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Fermentation of enset (*Ensete ventricosum*) in the Gamo highlands of Ethiopia: Physicochemical and microbial community dynamics

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ABSTRACT

Enset (*Ensete ventricosum*) provides staple food for 15 million people in Ethiopia after fermentation into kocho. The fermentation process has hardly been investigated and is prone to optimization. The aim of this study was to investigate the physicochemical and microbial dynamics of fermentation practices in the Gamo highlands. These practices show local variation, but two steps were omnipresent: scraping of the pseudostem and fermenting it in a pit or a bamboo basket. Enset plants were fragmented and fermented for two months in order to investigate the physicochemical (temperature, moisture content, pH and titratable acidity) and microbial dynamics (total viable aerobic counts, counts of Enterobacteriaceae, lactic acid bacteria, yeasts and moulds and *Clostridium* spores counts, and Illumina Miseq sequencing). 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 mesenteroides* initiated the fermentation. Later on, *Prevotella paludivivens, Lactobacillus* sp. and *Bifidobacterium minimum* dominated. These three species are potential candidates for the development of a starter culture.

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1. Introduction

Enset (*Ensete ventricosum* (Welw.) Chees man, Musaceae) is an important food security crop for 15 million Ethiopian people (Yemataw et al., 2014), where it is cultivated as a food, forage and fiber crop (Brandt et al., 1997; Nurfeta et al., 2008). In the highlands of the southern, southwestern and central part of Ethiopia, where population density is high, enset products are the main staple food and are especially important in the diet of women and children (Olango et al., 2014; Tsegaye and Struik, 2001). Moreover, due to high yield and drought tolerance, enset-based production systems are an interesting avenue to contribute to the United Nations sustainable development goals, especially in a context of global change.

Unlike other members of the Musaceae, enset does not bear edible fruit. Instead, the pseudostem and corm of the enset plant are traditionally processed into important starchy food products, being 'kocho' and 'bulla' (Atlabachew and Chandravanshi, 2008; Brandt et al., 1997; Pijls et al., 1995). To this end, the pseudostem and corm are pulverized manually and the solid fraction is fermented to obtain kocho. It is an age-old tradition, which is still used with little modification (Tariku and Mogessie, 2011). Enset can take up to 8 years to mature, but can be harvested at an earlier stage if necessary. The onset of flowering or the dry season are considered the best harvesting time for the production of kocho (Yemataw et al., 2014). The fermentation techniques and the tools used to process enset differ among regions and even among localities

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(Karssa et al., 2014; Yirmaga, 2013). Traditional methods of enset preparation are carried out in the backyard of the farmer's home (Fig. 1A) and are time consuming and labor intensive. The fermentation is by far the slowest step, as fermentation time varies from a month to a year, depending on incubation temperature, which is partly dependent on the altitude of the production site (Gashe, 1987).

The sensory quality of fermented enset is very variable and generally poor, which leads to a lower market price for kocho compared to other crops consumed in the country (Ashenafi, 2006; Brandt et al., 1997). It is also deficient in proteins and vitamin A (Tsegaye and Struik, 2001; Yirmaga, 2013). An accurate understanding of the microbial dynamics during the fermentation can help to optimize and standardize the process, but only a few studies have been carried out so far to characterize the microbiota involved in the traditional enset fermentation process. All of them exclusively use culture-dependent methods i.e. classical counts (Gashe, 1987; Karssa et al., 2014; Yirmaga, 2013). These studies indicate that lactic acid bacteria (LAB) are the dominant micro-organisms responsible for enset fermentation. However, culture-based methods often fail to characterize less abundant organisms or those organisms that require selective enrichment. Additionally, organisms that cannot be cultivated under traditional laboratory conditions remain undetected. These limitations can be met by metagenetic analyses such as Illumina sequencing (Ercolini, 2013).

The aim of this study is therefore to contribute to an improved nutritional and economic revenue for enset-dependent households by gaining insight in the enset fermentation process in the Gamo highlands of Ethiopia, and its relation to fermentation practices. To this end, based on the survey among 60 households, three different enset varieties were selected, processed and fermented in bamboo baskets or so-called 'erosas' to obtain kocho. Physicochemical parameters and microbial numbers were monitored. Furthermore, the composition of the microbial community was analyzed using Illumina Miseq sequencing.

2. Materials and methods

2.1. Enset preparation practices and experimental setup

A survey of fermentation practices conducted among 60 households in the Gamo Highlands (districts Chencha, Dorze and Bonke) in 2016 was conducted to design the experimental setup. Based on the survey, three varieties were selected, i.e. Gena, Maze and Ketishe, which are preferred by over 90% of the respondents for kocho and bulla preparation for their superior product quality and their shorter fermentation time. Processing techniques in the study area showed considerable variation, but nevertheless a general process flow could be distinguished, which is presented in Fig. 2. The first phase of the general process flow involves scraping of the pseudostem and corm with a sharp-edged utensil to separate the pulp from the long fibres (subsequently used to produce ropes and packaging sacks). The pulp is subsequently squeezed using a clean cloth or sack. The squeezed liquid is decanted and used to produce a starchy residue referred to as bulla. In the first phase of the fermentation, the pulp is placed loosely under wilted enset leaves

Fig. 1. Enset plant in the garden (A), traditional enset processing (B), bamboo basket or so-called 'erosa' filled with chopped enset and covered with dried enset leaves (C) and enset fermented for 60 days (D).

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