



**Josef G. Knoll-Europäischer Wissenschaftspreissträger 2020**

**Josef G. Knoll-European-Science Award Winner 2020**

**Addisu Fekadu Andeta “Optimization of enset fermentation: Physicochemical and microbial dynamics and development of starter cultures”, KU Leuven, Belgium, 2019**

### Summary

Enset (*Ensete ventricosum* (Welw.)) is a multipurpose crop widely cultivated in Ethiopia. It serves as a staple food for over 15 million Ethiopian people after fermentation into kocho. The fermentation of enset into kocho takes longer time and results poor sensory quality. The process has hardly been investigated and prone to optimization. The aim of the PhD research was twofold. First, the work wants to provide fundamental knowledge on the physicochemical and microbial dynamics during enset fermentation, taking into account a selected number of factors that possibly influence the dynamics, such as enset variety and fermentation system. Second, the aim of the work was to study possibilities to optimize the fermentation process by (1) considering a new fermentation system that is judged to be feasible to implement at large scale in the local households and by (2) developing and introducing a standardized starter culture. In order to achieve these objectives, the following major field activities were performed:

#### *Task 1: Survey of the traditional enset fermentation practices in Gamo highlands*

A detailed survey and field observations were conducted to generate information on traditional enset fermentation practices, storage conditions, the use of a starter culture, fermentation time, sensory properties of the fermented enset and the tools used to process enset. Enset producing households were asked different questions using an open-ended and semi-structured questionnaire. The study revealed that the traditional enset fermentation process is characterized by a wide variety of processing techniques and storage conditions across different districts. The sensory quality of the fermented enset was found to be very variable across the study area. The survey also showed that the fermentation time varied greatly depending on the altitude of the district, the season and the volume of enset to be fermented. However, the majority of the respondents mentioned that a minimum of two month is required for complete fermentation. None of the respondents use any traditional or standard starter culture to enhance enset fermentation. The survey further revealed that Gena, Maze and Ketishe varieties were the predominantly used enset plants for kocho and bulla preparations. Moreover, majority of the respondents prefer to ferment the processed enset in a bamboo basket also known as ‘Erosa’, followed by fermentation in a pit (Figure 1D). The study also showed that indicators like smell, color change and elasticity were used to check the completion of the fermentation process.



Figure 1: Enset plant in a garden (first row left), traditional enset processing (first row right), Squeezing and preparation for fermentation (second row left), Processed enset being fermented in underground pit (second row right)

*Task 2: Investigation of physicochemical and microbial dynamics in different enset fermentation systems in Gamo highlands*

A. Physicochemical and microbial community dynamics in controlled fermentation of three enset varieties

Based on the survey results and field observations, three predominantly used enset varieties were selected, processed and fermented in a bamboo-baskets or also called 'erosas'. Physicochemical parameters and microbial dynamics were monitored for two months. Furthermore, the microbial community composition was analyzed using Illumina Miseq sequencing platform. Samples were taken on days 1, 7, 15, 17, 31 and 60. The pH decreased, whereas the titratable acidity increased during fermentation. Of all counts, those of lactic acid bacteria and *Clostridium* spores increased during fermentation. *Leuconostoc* sp. initiated the fermentation. Later on, *Prevotella* sp., *Lactobacillus* sp. and *Bifidobacterium* sp. dominated. These three species are potential candidates for the development of a starter culture.

B. Physicochemical and microbial community dynamics in three fermentation systems using one variety

Three different fermentation systems, being the pit, the bamboo basket or 'erosa' and the Sauerkraut jar, were compared (Figure 2). The same physicochemical and microbiological parameters as mentioned above were monitored. Nine enset plants were processed and allowed to ferment for three months. Samples were taken on days 1, 7, 15, 31, 60 and 90. The results showed that moisture content and pH generally decreased and titratable acidity

increased during fermentation. Total viable aerobic counts were generally high for all samples and Enterobacteriaceae counts reduced to below the detectable level after day 1 for the pit and the jar fermentations and after day 7 for the erosa system. Illumina MiSeq sequencing of the 16S ribosomal RNA gene revealed that *Leuconostoc* and *Lactococcus* spp. were the most abundant lactic acid bacteria in the initial phases of the three enset fermentations. Later on, members of *Lactobacillus*, *Weissella* and *Bifidobacterium* spp. dominated the fermentations. A crucial finding was that jar fermentation led to slightly lower spore counts ( $2.81 \pm 0.30$  log cfu/g) of the potential human pathogen *Clostridium* as compared with the pit ( $3.26 \pm 0.15$  log cfu/g) and erosa ( $3.20 \pm 0.04$  log cfu/g) fermentation systems.



Figure 2: Fermenting enset within a pit (A), a bamboo basket or so-called ‘erosa’ (B) and a Sauerkraut jar (C).

*Task 3: To characterize the microbial community of the traditional starters made for enset fermentation*

In some enset growing regions of Ethiopia traditional starters are being used to aid enset fermentation. However, preparation of the starters differs among individual ethnic groups. Thus, the aim of the study was twofold. The first objective was to obtain information on traditional starter preparations and their usage. To this end, a survey was conducted in 18 districts of Southern Ethiopia. The second objective was to collect and characterize the microbial community of the diverse types of starters via culture-based methods and using Illumina Miseq sequencing. For the microbial load determination, traditional starters were collected from the seven zones. These samples were taken in sterilized beakers, three samples of already prepared traditional starter (about 100g) were collected from each household and pooled to one sample (300g). The microbial characterization results showed that lactic acid bacteria were found in high numbers in all samples collected (up to  $9.07 \pm 0.0$  log cfu/g). Overall, the most dominant bacterial genera in the traditional starters belonged to *Lactobacillus*. The universal presence of *Clostridium* spores at relatively high numbers in the traditional starters highlights the urgency of monitoring of microbial safety risks and implies another argument for the development of a commercial, and hence safe starter.

*Task 4: Development and validation of lactic acid starter cultures for enset fermentation*

Isolates which were obtained from the different fermentation systems (Task 1 and Task 2) and from the traditional starters (Task 3) were screened based on their ability to degrade starch, to tolerate different temperature ranges (5, 10, 15 and 45 °C) and different salt concentrations (2% and 4%) and on their growth at pH 4. Three promising strains, *Lactobacillus plantarum* (two strains) and *Leuconostoc mesenteroides* (one strain) were selected and evaluated under field and laboratory conditions. Aliquots of pulverized enset were inoculated with one of the isolates at a cell density of 3 log cfu/g and one uninoculated

portion was used as control. Fermentation was performed in sauerkraut jars. Samples were taken on days 0, 7, 15, 30 and 75. On day 7 and onwards, the inoculated enset samples showed a larger pH reduction compared to the control. Total aerobic counts were generally high for all samples and Enterobacteriaceae counts were reduced to below the detectable level on day 7 and onwards. *Clostridium* spore counts were significantly lower for the inoculated than for the uninoculated samples on day 75. *Leuconostoc* and *Lactobacillus* spp. were the most abundant lactic acid bacteria in both inoculated and uninoculated samples. Inoculated enset showed a faster colonization of lactic acid bacteria than the uninoculated biomass. Moreover, a sensory panel agreed that kocho prepared with this strain was the best in terms of color, taste and smell. The three starters selected appeared to be suitable as starter culture for fermentation of enset.



Figure 3: Enset fermented with starter culture microorganisms in a Sauerkraut jar (left), Kocho bread prepared using traditional method (middle), and Kocho bread fermented with the identified starter culture and baked (right)

Articles published in an internationally recognized journal from the PhD research work

Andeta, A. F., Teffera, F. E., Misganaw, F. W., Borremansa, A., Vandeweyer, D., Smedt De, Bossaert, S., Crauwels, S., Lievens, B., Vancampenhout, K., Van Campenhout, L. (2019b). Development and validation of lactic acid starter cultures for enset fermentation. *LWT - Food Science and Technology*, 115, 108462. <https://doi.org/10.1016/j.lwt.2019.108462>.

Andeta, A. F., Vandeweyer, D., Teffera, E. F., Woldesenbet, F., Verreth, C., Crauwels, S., Lievens, B., Vancampenhout, K., Van Campenhout, L. (2019a). Traditional starter cultures for enset fermentation: Unravelling their production and microbial composition. *Food Bioscience*, 29, 37-46. <https://doi.org/10.1016/j.fbio.2019.03.006>.

Andeta, A. F., Vandeweyer, D., Teffera, E. F., Woldesenbet, F., Verreth, C., Crauwels, S., Lievens, B., Vancampenhout, K., Van Campenhout, L. (2018b). Effect of fermentation system on the physicochemical and microbial community dynamics during enset (*Ensete ventricosum*) fermentation. *Journal of Applied Microbiology*, 126(3), 842–853. <https://doi.org/10.1111/jam.14173>.

Andeta, A.F., Vandeweyer, D., Woldesenbet, F., Eshetu, F., Hailemichael, A., Woldeyes, F., Crauwels, S., Lievens, B., Ceusters, J., Vancampenhout, K., Van Campenhout, L. (2018a). Fermentation of enset (*Ensete ventricosum*) in the Gamo highlands of Ethiopia: Physicochemical and microbial community dynamics. *Food Microbiology*, 73, 342–350. <https://doi.org/10.1016/j.fm.2018.02.011>.