A proteomics-based study of endogenous and microbial xylanases and xylanase inhibitors associated with barley grains used for liquid feed

The mature barley grain contains a complement of enzymes that are synthesized during seed development for degradation of seed storage reserves during germination. These enzyme activities (first wave enzymes) are considered important for maximizing nutrient digestibility in food and feed. Several strategies, such as liquid feed and supplementation of amino acids and microbial exogenous enzymes, are applied to improve protein absorption. A diverse commensal microbial community populates the cereal grains. The colonizing microflora constitute an integrated part of the seeds and interact/influence the plant and/or competitors via secretion of an array of enzymes and compounds/metabolites. The occurrence of these enzyme activities both of plant and fungal origin present a great potential for improvement of grain nutritional components for feed applications. Knowledge is lacking in the variation of these enzyme activities between barley cultivars, as well as the distribution and composition of the residing commensal fungal community and the grain surface associated proteins. To obtain new insight into the interplay between barley grains and the colonizing fungi, we set out to study the activities of barley grains as well as the populating fungal community using different approaches.

The present thesis aimed at profiling the changes in proteome of liquid feed using gel based proteomics. Comparative proteomics analysis was performed on liquid feed supplemented with protease inhibitors and/or the thioredoxin system (NTR/Trx). Addition of protease inhibitors to liquid feed seemed to some extent hamper protein degradation. With the NTR/Trx, the 2D-gels clearly showed an altered protein pattern and increased solubility of storage proteins, CM -proteins and protease inhibitors.

The variability of microbial and endogenous xylanase, as well as xylanase inhibition activities across a set of barley cultivars were determined using activity assays and gel-based proteomic analysis. Considerable inter-cultivar variability was found in the level of both microbial and endogenous xylanase, as well as xylanase inhibition activity levels. Harvest year and cultivar/genotype had a significant impact on the variability of all three parameters. Harvest year/weather conditions seem to largely affect xylanases and inhibition activity levels, thus indicating the instability of these parameters. A Reference map over the surface-associated proteins (surfome) constitutively present on barley grains of two barley cultivars were established using 2-DE and mass spectrometry. The majority of the identified proteins was of plant origin and ascribed to play a role in defense and/or oxidative stress mechanisms. A metaproteomics approach was applied to profile and characterize the composition of the fungal community populating the surface of barley grains across different cultivars and their secretomes. It was revealed, the barley cultivars with high microbial xylanase activity levels were found to contain high numbers of storage fungi e.g. Aspergilli and Penicillia. A detailed analysis of the secretomes of two of the isolated fungi, i.e Aspergillus niger and Fusarium poae, grown on barley flour and wheat arabinoxylan enabled identification of a collection of amylopectic and xylanolytic enzymes involved in cell wall degradation and carbohydrate catabolism.

To gain a better understanding of the role of these xylanase inhibitors, the barley XIP III was expressed in a secretory Pichia pastoris system. The expressed rXIP-III with a HIS6-tag at the C-terminal was purified from the culture medium using metal affinity chromatography. Varying degree of inhibitory activity was found against fungal xylanases, xylanases recovered from the surface of barley grains, and secretomes of grains-associated fungi grown on WAX and barley flour. The secretomes of Fusarium tricinctum were found to be moderately resistant to the rXIP-III, with high residual xylanase activity levels.