HORTINLEA
DIVERSIFYING FOOD SYSTEMS

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

JOINT INTERIM REPORT (SP4)

01/01/2014 – 31/12/2014
FORMAL REPORT

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Description of activities/milestones
SP4: Quality assurance and preservation of African vegetables during postharvest for reducing food losses and improving nutritional value, storability and food safety
PH 1: To evaluate optimum development stage, harvest time and techniques of ALVs for reducing quality losses and optimizing storability and transport focusing on: Morphological parameters, Physiological parameters, Biochemical composition, Analysis of gene expression - Morphological parameters - Physiological parameters - Biochemical composition - Analysis of gene expression Knowledge of optimum harvesting procedures in terms of morphological, physiological, biochemical and genetic characteristics for improving postharvest behavior of ALV has been elaborated
PH2: To assess appropriate cooling, MAP and CA storage conditions for the core ALVs to improve storability in terms of quality attributes, quality losses (nutritional and health-promoting properties, microorganism decay) at different stages of plant development Progress under this activity is on track. First storage trials at different temperatures and quality determination (basic chemical analyses) were conducted; no delays from the original time frame are anticipated. Progress under this activity is on track. First storage trials at different temperatures and quality determination (basic chemical analyses) were conducted; no delays from the original time frame are anticipated.
PH 3: To evaluate the effects of postharvest treatments (UV irradiation, heat treatment, electrical impulses) on nutrient quality, health-promoting properties, microorganism decay and shelf-life of ALVs during food supply chain (harvest, handling, storage, transportation, marketing (open markets, supermarkets), and processing industries This activity started 4 months before its planned commencement date. Following the employment of the PhD student the first experiments on postharvest treatments (UV-C, direct electric current, UV-B) and shelf-life/storage and their impact on nutritional and health promoting properties of ALV's were conducted.
PH 4: To evaluate the effects of processing technologies (blanching, solar drying and fermentation) on quality attributes, microbial quality and safety (absence of pathogens, mycotoxins) of ALVs. After receiving the official AVRDC seeds from IGZ in February 2014 we started immediately with plant cultivation. Therefore, this activity (fermentation trials) started with 7-8 weeks old AIV leaves (because of missing information about development stages, optimal harvest time) 18 months earlier than written and is on track. Solar drying will be performed by the Phd-student during his stay in Kenya. Influence of fermentation on pathogen development was investigated for the core cultivars. The identification and evaluation of critical control points in terms of quality attributes and quality losses at harvest and during handling and storage is in progress.
PH5: To analyze, evaluate and document critical control points in terms of quality attributes, quality losses (nutritional and health-promoting properties, microorganism decay) of the core ALVs during postharvest (harvest, handling procedures, storage, transportation, marketing (open markets, supermarkets), processing industries The identification and evaluation of critical control points in terms of quality attributes and quality losses at harvest and during handling and storage is in progress. Progress under this activity is on track, no delays from the original time frame are anticipated.

The acquisition and distribution of uniform seeds (purchased by AVRDC) was finalized. Now it is assured that all collaborates in SPs 4 and 5 (see below) are using identical seed material of the so-called core plants. Thus, the obtained results will be comparable and lead to a comprehensive picture (**H1 IGZ**). A literature review on various aspects of pre-harvest and post-harvest factors affecting quality of produce along the field to consumer chain has been completed with focus on maturity stages, harvesting practices, as well as post-harvest handling, losses and available strategies to reduce losses. Applying established experimental methods including carbohydrates analysis via photometric and enzymatic methods. Preliminary results indicated that the levels of starch and sugars (glucose, fructose and sucrose) increase with plant age while the levels in old leaves are the lowest. Additionally protocols on chlorophyll analysis and carotenoids measurement resulted in high levels of carotenoids and chlorophyll immediately after harvesting but storing the vegetables at 20° Celsius significantly decreases these levels. Further, three harvesting methods have been investigated (continuous, uprooting and pinching) and the results indicated that pinching gives the highest yield in terms of dry weight per hectare in nightshades, spider plants and amaranths while in cowpeas continuous harvesting gives the highest yield. This is because after pinching the plants produced more shoots which yielded more than unpinched plants. It was determined that harvesting early in the morning or late in the evening ensures longer storability than in the afternoon. With regard to Polysaccharides and saccharides: Photometric and enzymatic measurements of polysaccharides and saccharides (glucose, fructose, sucrose and starch) were done to compare different concentrations at different stages of plant growth, different leave positions and storage temperatures. It was noted that the level of polysaccharides and saccharides was higher in 30 days old plants than in 90 days old plant. Similar results were found with regard to chlorophyll and carotenoids. Additionally the levels of polysaccharides were higher in younger leaves than in the older lower leaves of the plant. This indicates that the polysaccharides and saccharides are redirected to other vigorously growing plant parts. Ethylene measurements were conducted to determine the effects of plant age and storage temperature to storage longevity. The results indicated higher ethylene gas production by the vegetables stored at 24˚C compared to those stored at 4˚C, however, the concentrations on the 90 days old plants was less than 30 days old plants, further experiments will be conducted to determine the stage at which nutrients are high and the ethylene gas production is lower during storage to enhance storability (**PH1, LUH**). Within the framework of activities **PH1, PH3 and PH5 (HUB**), Nightshade, Vegetable Amaranth and Ethiopian kale were grown with seeds from AVRDC under greenhouse conditions in two different cultivation sets.[1] Treatment of Nightshade plants with direct electric current (12V/200µA and 20V/200µA) had effects on growth and plant development. Application of weak DEC promoted plant growth, while higher DEC dosage negatively affected the development. Applying 12V/200µA increased bioactive plant substances, e.g. carotenoid and chlorophyll content of both leaves and stems of Nightshade plants. Thus, application of weak DEC dosage to plants activated physiological responses leading to enhanced growth, yield and crop quality. Post-harvest treatment of Amaranth and Ethiopian kale leaves with UV-C showed similar physiological reactions. Hormic dosage of 1.7 kJm-2 revealed significant reduction in weight loss, and increase in carotenoid and chlorophyll content compared with the control. Higher dosage (3.4 kJm-2) was found to negatively affect quality of both vegetables. Cold temperature (5 °C) storage also enhanced effectiveness of UV-C effect compared with retailer's simulation storage conditions (20 °C). Thus, results of these studies may provide environmentally safe, relatively cheap and easy to apply methods of enhancing yield, improving nutritional important quality attributes and reducing post-harvest losses of ALVs.

Under the activities **PH3, PH4 and H1 at Hamburg University**, the efforts already done in the first project period have been continued. Extraction, further sample preparation and analytical methods have been established in close cooperation with the German partner IGZ. First results indicate that especially the core plants amaranth and cowpea seem to be very promising in terms of the profile of the secondary plant metabolites flavonoids and saponins. Here, a deeper characterization will be

[1] Climate conditions, i.e. temperature, rel. air humidity and light intensity during the experiments are presented in Fig. 2 in the Annex.

forced in the next project periods. In order to assess appropriate cooling, MAP, CA-storage (**PH2 - MRI**), cowpea plants in 5L pots in a peat-based substrate (low fertilized) were cultivated using the official AVRDC seeds.[2] At the **University of Nairobi**, preliminary data on cold storage and MAP studies show efficacy of the two storage technologies to preserve quality and extend the shelf life of leafy vegetables. Storage of the vegetables in evaporative coolers (charcoal and brick) without packaging preserved the fresh state of the vegetables by 4 to 6 days. A combination of cold storage and MAP using Xtend resulted in a prolonged shelf life of up to 18 days. The temperatures in the evaporative coolers were 10 – 15 ^{0}C lower than the ambient room conditions. The relative humidity in the chambers ranged between 90 – 99.5% which was almost half that of ambient air (40-55%).

Lactobacillus (L.) plantarum BFE 5092, L. fermentum and Leuc. mesenteroides ssp. mesenteroides BFE 7668 were proved as the best starter cultures for fermentation of cowpea and nightshade leaves in 3% salt and 3% salt/3% sugar solution. Determination of CFU/ml on different agar media showed that the inoculated starter strains performed well during the fermentation period (MRS agar) whereas enterobacteria, yeast and molds (VRBD, MG) were inhibited in the batches inoculated with the starter cultures (**PH4 - MRI**). Determination of CFU/ml on different agar media showed that the inoculated starter strains performed well during the fermentation period (MRS agar) whereas enterobacteria, yeast and molds (VRBD, MG) were inhibited in the batches inoculated with the starter cultures (Fig. 3 in the Annex). Fig. 4 shows the determined pH values in the inoculated batch with starter cultures in comparison to the uninoculated control. The starter culture combination Leuc. mesenteroides ssp. mesenteroides BFE 7668 and L. plantarum BFE 5092 resulted in a quick drop of the pH to lower than 4.0 within 24 h, whereas the pH in the control was still above 6.0 after 24 h (Fig. 4). A pH value below 4.0 in the control was achieved only after 144 h at the end of the fermentation which is too slow to inhibit spoilage and pathogenic microorganisms which may present a risk for human health. With regard to PH4 “microbial quality and safety” the objectives were to determine the microbial state of seeds and leaves of cowpea, spider plant, and amaranth and detect the total microbial counts of the natural occurring aerobe microorganisms, enterobacteria, lactic acid bacteria, yeasts and moulds on the plant material. Occurrence of pathogens on fresh and fermented AIVs was also investigated. Three challenge tests with the pathogens Listeria monocytogenes and Salmonella Enteritidis were conducted with cowpea leaves under optimized fermentation conditions. Results: Inoculated listeria could not be detected after two days of fermentation with selected starter cultures. Salmonella were completely reduced after three days. During the six days of fermentation the samples were strongly acidified by the starter cultures and pH decreased from 6.76 to 3.9. At the end of fermentation the pH-value amounted to 3.55. A fermentation trial without starter cultures and pathogens served as control. It could be clearly recognized that the pH-value was dependent on the natural occurring lactic acid bacteria. The final pH-value of cowpea leaves samples without detectable lactic acid bacteria amounted to 6.44. This pH-value was not low enough to reduce the natural existing enterobacteria. Obvious growth of enterobacteria (2,07x104 to 1,15x109 CfU) was observed. In control trials with natural occurring lactic acid bacteria on the leaves, the pH-value dropped to 3.96. The detected acidification led to a significant reduction in enterobacteria from 9.01 to 2.2 log CfU/ml. A complete reduction of enterobacteria and pathogens could only be observed in fermentation trials with added starter cultures. Conclusion: the selected starter cultures Lactobacillus plantarum and Leuconostoc mesenteroides ssp. mesenteroides strongly acidified the fermentation samples and

[2] Growing conditions: 4 weeks in a climatic chamber with temperature day/night of 25°C/20°C, relative humidity of 40% during day time and 70% during night time; 12.5 hours light

could inhibit possibly existing pathogens on the leaves completely.