, Validation, Writing - original draft, Writing - review & editing. **Michiel Wijtten:** Formal analysis, Writing - review & editing. **Yannick Weesepoel:** Conceptualization, Funding acquisition, Resources, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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