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Data Article

Dataset on proteomic changes of whey protein after different heat treatment

Ling Xiong ^a, Sjef Boeren ^b, Jacques Vervoort ^b,
Kasper Hettinga ^{a,*}^a Dairy Science and Technology, Food Quality and Design, Wageningen University and Research, P.O. Box 17, 6700AA, Wageningen, the Netherlands^b Laboratory of Biochemistry, Wageningen University and Research, P.O. Box 8128, 6700ET, Wageningen, the Netherlands

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ABSTRACT

Hereby we provide data from a shot-gun proteomics experiment, using filtered-aided sample preparation (FASP), and liquid chromatography with tandem mass spectrometry (LC-MS/MS), to relatively quantify the changes in the protein profile of whey proteins after heating milk at either 65 °C, 70 °C, 75 °C, 80 °C, or 85 °C for 30 min. The data supplied in this article supports the accompanying publication [1]. The raw mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier "PXD016436".

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1. Data

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD016436. Data analysis output from MaxQuant is made available as Excel document, both as raw output as well as filtered and annotated

* Corresponding author.

E-mail address: kasper.hettinga@wur.nl (K. Hettinga).

Specifications Table

Subject	Agricultural and Biological Sciences
Specific subject area	Food Science - Heat treatment induced changes of whey protein
Type of data	Raw LC-MS/MS datafiles, spreadsheets containing raw output from MaxQuant as well as filtered and annotated output.
How data were acquired	Filtered-aided sample preparation (FASP), LC-MS/MS (Thermo Orbitrap-XL).
Data format	RAW files (Thermo proprietary) and Excel files with data analysis output.
Parameters for data collection	Whey protein from milk heated at 65 °C, 70 °C, 75 °C, 80 °C, or 85 °C for 30 min.
Description of data collection	Skim milk samples were heated at 65 °C, 70 °C, 75 °C, 80 °C, or 85 °C for 30 min. pH of heated skim milk samples was adjusted to 4.6 and samples were then centrifuged at 100,000 g for 90 min to remove the casein and denatured whey proteins. Milk serum containing native whey protein was prepared with FASP prior to analysis by LC-MS/MS. Wageningen University and Research, Wageningen, The Netherlands.
Data source location	The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD016436 (https://www.ebi.ac.uk/pride/archive/projects/PXD016436). An Excel document with data output is linked directly to this article (Supplementary table 1).
Data accessibility	
Related research article	Xiong, L. et al., <i>Effect of heat treatment on bacteriostatic activity and protein profile of bovine whey proteins</i> . Food Research International, 2020. 127 : p. 108688.

Value of the Data

- This dataset shows quantitative changes of bovine whey protein after heating at different temperatures within the pasteurization range, focusing on low abundant proteins.
- Researchers who study denaturation of whey protein can use this data as a comparison.
- Starting from these data, the effect of different components in milk on heat induced changes of individual proteins could be studied further, focusing on both interaction between milk components and their biological activity.
- These data provide information about changes of whey proteins induced by heat treatment, also in relation to their biological activity, which can help to optimize the processing of bio-functional dairy products.

output ([Supplementary table 1](#)). The raw MaxQuant output contains all the distinct proteins that were identified and quantified along with the number of peptides detected. The dataset was filtered to show only proteins reliably identified by minimally 2 peptides of which at least 1 was unique and 1 was unmodified. This filtered and annotated dataset is the basis of the accompanying publication “Effect of heat treatment on bacteriostatic activity and protein profile of bovine whey proteins” [1].

2. Experimental design, materials, and methods

2.1. Material

Bovine raw tank milk was collected from clinically healthy cows, which were all housed on the Wageningen University farm (Wageningen, Netherlands).

2.2. Heat treatment

Milk fat was removed from the raw unheated milk by centrifuging milk at 1500×g for 20 minutes at 4 °C (with rotor 16.250, Avanti Centrifuge J-26 XP, Beckman Coulter, USA). After that, 50 ml of the unheated skim milk samples was heated at 65 °C, 70 °C, 75 °C, 80 °C or 85 °C, in triplicate, for 30 min, in a water bath. After this heat treatment, samples were cooled with ice water to room temperature before further analysis was conducted.

2.3. Native whey protein preparation

Casein and denatured proteins were removed from the skim milk samples by acidification and ultracentrifugation according to a previous paper [2]. The pH of all heated skim milk samples was adjusted to 4.6. This causes a precipitation of the casein together with the denatured/aggregated whey proteins. After equilibrating the acidified samples at 4 °C for 30 min, the samples were ultracentrifuged at 100,000×g for 90 min at 30 °C (with rotor 70 Ti, Beckman L-60, Beckman Coulter, USA). After ultracentrifugation, the top layer contained some traces of milk fat, whereas the micellar casein with the denatured/aggregated whey proteins were present in the pellet in the bottom of the tube. The supernatant, which is including the native whey proteins, was in the middle layer of the ultracentrifugation tube. This supernatant fraction was collected for further analysis.

2.4. Proteomics analysis

The Filter Aided Sample Preparation (FASP) method was carried out according to published articles [3–5]. Native whey protein samples of 20 µL (with 116–200 µg protein) were diluted in 180 µL SDT-lysis buffer (100 mM Tris/HCl pH 8.0 + 4% SDS + 0.1 M dithiothreitol) to get an approximately 1 µg/µL protein solution. After incubation at 95 °C for 10 min, samples were centrifuged at 13524×g for 10 min in an Eppendorf 5424 centrifuge (Eppendorf, Hamburg, Germany). The centrifuged samples were cooled down to room temperature. Then, 20 µL (11.6–20 µg protein) of the sample in the SDT buffer was mixed with 180 µL 0.05 M IAA (Iodoacetamide)/UT (100 mM Tris/HCl pH 8.0 + 8 M urea) in a low binding Eppendorf tube. This sample was then incubated for 10 min while mild shaking at room temperature. Part of the sample (100 µL, containing 5.8–10 µg protein) was transferred to a Pall 3K omega filter (10–20 kDa cutoff, OD003C34; Pall, Washington, NY, USA) and the filter was then centrifuged at 13524×g for 30 min. The same centrifugation step was repeated three more times after adding 100 µL UT. After this centrifugation with UT, 110 µL 0.05 M ammonium bicarbonate (ABC) was added to the filter unit and centrifuged at 13524×g for 30 min. The filter was then transferred to a new low-binding Eppendorf tube, after which 100 µL ABC containing 0.5 µg trypsin was added. The tube with filter was centrifuged at 13524×g for 30 min after incubation for 2 hours at 45 °C. The tryptic peptides were in this way collected in the low-binding tubes. Finally, 4–6 µL 10% trifluoroacetic acid (TFA) was added to the tryptic peptides to adjust the pH to around 2 for LC-MS/MS analysis.

The LC-MS/MS parameters are the same as described previously [6]. First, 18 µL of the trypsin-digested peptides was injected onto a 0.10 * 30 mm Magic C18AQ 200A 5 µm beads (Michrom Bioresources Inc., USA) pre-concentration column (prepared in house) at a maximum pressure of 800 bar. Peptides were eluted from the pre-concentration column onto a 0.10 * 200 mm ReproSil-Pur 120 C18-AQ 1.9 µm beads analytical column with an acetonitrile gradient at a flow of 0.5 µL/min, using gradient elution from 8% to 33% acetonitrile in water with 0.5 v/v % acetic acid in 50 min. The column was washed using an increase in the percentage acetonitrile to 80% (with 20% water and 0.5 v/v % acetic acid in the acetonitrile and water) in 3 min. Between the pre-concentration and analytical column, an electropray potential of 3.5 kV was applied directly to the eluent a stainless steel needle fitted into the waste line of the micro cross. Full scan positive mode FTMS spectra between *m/z* 380 and 1400 were measured on an LTQ-Orbitrap XL (Thermo Electron, San Jose, CA, USA). CID-fragmented MSMS scans of the four most abundant doubly- and triply-charged peaks in the FTMS scan were recorded in data-dependent mode in the linear trap (MSMS threshold = 5.000).

For protein identification and quantification, each run with all MSMS spectra obtained was analyzed with Maxquant 1.6.0.1 with the Andromeda search engine. The digestion of enzyme was set as trypsin. Carbamidomethylation of cysteines was set as a fixed modification, and Oxidation of methionine, N-terminal acetylation and de-amidation of asparagine or glutamine were set as variable modifications. The bovine (taxonomy ID: 9913) reference database for peptide and protein searches was downloaded as fasta file from Uniprot with reverse sequences generated by Maxquant. All fasta files were downloaded from <http://www.uniprot.org/> (accessed Dec 2013). A set of 31 protein sequences of common contaminants was used as well, which included Trypsin (P00760, bovine), Trypsin (P00761, porcine), Keratin K22E (P35908, human), Keratin K1C9 (P35527, human), Keratin K2C1 (P04264, human), and Keratin K1C1 (P35527, human). A maximum of two missed cleavages were allowed, and a mass

deviation of 0.5 Da for fragment MS/MS peaks and 20 ppm and 4.5 ppm for the peptide MS peaks during the first and main search, respectively. The false discovery rate (FDR) was set to 1% on both peptide and protein level. The length of peptides was set to at least seven amino acids. Finally, proteins were identified based on minimally 2 distinct peptides of which at least one unique and at least one unmodified. The intensity based absolute quantification (iBAQ) algorithm calculates the sum of all peptide intensities divided by the number of theoretically observable tryptic peptides.

The relevant MS data have been deposited to the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) via PRIDE with the identifier PXD016436 [7].

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dib.2020.105227>.

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