HORTINLEA
DIVERSIFYING FOOD SYSTEMS

"Diversifying Food Systems: Horticultural Innovations and Learning for Improved Nutrition and Livelihood in East Africa" (HORTINLEA)

JOINT INTERIM REPORT (SP5)

01/01/2014 – 31/12/2014

FORMAL REPORT

Description of activities/milestones
SP5: Impact of fresh and processed African leafy vegetables on human health
H1: To identify and quantify protective health-promoting compounds in fresh ALV influenced by African genotype, cultivation, postharvest treatment Resulting from the first experiments at the IGZ and using material from MRI important knowledge could be gained on the stability of important secondary plant compounds. Based on these findings material from further experiments in Kenya and Germany are handled.
H2: To investigate and quantify changes within the level of nutrients and health-promoting compounds, anti-nutritive components and carcinogenic mycotoxins in ALV under typical African and improved cooking/ processed procedures The work to this activity is still in an early phase. For the identification of true health effects the relevant health promoting substances and biological markers need to be identified. This work is currently going on. Basic chemical analyses were performed with fresh and fermented cowpea leaves after 7-8 weeks of cultivation in a climatic chamber and the greenhouse. Due to the delayed purchase of uniform seed material not many results are gained yet. First analyses of processed (fermented) plant material have been carried out however in how far this delay might impact the timely progress in this activity cannot be precisely estimated to date.
H3: To identify and quantify carcinogenic mycotoxins in ALV matrix in order to provide recommendations for mycotoxin reduction during processing Leaves of core AIVs were investigated for the appearance of potential mycotoxin producing fungi. Fungi were isolated and identified; mycotoxin production was determined by leaf infection assays. Progress under this activity is on track, no delays from the original time frame are anticipated.
H4: To evaluate the antigenotoxic/antimutagenic potential of ALV against major African environmental carcinogens using human call culture models African environmental carcinogens using human cell culture models; Determination of optimized cultivation conditions and, cooking/processing procedures for a protective effect of ALV against carcinogenic substances.
H5: To evaluate the efficacy of the ALV' protective potential against carcinogens using cancer biomarkers in human intervention trials based on the cell culture Studies Determination of the protective efficacy of ALV against carcinogens in humans
H6: To determine underlying antimutagenic/ anticancer mechanisms of ALV *in vitro* and *in vivo* Identification of antimutagenic/anticancer mechanisms

As a prerequisite, it was made sure that uniform starting conditions and plant material were present for all partners in Germany and Kenya. Subsequent to the first growing experiments at **IGZ**, simple investigations addressing the effect of cooling directly after harvest were conducted. Additionally the effect of a treatment comparable to "solar drying" was included. Qualitatively it could be shown that the amount of some secondary plant metabolites was dramatically reduced by a heat treatment (up to 70°C similar to solar drying). Such effects were most obvious for carotenoids and flavonoids. By contrast, the secondary metabolite content was not significantly altered by immediate deep freezing compared to 6 hr storage at +4°C prior to freezing. In first chemical analyses the spectrum of flavonoids as one major health promoting class of substances was investigated. Existing methods were adapted and extended to the novel metabolites present in the core plants. The pattern of flavonoids is very complex and clearly different from European leafy vegetables. Consequently, it will not by feasible to quantify the substances using commercial reference compounds. It will be necessary to obtain self-isolated substances by extraction and separation from plant material. Both

mentioned results have an impact on the nutritional and health quality of ALV by selecting most effective eco-physiological conditions of chain management (**H1 IGZ**).

Activity **H1** and **H2 (MRI)**: Basic nutrient analyses of fresh and fermented AIVs included the determination of moisture/dry mass, protein, fat, soluble sugars (such as glucose, fructose and sucrose), ash, minerals, and total fibre. Method optimisation and hence analyses of vitamin B_1 and B_2 were conduction. Determination of vitamin C and vitamin E. LC-MS Screening of fresh plant extracts for phenolic acids and their derivatives. The composition of basic nutrients and the concentration of vitamin E did not change significantly after the fermentation process. However, a loss of water-soluble vitamins (up to 75 % vitamin C) was observed. This can be explained by the unavoidable degradation of these vitamins as well as their dissolving in the fermentation liquid. Basic nutrient analyses of fresh and fermented AIVs are connected to activities H1 and H2. Activity **H3 (MRI)**: In the previous reporting period a first overview about the fungal contamination load occurring on cowpeas have been established. In the current reporting period, single colonies were isolated from the surfaces of cowpeas and taxonomically identified by cloning and sequencing of the ribosomal ITS regions. The ITS sequences were compared to the sequences in the GeneBank database by BLAST analysis. By this approach, the main types of species occurring on cowpea plants could be identified. The most important group are Penicillia, followed by Cladosporium, Aspergillus and Fusarium. In a first comparison the fungal population on Amaranth leaves, grown in the same environment than the cowpeas was similar, however in contrast to the situation on cowpeas more Cladosporium strains could be found on Amaranth compared to cowpeas. Until now two potential mycotoxin producing species have been identified: one strain of Aspergillus niger and another strain of A. oryzae. Both are potential but not necessarily ochratoxin A producing organisms. These two strains were grown on YES medium for 7 days. These conditions are usually very supportive for ochratoxin A biosynthesis. However even at these conditions no production of ochratoxin A could be detected. Furthermore, a first correlation between different varieties of cowpeas and growth of potential mycotoxin producing fungi, performed by leaf infection assays have been carried out. These investigations on fungal contamination of AIVs with potential mycotoxin production were performed under the point of view of activity H3.

Initial experiments to determine bioactivity in relation to human liver tumour cells were conducted with plant material from MRI in Karlsruhe. Other partners provided the first plant materials towards the end of 2014. The amount of material made available turned out to be very limited factor. Therefore up until the end of 2014 a systematic assessment of suitable extraction conditions was carried out of which a possibly good yield could occur with the least possible plant consumption (**Act. H4-6 - ALU**).