**Supplementary information**

**A portable dry film FTIR instrument for industrial food and bioprocess applications**

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**Supplementary Materials**

**S1. Chemicals, standards, and samples**

Chemicals and standards were purchased and used in different experiments throughout this study.A range of analytical grade molecular weight standards consisting of bovine serum albumin (BSA), albumin from chicken egg white, carbonic anhydrase from bovine erythrocytes, lysozyme, cytochrome c from bovine heart, aprotinin from bovine lung, oxidized insulin chain B from bovine pancreas, human angiotensin II, bradykinin fragment 1-7, [D-Ala2]-leucine enkephalin, Val-Tyr-Val, and L-tryptophan was procured from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). Glucose was obtained from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). Fetal bovine serum was purchased from Thermo Fisher Scientific (Waltham, MA, USA), Ultroser G was purchased from PALL Corporation (NY, USA) and Cytodex 1 microcarrier was purchased from Sigma-Aldrich (Merck, KGaA, Darmstadt, Germany). Egg white powder was obtained from Norilia (Oslo, Norway), and pork plasma was obtained from the pork blood in a commercial slaughter abattoir in Flesland (Hvalstad, Norway). Alcalase was purchased from Novozymes A/S (Bagsværd, Denmark). HPLC-grade acetonitrile was supplied by VWR (Radnor, PA, USA). Purified water was prepared by deionization and membrane filtration (0.22 μm) using a Millipore Milli-Q purification system (Merck Millipore, Burlington, MA, USA).

**S2. Enzymatic protein hydrolysates**

Enzymatic hydrolysis of residual raw materials sourced from chicken and turkey processing took place at the Bioco enzymatic protein hydrolysis production plant (Hærland, Norway). The hydrolysis process is a multi-stage process and includes grinding, water addition, preheating, enzyme introduction, and enzyme deactivation. Usually, an uncontrolled mixture of chicken and turkey carcasses is used as feed which subsequently is hydrolyzed with one enzyme. For this study, however, the feed and enzyme types were varied according to an experimental plan, creating a large variation in protein composition. A more detailed description of the sampling process and the experimental plan is mentioned in Kafle et al., 2024.1

A total of 463 samples were collected from the enzymatic hydrolysis process line during November and December 2022. After each sampling, centrifugation for 4 min at 4100 rpm (MEGA STAR 600, VWR, Oslo, Norway) was carried out for phase separation. The phase-separated samples were frozen down to -20 °C and sent to the laboratory for further analysis. The FTIR acquisition of these protein hydrolysates was performed using both portable dry film FTIR and benchtop FTIR in a controlled laboratory environment. Out of 463 protein hydrolysate samples, a subset of 200 samples were selected for reference analysis and development of calibration models for predicting AMW, whereas 68 samples were selected for developing calibration models for collagen content. Sample selection was performed using a space-filling algorithm (the Kennard-stone algorithm) on the first two components from the principal component analysis (PCA) of the FTIR spectra.2

Additional sampling was performed in May 2023 collecting 86 protein hydrolysate samples. At this time, a different enzyme than in the previous sampling was used in the process. These samples were measured fresh (i.e., not previously frozen) with the portable dry film FTIR system at-line to the process lines in the industrial environment. Later, the samples were frozen and carried to the laboratory for measurements with the benchtop FTIR system. Out of these 86 protein hydrolysate samples, 60 samples were chosen to develop calibration models for the prediction of AMW, and the remaining 26 samples were reserved for model validation.

**S3. Lactate-containing media**

Bovine muscle satellite cells (MuSCs) were isolated from freshly slaughtered cattle at an industrial abattoir using a well-established method.3, 4 Extracted from *Longissimus thoracis* (beef sirloin, Nortura AS, Rudshøgda, Norway), the cells originated from young bulls of the Norwegian Red breed. No Animal Procedure approval was necessary, as confirmed by the Norwegian Food Safety Authority under Norwegian Law. Additional details about the procedure can be found in Tzimorotas et al., 2023.5

MuSCs expansion potential was examined in a spinner flask under varied conditions. The experimental setup included a proliferation medium with serum (2 % Fetal Bovine Serum, FBS + 2 % Ultroser G), a cell density of 900 cells/cm2, and Cytodex 1 microcarriers. Cultures were triplicated, employing 150 mL antibiotic-free media, stirring at 50 rpm for eight days, using DWK Life Sciences Wheaton Magna-Flex spinner flasks (250 mL, Thermo Fisher Scientific, MA, USA). Sampling took place on days 1, 3, 5, 7, and 9. An alternative experiment maintained the same parameters with a temperature shift (37 °C) and included various hydrolysates (described previously)6 from the Norwegian food industry as serum replacements. These hydrolysates, individually added and compared, comprised 2 % serum, 2 % FBS, Alcalase-treated egg white powder (high and low molecular weight fractions), and Alcalase-treated pork plasma (high and low molecular weight fractions). Day 0 samples were collected 20 min after inoculation (no stirring), followed by samples at 2, 4, 7, and 9 days. From both experimental setups, a total of 59 samples were collected for FTIR analysis.

**S4. Average molecular weights (AMW) of protein hydrolysates**

The AMW calculations of protein hydrolysates were carried out following a published protocol using SEC analysis with some adjustments.7 The molecular weight standard solutions were prepared at 2 mg/mL in ultrapure water. The mobile phase was a 30:70 (V/V) mixture of acetonitrile and ultrapure water with 0.05 % trifluoroacetic acid (TFA). For both standards and samples, an injection volume of 10 μL was used. The chromatographic separation was performed using a BioSep SEC-S2000 column, 300 mm long with an inner diameter of 7.8 mm (Phenomenex, Torrance, CA, USA). Isocratic elution was maintained at a 0.9 mL/min flow rate for 20 min, followed by a switch to NaH2PO4 (0.1 M) for 3 minutes (for cleaning). The column was equilibrated for 27 min before the subsequent run. The sample compartment was maintained at a temperature of 40 °C. The detection of the eluents was achieved by monitoring absorbance at 214 nm. The size exclusion chromatographic traces were acquired using a Dionex UltiMate 3000 instrument (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a quaternary pump and photodiode array detector.

The initial chromatograms obtained from the size exclusion chromatography (SEC) were used for generating molecular weight distributions and calculating the AMW through the application of established calibration standards.7 All these calculations were carried out using the openly available MATLAB toolbox SEC2MWD.8

**S5. Collagen content**

The collagen content was quantified by measurement of the amino acid Hyp using a Hydroxyproline assay and assuming 13.5 % Hyp per weight in collagen.9 A subset of 68 protein hydrolysate samples underwent Hyp content analysis using the Hydroxyproline assay kit MAK008-1KT (Sigma-Aldrich, St. Louis, MO, USA), following the methodology outlined by Kristoffersen et al., (2022).10 The analysis was performed in triplicates. The freeze-dried samples were weighed and dissolved in 1.0 M HCl to prepare a 1 mg/mL solution. The samples were further diluted with 50 % v/v of concentrated HCl (~12 M) in Wheaton sample vials with polytetrafluoroethylene (PTFE) solid caps. The samples were hydrolyzed for 3 hours at 110 °C in a heating block from VWR (Radnor, PA, USA). After hydrolysis, 10 µL of the samples and various concentrations of Hydroxyproline standards 0, 0.2, 0.4, 0.6, 0.8, and 1 µg/well were transferred to a Pierce 96-well Polystyrene plate (Thermo Fisher Scientific, Waltham, MA, USA). After heating at 60 °C for around 30-40 min, 100 µL of the reagent mix containing Chloramine-T in oxidation buffer was added to all samples and standards. Following a 5-min mixing and incubation, 100 µL of Dimethylamine borane (DMAB) reagent containing 50 % of Perchloric acid, was added to the wells and mixed. The plate was again incubated in a heated oven at 60 °C for 90 min. The absorbance was measured at 560 nm using a BioTek Synergy H1 spectrophotometer (BioTek Instruments, VT, USA). The Hydroxyproline standard curves were used to determine the Hydroxyproline content as described in the Sigma-Aldrich protocol.

**S6. Lactate content in cultivation media**

Lactate production from MuSCs in the cultivation medium was assessed using the Reflectoquant test developed by Merck KGaA using the RQflex 10 instrument (Darmstadt, Germany). The Reflectoquant test relies on the oxidation of lactate by nicotinamide adenine dinucleotide (NAD) catalyzed by lactate dehydrogenase, resulting in the formation of pyruvate. In the presence of diaphorase, the NADH reduces a tetrazolium salt to a blue formazan, which is quantified reflectometrically. The reflectometric test strips were immersed in an appropriately diluted supernatant of the samples, with a measuring concentration range of 3 to 60 mg/L, and then carefully positioned in the instrument for analysis. The obtained results were used as a reference for developing calibration models for predicting lactate content in the cultivation medium.

**S7. Dark noise and noise estimate**

The noise discussed here pertains specifically to processes related to the instrument itself, such as the source, detector, and other components in the set-up, excluding any signal variation that could be attributed to environmental factors water vapor or carbon dioxide.

We have conducted a comprehensive investigation into the dark noise of the system across the entire spectral range (Fig. S3). The dark noise can influence the signal-to-noise ratio, but it can efficiently be reduced by averaging, for example, taking 40 spectra. There is not much to gain from taking more than 40 spectra. Dark noise is somewhat larger than the noise shown in the other images (Fig. S4), with approx. 1 count at each wavenumber and remains fairly constant throughout the entire spectrum.

Additionally, we examined the noise in the instrument in various regions across the spectrum to ensure that this is not dependent on the signal level. For example, we have chosen the regions from 5000 cm-1 to 4800 cm-1 and from 2640 cm-1 to 2500 cm-1.

At first,

* Five repetitions of 40 averages of the reference well of the silicon wafer were taken
* Two regions were chosen from 5000 cm-1 to 4800 cm-1 and from 2640 cm-1 to 2500 cm-1
* For each region

1. subtract the mean of those regions of these five runs
2. subtract a linear trend from (a)
3. plot the residuals

The signal level is quite different in those regions, but both are free of absorption when measuring laboratory air (Fig. S4). The noise is practically identical in the two regions (thus, we assume the whole spectrum), with a standard deviation of approx. 0.1 counts.

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**Supplementary Tables**

**Table S1:** Analytical performance of the portable dry film FTIR system for BSA standard proteins in glucose solution

|  |  |
| --- | --- |
| **Portable dry film FTIR** | **Values** |
| Concentration range | 0.1 – 10 µg/well |
| Calibration equation | y = 0.2561x + 0.1062 |
| R2 | 0.99 |
| SD of intercept | 0.046 |
| LOD (µg/well) | 0.6 |
| LOQ (µg/well) | 1.8 |

**Table S2**: The second derivative bands for protein hydrolysates in the spectral region from 1800 cm-1 to 834 cm-1

|  |  |  |
| --- | --- | --- |
| **Band Positions (cm-1)** | | **Annotation** |
| **Benchtop FTIR** | **Portable dry film FTIR** |  |
| 1690–1670 | 1690–1670 | C=O amide I: β turns |
| 1656 | 1656 | C=O amide I: α-helix |
| 1631 | 1634 | C=O amide I: β sheet |
| 1593 | 1584 | COO− (asym stretch) |
| 1548 | 1548 | Amide II: α-helix |
| 1517 | 1516 | –NH3+ (scissor) |
| 1454 | 1452 | CH2 (scissor) |
| 1402 | 1398 | COO− (sym stretch) |
| 1313 | 1310 | Amide III, CH2 (def, rock), OH (def, bend) |
| 1242 | 1238 | Amide III, C–O (stretch) |
| 1124 | 1122 | CNH3 (rock), CH2 (wag |
| 1083 | 1082 | C-O stretching |
| 1045 | 1042 | CO, CC, CN (stretch) |
| 977 | 976 | CCOO (wagging) |
| 928 | 924 | CH2 (twist) |
| 851 | 850 | Not Assigned |

**Supplementary Figures**

A graph showing a blue and red line

Description automatically generated

**Fig. S1**. Mean spectrum of PET foil (in transmission mode) measured at different environments using a portable dry film FTIR system (T = mean temperature, RH = mean relative humidity).

**A diagram of different colored dots

Description automatically generated**

**Fig. S2.** The measured relative humidity and temperature measured in laboratory and industrial environment.

A red and blue graph

**Fig. S3.** The dark noise in the detector for a single spectrum and the average of 40 and 100 spectra.

A group of graphs showing different types of data

Description automatically generated

**Fig. S4.** The noise in two wavenumber regions, A) 5000 cm-1 to 4800 cm-1 and B) 2640 cm-1 to 2500 cm-1.

**A graph of different types of waves

Description automatically generated with medium confidence**

**Fig. S5**. Predicted vs. reference plot of PLSR model for calibration of AMW of protein hydrolysates with corresponding regression coefficients, using data measured with the portable dry film FTIR system (A) and the benchtop FTIR (B).

**A group of graphs showing different types of data

Description automatically generated**

**Fig. S6**. Predicted vs. reference plot of PLSR model for calibration of collagen content in protein hydrolysates with corresponding regression coefficients, using data measured with the portable dry film FTIR system (A) and the benchtop FTIR (B).

**A graph of a wave number

Description automatically generated**

**Fig. S7**. Second derivative of FTIR absorbance spectra (1800 cm-1 to 834 cm-1) of representative cultivation media samples, measured with the portable dry film FTIR system (A) and the benchtop FTIR (B).

**A group of graphs showing different types of data

Description automatically generated with medium confidence**

**Fig. S8**. Predicted vs. reference plot of PLSR model for calibration of lactate content in cultivation media with corresponding regression coefficients, using data measured with the portable dry film FTIR system (A) and the benchtop FTIR (B).