Detection of fresh, chilled and frozen-thawed meat by means of metabolite profiling and HADH-assay

Manuela Peukert [1], Buesra Canbaz [2], Dagmar Adeline Brueggemann [1]

[1] Max Rubner-Institute, Germany, [2] University of Ankara, Turkey

Objectives: The discrimination of fresh and frozen-thawed raw meat has been an issue in relation to meat safety, meat quality and consumer deception for many years. The enzymatic detection of frozen meat by HADH assay is widely accepted, especially when the meat was deep frozen (> -18°C)[1]. However, temperature dependent limitations of HADH assay validity between chilling (2°C) and deep freezing (> -18°C) have yet been barely studied. Alternative markers for fresh and frozen-thawed meat distinction are needed that cover various temperature regimes and freezing cycles. We investigate storage time and temperature dependent patterns of metabolites in meat (i) to better understand ripening processes in relation to meat quality as well as matrix and microbial depen- dent spoilage, and (ii) to elucidate metabolite patterns, which correspond to varying storage conditions. As an initial study, the pre- sented work focusses on metabolite changes during cold storage of pork meat (*M. Longissimus dorsi*) at 2°C, -3°C and -28°C. In addition, the HADH assay has been applied to validate the test performance under different freezing temperatures.

Materials and Methods: Pork *M. longissimus dorsi* were purchased from a local slaughterhouse 24h *post mortem*. Vacuum packed samples were transferred to different storage conditions - control samples were directly frozen in liquid nitrogen and subsequently frozen at -80°C. Chill samples were stored at 2°C for 2, 5, 8 and 13 days, superchill (-3°C) and freeze samples (-28°C) were stored for 5 and 13 days. Before extraction, meat samples were lyophilized and homogenized using a ball mill. Extraction was carried out in two steps: First, a polar extraction with 80% methanol and second, an extraction using methanol:chloroform. Combined extracts were dried in glass vials by vacuum centrifugation. Before GC-MS measurement, samples were derivatized by methoxyamination followed by silylation with MSTFA. The GC-MS system was a Shimadzu GCMS QP2010 Ultra, equipped with a non-polar Rxi-5SilMS (Restek, Bellefont, USA) column. For data acquisition and data integration the GCMS Solution software from Shimadzu was used. Further statistical analyses and data visualization were performed with the software tools SIMCA (Umetrics, Malmö, Sweden) and JMP (SAS, Heidelberg, Germany). The HADH assay was performed according to Gottesmann and Hamm (1982)[2] with adaptions in terms of volume reduction and dilution of the meat extracts.

Results and Discussion: Principal component analysis (PCA) revealed a huge shift in the overall pattern of metabolites during stor- age at 2°C. This shift was mainly caused by increasing free amino acid, fatty acid and monosaccharide contents due to proteolytic breakdown, sugar hydrolysis and lipid degradation processes during the ripening process. Also, degradation products from energy metabolism accumulated continuously during the two weeks sampling period. Superchilling at -3°C slowed down the breakdown processes. After two weeks at -3°C a similar metabolite pattern compared to 8 days at 2°C was observed, and at -28°C storage temperature the original pattern was nearly conserved. However, some differences between controls and freezing at -28°C were found. For example, small organic acids such as glycerate and malonate, fatty acids and the amino acid phenylalanine slightly increased. Besides being temperature dependent, metabolic shifts are strongly related to the time component. To classify metabolic markers into a time-temperature map, compounds independent of feeding, gender, genotype and rearing conditions need to be determined at first. A detection of frozen-thawed meat would then be possible if slaughter time is (correctly) known. The increase of free fatty ac- ids and precursors of lipid biosynthesis (malonate and glycerate) is suspected to result from lipid structure damage due to ice crystal forming and oxidative processes. Though amount and state of most metabolites has been preserved during frozen storage, substrates sensitive to oxidation are still susceptible and represent a target to oxidative damage. However, under chilling and superchilling conditions the degradation and oxidation processes are accelerated and detectable even after short storage times. Therefore, it can be suspected that a lower amount of oxidation products might be a hint for frozen storage. To address this hypoth- esis, further studies will use induced oxidative reactions, and involve longer frozen storage times and varying storage temperatures in the range between superchilling and - 40°C compared to chilling at 2°C. In parallel, we tested applicability of the HADH assay at -3°, -10° and -28°C. Enzyme activity was found to be highly temperature dependent. At superchilling, no differentiation to fresh control samples was possible. At -10°C an intermediate activity of HADH was obtained, while at -28°C a clear distinction was pos- sible. The results indicated that application of the HADH assay is only valid when the meat was deep frozen (< - 18°C).

References:

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Key words: Frozen-thawed meat, Metabolomics, Oxidation