Quantification of oat DNA:

1. Feasibility on absolute quantification on spike samples: experimental data and repeat measurements, statistical evaluation. Compilation of quantitative data

We have continued the feasibility study on absolute quantification of oat in spiked corn, soja, and yoghurt samples. According to the milestone and deliverables plan, we have carried out a repeatability study in order to statistically evaluate the quantification results in terms of repeatability and precision. This repeatability study included a comparative analysis of quantification results obtained with a genomic oat standard curve, and a recombinant oat plasmid standard curve. For both standard curve types, we have compiled and statistically evaluated all exploitable quantification data generated for 3 spike levels (10ppm, 50ppm, 500ppm) per matrix (soja, corn, yoghurt). Quantification results were expressed in "oat genome equivalents (ge)" for the genomic standard curve, and "DNA copies" for the plasmid standard curve.

Based on the results obtained, we tried to calculate conversion factors in order to establish (→ rough estimation) a link between the spike level (mg oat per kg matrix), the amount of genome equivalents measured, and the respective DNA copy number calculated. The following observations were made :

- we measured low genome equivalent values in all spike samples, whereas the result expressed in copy numbers was significantly higher. This may be explained by the fact, that the target sequence is present in multiple copies per cell. The ratio ge/copy number was calculated for each matrix and each spike level, which may be an indicator (rough estimation) of the ratio target copies / ge per cell, and target copies / ppm.
- we measured relatively high RSDr values which may be explained by the fact that absolute quantification is much more prone to errors than relative quantification, where DNA extraction effeiciencies, pipetting errors etc are factored out.

2. Comparative analysis of spike samples analysed with the qualitative oat-discriminating PCR system and the quantitative cereal-specific PCR system. Compilation of qualitative data

A statistical evaluation of the results of the oat-discriminating standard PCR system and the cereal-specific quantitative Real-time PCR system has been carried out. For this purpose we have determined the false positive rate for soya and corn samples spiekd with 500, 50 and 10ppm oat respectively. The following results were obtained :

- Oat-discriminating standard PCR system Standard PCR :
    - No false positives for all repeat measurements at the 500, 50 and 10ppm spike level (soja and corn)
- Cereal-specific quantitative Real-time PCR system:
    - Soja :
        - 10ppm : 86% false negatives
        - 50ppm: 14% false negatives
        - 500ppm: 14% false negatives
    - Mais :
        - 10ppm : 43% false negatives
        - 50ppm: 43% false negatives
        - 500ppm: 43% false negatives

The results show a difference in sensitivity of the qualitative and the quantitative PCR systems. The oat-discriminating standard PCR system shows better repeatability and sensitivity at the tested spike levels.

3. Compilation of quantitative and qualitative data for oat and T dicoccum (Farro della Garfagnana

First experiments on pasta samples labelled “without gluten”, and maize starch samples, spiked with different amounts of Farro Della Garfagnana (500mg/kg, 50mg/kg, 10mg/kg), have been presented in the 15 months report. The results showed, that all spiked gluten free pasta samples contained cereal DNA, and the fluorescence signal measured did not correlate with the amount of Farro Della Garfagnana added. The signal most probably corresponded to the initial contamination of the sample with cereals. As a consequence, these results can not be interpreted correctly.

In the oat-quantification assays described above, we also frequently observed false positive results in the unspiked soja and corn samples, demonstrating the difficulty to obtain cereal validation samples free of wheat, rye, barley or oat. For this reason, the

results presented in this study need to be interpreted with care, since quantification results may be biased by residual contamination of the samples. This holds true in particular for samples spiekd with very low amounts of oat. The quantification data presented here therefore may not represent “true” values, but are to be considered as the result of a feasibility study for a absolute quantification by PCR.