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Fermentation of Enset (*Ensete ventricosum*) in the Gamo Highlands of Ethiopia

Introduction of Sauerkraut Jars

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Preface

The result of 10 weeks of research at the Arba Minch University in Ethiopia is written down in this master thesis, that forms the closure of my education as ‘Master in the Bioscience option food industry’. I got the opportunity to do an internship in Ethiopia on an interesting topic. During my research and the writing of this master thesis, I received help from several people I would like to acknowledge.

First, I wish to express my thanks to my co-promotor and PhD student Addisu Fekadu Andeta. He shared his experience and knowledge on the topic with me and created a positive work environment. I am also very grateful to my promotor Ir. Anneke De Smedt for her advice and support. I also want to thank her for reading and correcting my thesis. Guided by her correct and constructive feedback, I have been able to bring this to a good end. Next, I would like to thank both Prof. Dr. Ir. Leen Van Campenhout and Ing. Dries Vandeweyer for reading my long emails during my stay in Ethiopia and providing me with advice, both in Ethiopia as in Belgium.

I also want to thank Lisanne Chen for being a good roommate and friend. The driving skills of John and his effort to teach me some Amharic deserve a special mention, just as the always positive mood of Sabura Shara. I am also very grateful to Dr. Fassil Teffera for his guidance and his investment to keep the team spirit up. Also thanks to Gemachu for his assistance in the lab.

I have no words to explain my gratitude towards the locals of Dorze for their willingness to participate in the research, their hospitality (giving me a lot of kocho when I came around) and their never-fading smiles. They made me feel welcome, even though communication was difficult. I also want to thank the personnel of Paradise Lodge in Arba Minch for their sense of humor and pleasant moments. Thanks a lot to the guards for their greets over and over again and their big smiles when I tried to greet them back in Amharic.

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I would like to express my enormous gratitude to my lovely parents and my sisters Karen and Lore for their motivational words and moral support. That does not only apply to the present, but also through all the years I have been studying. And last but not least: special thanks to my boyfriend Kevin for his support and the hot chocolates he made for me during long work evenings.

Ellen Gorrens

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Samenvatting

Meer dan 15 miljoen mensen in Ethiopië zijn afhankelijk van enset, een inheemse plant met meerdere doeleinden. De bijnaam van enset is ‘de boom tegen honger’ vanwege zijn sleutelrol in het garanderen van voedselzekerheid. Zijn pseudostam en knol worden lokaal verwerkt en gefermenteerd tot kocho. De verwerking is arbeidsintensief en tijdrovend. De kwaliteit van kocho is laag en variabel. Deze masterproef, uitgevoerd in het kader van een VLIR-UOS project, focust zich op de verbetering van het traditionele fermentatieproces in de Gamo Hooglanden van Ethiopië. Door de beperkte kennis omtrent de fermentatie van enset, was het nodig om spontane fermentaties zowel fysicochemisch als microbiologisch te beschrijven. Putten, erosas (bamboemanden) en zuurkoolpotten werden gebruikt als fermentatiesystemen. De eerste twee komen algemeen voor in de Gamo Hooglanden. De potten daarentegen werden geïntroduceerd omdat de fermentatie van zuurkool en enset veel gelijkenissen vertonen. Tijdens de fermentatie daalde de pH initieel in de drie systemen, maar dat gebeurde trager in de potten. Na 15 dagen was er een stijging in pH bij de putten en erosas. De oorzaak ervan is niet geheel duidelijk, maar saccharolytische clostridia, zoals *Clostridium tyrobutyricum*, zijn mogelijk betrokken. Mogelijks was er ook interferentie van grote stukken plantenmateriaal tijden de meting. Ook het vochtgehalte daalde tijdens de fermentatie. Na zo’n 12 uur was er al een zeer grote daling in de putten en erosas. Die waren voor een stuk permeabel terwijl evaporatie in de potten minder mogelijk was door het waterslot en het geglazuurd materiaal. Tijdens de hele fermentatie was het vochtgehalte in de potten consistent en significant hoger dan in de andere systemen. Dat leek niet voor problemen te zorgen, wat te verwachten was, want in zuurkoolfermentaties moet het vochtgehalte hoog zijn om anaerobiose te vormen. Dat creëert mogelijkheden om het handmatig of mechanisch persen voorafgaand de fermentatie over te slaan. De fermenterende enset had een hoge microbiële belading tijdens heel de fermentatie. Melkzuurbacteriën waren dominant aanwezig en worden verantwoordelijk geacht voor de reductie in pH. Het aantal gisten en schimmels vertoonde een dalende trend. Gisten zijn mogelijks verantwoordelijk voor de hydrolyse van zetmeel, zodat fermenteerbare suikers worden vrijgesteld voor de melkzuurbacteriën. De tellingen van Enterobacteriaceae waren initieel hoog, maar daalden na 15 dagen overal beneden de detectielimiet. *Clostridium* endosporen waren aanwezig in hoge aantallen, wat vragen doet rijzen over de voedselveiligheid. De aantallen in de potten waren wel consistent lager dan in de andere systemen. Op de bekomen kocho werd sensorisch onderzoek uitgevoerd. Zowel ongebakken als gebakken kocho van de zuurkoolpotten behaalden de hoogste scores voor geur, hardheid, smaak en algemene acceptatie. Alleen de kleur van de kocho uit de potten werd te licht bevonden, maar een verkeerde interpretatie van de vertaling kan daar aan de basis van liggen. Tussen de scores van de ongebakken en gebakken kocho waren er kleine, positieve correlaties. Zuurkoolpotten zijn dus een goed alternatief voor de putten en erosas. Ze waren de beste in termen van pH daling, telling van *Clostridium* endosporen en sensorische eigenschappen. Het financieel plaatje voor de aankoop van potten door families in de Gamo Hooglanden en de haalbaarheid van de lokaal gemaakte potten zonder glazuurlaag moeten nog uitgeklaard worden.

Abstract

Enset (*Ensete ventricosum* (Welw.) Cheesman, *Musaceae*) is an indigenous, multipurpose plant on which more than 15 million people from the Southern and Central Ethiopia rely for subsistence. Enset is nicknamed 'the tree against hunger' since it plays a key role in assuring food security. It does not produce edible fruit, but its pseudostem and corm are traditionally processed and fermented to obtain kocho. The processing is labor and time intensive. The quality of kocho is reported to be poor and variable and is therefore sold for a lower price compared to other crops in Ethiopia. This master thesis, carried out in the framework of a VLIR-UOS project, focusses on the optimization of the traditional fermentation of enset in the Gamo highlands of Ethiopia. Since the microbial knowledge of the enset fermentation is limited, it was necessary to first characterize different spontaneous enset fermentations. Three fermentation systems were used, namely pits, erosas (bamboo baskets) and sauerkraut jars. The use of pits is common throughout Ethiopia and erosas are commonly used in the Gamo Highlands. Sauerkraut jars on the other hand were introduced since the sauerkraut and enset fermentation share a lot of similarities. The use of it may lead to a more standardized, controlled process with less variation in the fermentation. During fermentation, the pH dropped initially in all the three fermentation systems, but the rate in the sauerkraut jars was slower. After 15 days, a pH increase until at least day 60 was observed in the pits and erosas. The exact cause for it is unknown, but saccharolytic clostridia, such as *Clostridium tyrobutyricum*, might be involved. Possibly, interference of pieces of plant material during pH measurement may have occurred. The pH ended at 4,35 ± 0,02 for pits, 4,55 ± 0,05 for erosas and 4,29 ± 0,02 for jars after 90 days of fermentation. The moisture content also dropped in all three fermentation systems during fermentation time. After approximately 12 hours, a major decline in moisture content was noticed in the pits and erosas. The pits and erosas were not completely closed and to some extent permeable for water, while evaporation in the jars was less possible due to the water lock and the glazed in and outside material. Throughout the fermentation, the moisture contents in the jars were consistently and significantly higher than in the other systems; but that did not seem to cause a problem. In fact, in sauerkraut fermentations, the moisture content needs to be sufficiently high so anaerobic conditions can develop. That may create opportunities in the future to omit the manual or mechanical squeezing prior the fermentation of enset. The fermenting enset had a high microbial load during fermentation. The total viable counts (both aerobic and anaerobic) were around 9 log cfu/g for all systems throughout the whole fermentation. Lactic acid bacteria were the most dominant group. They are held responsible for reducing the pH. The counts of yeasts and molds showed a declining pattern. The role of yeasts in the fermentation is not clear yet. They may hydrolyze starch, providing fermentable sugars for lactic acid bacteria. High counts of Enterobacteriaceae were initially observed, but they decreased after 15 days below the detectable level in all the fermentation systems. *Clostridium* spores were also present in high amounts throughout the fermentation, evoking questions about food safety. The counts of the jars were consistently lower than in the other fermentation systems, which indicates that the jars provide a safer alternative even in case of consumption of not fully fermented kocho.

Thereafter, sensory analysis was performed on the obtained kocho. Both the unbaked and baked kocho derived from the jars obtained the highest mean scores for smell, hardness, taste and overall acceptance. Only the color from the jars was considered too light, but a wrong

interpretation of the translation could be the cause. Small, positive correlations between the scores of the unbaked and baked kocho have been noticed for all questioned features.

Sauerkraut jars are thus a good alternative for pits and erosas. They were the best in terms of pH reduction, counts of *Clostridium* endospores and sensory properties. The financial impact of the purchase of locally produced sauerkraut jars by families in the Gamo Highlands and the feasibility of the locally made jars without glazed layer, still need to be examined.

Keywords: Enset, Kocho, Sauerkraut jars, Gamo Highlands, Ethiopia

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List of abbreviations

AMU = Arba Minch University

AOAC = Association of Official Analytical Chemists

a_w = water activity

BE = baked aradisame deriving from erosas

BJ = baked aradisame deriving from jars

BP = baked aradisame deriving from pits

cfu = colony forming units

CSA = Central Statistical Agency of Ethiopia

DW = dry weight

EFSA = European Food Safety Authority

e.g. = example given

EHNRI = Ethiopian Health and Nutrition Research Institute

FAO = Food and Agriculture Organization

GRAS = generally considered as safe

LAB = lactic acid bacteria

MRS = de Man Rogosa Sharpe Agar

ND = not detected

PCA = Plate Count Agar

psi = pound per square inch

RCA = Reinforced Clostridium Agar

SNNPR = Southern Nations, Nationalities and Peoples' Region

ssp. = species pluralis

TA = titratable acidity

UE = unbaked kocho deriving from erosas

UJ = unbaked kocho deriving from jars

UNU = United Nations University

UP = unbaked kocho deriving from pits

VRBG = Violet Red Bile Glucose Agar

WHO = World Health Organization

1 INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Cheesman, *Musaceae*) is an indigenous food security plant for more than 15 million people of Southern and Central Ethiopia (Yemataw et al., 2014). It is a drought tolerant crop, providing food, animal fodder, fibers and traditional medicine (Mohammed et al., 2013). Enset is often nicknamed 'the tree against hunger'. It does not bear edible fruit, but the pseudostem and corm are traditionally processed and fermented to kocho (Brandt et al., 1997). The processing of enset for fermentation of kocho is labor and time intensive and mainly done by women (Negash & Niehof, 2004). The quality of kocho is reported to be poor and is sold on the local market for a lower price compared to other crops in Ethiopia (Brandt et al., 1997; Ashenafi, 2006).

Therefore, this thesis research, carried out in the framework of a VLIR-UOS project (namely 'Sustainable Enset farming systems for food security and livelihood improvement in Gamo highlands', grant number ZEIN2015PR407), aims at optimizing the traditional fermentation of enset to improve the food security. The research was performed in collaboration with Mr. Addisu Fekadu Andeta, an Ethiopian PhD student from Arba Minch University. The study took place in Dorze in the Gamo highlands of Ethiopia, one of the enset growing areas in the Southern Nations, Nationalities and Peoples' Regional State (SNNPR).

This master thesis consists of two main parts, namely a literature study (part 1) and the practical work (part 2). The literature study focusses on lactic acid fermentation (with sauerkraut as detailed example), the significance of enset and the traditional fermentation of enset to kocho, including fermentation practices, the microbiological and physicochemical dynamics, sensory quality and nutritional values of kocho.

The practical work consists of two experiments. The aim of the first experiment is to characterize different spontaneous enset fermentation with the help of culture dependent analyses since the microbial knowledge about it is limited. Three fermentation systems (pits, erosas and sauerkraut jars) are used. The use of pits is distributed throughout Ethiopia (Brandt et al., 1997) and erosas are commonly used in the Gamo Highlands (Andeta et al., 2018). Sauerkraut jars on the other hand are introduced as a fermentation system for enset. The use of it may lead to a more standardized, controlled process with less variation in the fermentation. The second experiment is to perform sensory analysis on kocho obtained by the three fermentation systems. Women sell kocho on the local market (Assefa & Fitamo, 2016), providing a primary source of income (Shank & Ertiro, 1996). If the fermentation and sensory properties improve, the food security will not only ameliorate, but it is also a strategy in poverty reduction.

Part 1: Literature

2 LACTIC ACID FERMENTATION

2.1 Fermentation

Fermentation is an ancient preservation method which involves activity of microorganisms. It extends the shelf life of the product considerably over that of the raw materials, enhances the nutritive value, enriches the diet by the development of attractive flavors, aromas and textures and may reduce the toxicity of some foods (Steinkraus, 1996; Jay et al., 2005; Ashenafi, 2006). In developing countries, fermentation serves as a manageable and affordable technique for food preservation and for increasing food safety (Motarjemi, 2002). Fermentation can occur spontaneous or by adding a starter culture that accelerates the process (Holzapfel, 2002). Industrial fermentations include for instance the production of amino acids (D'Este et al., 2017), enzymes (He & Chen, 2013), organic acids and antibiotics (Chen, 2013). Well-known examples in the food industry are salami, cheese, beer and wine (Jay et al., 2005). There are four kinds of fermentation processes, being lactic acid, alcoholic, acetic acid and alkaline fermentations (Steinkraus, 1996; Ashenafi, 2006). In this master thesis, only the lactic acid fermentation will be discussed as kocho is a lactic-acid fermented product (discussed in 4.3).

2.2 Lactic acid fermentation

Lactic acid bacteria (LAB) are a group of thirteen genera of Gram-positive, usually catalase-negative, generally non-sporulation, usually non-motile rods and cocci. They can ferment carbohydrates under microaerophilic to strictly anaerobic conditions. They are divided into two major groups, being homofermentative and heterofermentative. Homofermentative LAB produce lactic acid as the major or sole end product, while heterofermentative LAB also yield a variety of fermentation by-products, such as carbon dioxide, ethanol and acetic acid. They are more important than homofermentative LAB in terms of bringing flavor and aroma components to the fermented product (Jay et al., 2005; König & Fröhlich, 2009; Chen, 2013; Zhang & Zhang, 2014). Table 2.1 shows an overview of the thirteen genera of LAB.

Table 2.1 Overview of the thirteen genera of LAB (Jay et al., 2005)

Genus	Homo- or heterofermentative
Carnobacterium	Heterofermentative
Enterococcus	Homofermentative
Lactococcus	Homofermentative
Lactobacillus	Homofermentative/Heterofermentative
Lactosphaera	Heterofermentative
Leuconostoc	Heterofermentative
Oenococcus	Heterofermentative
Pediococcus	Homofermentative
Paralactobacillus	Homofermentative
Streptococcus	Homofermentative
Tetragenococcus	Homofermentative
Vagococcus	Homofermentative
Weissella	Heterofermentative

LAB have complex nutritional requirements. For instance, vitamin B_3 and vitamin B_5 are required by most LAB species and vitamin B1 by heterofermentative species (König & Berkelmann-Löhnertz, 2009). Some LAB are considered as probiotics (Salminen et al., 2004; Jay et al., 2005), but not all LAB have positive effects. Some LAB can cause food spoilage, while others are pathogenic to human (Price et al., 2012). LAB used in food fermentations are generally considered as safe (GRAS) (Adams, 1999; Holzapfel, 2002). Some of them were given the 'Qualified Presumption of Safety' status by European Food Safety Authority (EFSA, 2007).

LAB are responsible for lactic acid fermentations of plant and animal materials. In the Western world, sauerkraut (discussed in 2.2.1), yoghurt and cucumber pickles are well-known examples, but lactic acid fermentation occurs worldwide (Steinkraus, 1996). LAB are able to inhibit or kill other microbes by a number of factors. The conversion of sugars to organic acids and thereby the reduction of the pH is the primary preserving action (Belitz et al., 2004; Suskovic et al., 2010). A low pH inhibits the growth of undesirable microorganisms. It also inhibits the growth and toxin production of *Clostridium botulinum* (Belitz et al., 2004; Jay et al., 2005; Bhunia, 2008). LAB also produce bacteriocins. These are ribosomally synthesized peptides which are often active against bacteria closely related to the producer (Kjos et al., 2009). Bacteriocins are GRAS, usually pH and heat tolerant and they have a relatively broad antimicrobial spectrum against many food poisoning and food spoilage organisms (Gálvez et al., 2014). The best known bacteriocin is nisin. It is produced by some strains of *Lactococcus lactis* (Cotter et al., 2005; Jay et al., 2005). So far, only nisin is licensed as a food preservative as number E234 (Regulation (EC) No 1333/2008). Furthermore, LAB can inhibit other microbes by nutrient depletion and the production of hydrogen peroxide and diacetyl. All the factors above are referred as lactic antagonisms (Jay et al., 2005).

2.2.1 Sauerkraut fermentation

One of the best studied lactic acid fermentation of plant material is sauerkraut. It is a product obtained by lactic acid fermentation of cabbage. The microbiota naturally occurring on the cabbage, starts the fermentation. The outer green and dirty leaves of the cabbage and the cores of the heads are removed. After shredding the cabbage, salt is added at a level between 1,8 and 2,5% ($^{w}/_{w}$) (Pederson & Albury, 1969; Arthey & Dennis, 1991; Holzapfel et al., 2003; Belitz et al., 2004; Jay et al., 2005). The use of low levels of salt has a preservative effect and favors the growth of LAB (Belitz et al., 2004; Jay et al., 2005). Afterwards, the cabbage is tamped into barrels under weighted covers to create anaerobic conditions. Sufficient brine, formed by tissue fluid loss from the shredded cabbage due to the addition of salt, covers the cabbage. It supports the development of anaerobic conditions and forms thereby the selective basis for lactic acid fermentation (Arthey & Dennis, 1991; Holzapfel et al., 2003; Belitz et al., 2004). The temperature during fermentation is between 15 and 24 °C (Holzapfel et al., 2003; Belitz et al., 2004). The fermentation takes three to six weeks (Arthey & Dennis, 1991; Belitz et al., 2004). During that time, particularly CO_2 and lactic, acetic and other acids are formed (Russell & Gould, 1991), but most importantly, aroma substances are produced. Sauerkraut is known for its high vitamin C content, from 10 to 38 mg per 100 g (Belitz et al., 2004).

Heterofermentative *Leuconostoc mesenteroides* initiates the fermentation and predominates the early phase due to its high initial number and its shorter generation time at 18 to 20 °C than other LAB (Pederson & Albury, 1969; Arthey & Dennis, 1991; Belitz et al., 2004; Jay et al., 2005; Plengvidhya et al., 2007; Beganovic et al., 2014). As the pH decreases below 4,5, more

acid tolerant, homofermentative species take over after three to seven days of the process, dominated by *Lactobacillus plantarum* and to lesser extent by *Pediococcus* spp. The pH of the end product ranges between 3,0 and 4,1 (Pederson & Albury, 1969; Arthey & Dennis, 1991; Belitz et al., 2004; Jay et al., 2005; Johanningsmeier et al., 2007; Plengvidhya et al., 2007; Beganovic et al., 2014). The formed products, being lactic acid, ethanol, carbon dioxide, mannitol and aroma substances, give sauerkraut the desirable taste (Johanningsmeier et al., 2007; Plengvidhya et al., 2007). According to Daeschel and Nes (1995), *Lactobacillus plantarum* is one of the most tolerant to low pH and high acidity compared with other LAB. That might explain why it is found as the terminal participant in natural lactic acid fermentations of plant materials. On the other hand, many other bacteria species, such as *Weissella* spp. and *Leuconostoc fallax* (Barrangou et al., 2002; Breidt, 2004; Jay et al., 2005; Plengvidhya et al., 2007) and *Leuconostoc citreum* (Breidt, 2004; Plengvidhya et al., 2007), have been detected in spontaneous sauerkraut fermentations. Yeasts are also involved (Belitz et al., 2004; Pundir & Jain, 2010). Different authors report various stadia where *Lactobacillus brevis*, thought to be one of the dominant species during sauerkraut fermentation, is present. Some authors (Belitz et al., 2004; Jay et al., 2005) report that *Lactobacillus brevis* helps initiating the fermentation, while other authors (Pederson & Albury, 1969; Tamminen et al., 2004; Plengvidhya et al., 2007) observed *Lactobacillus brevis* after the initiation, but before or simultaneously with the dominance of *Lactobacillus plantarum*. *Lactobacillus brevis* is even regarded as the terminal organism, which makes that the sauerkraut fermentation appears in three stadia instead of two (Arthey & Dennis, 1991; Holzapfel et al., 2003). Not every study found *Lactobacillus brevis* in high numbers. Hence, the ecology of sauerkraut fermentations is complex. The diversity of the microorganisms involved still needs further research (Breidt, 2004; Plengvidhya et al., 2007).

When the fermentation is considered as finished, sauerkraut can be preserved for a long time if anaerobic conditions are maintained (Sperber & Doyle, 2009). During storage, the counts of aerobic mesophilic bacteria and LAB decrease (Viander et al., 2003; Pundir & Jain, 2010; Palani et al., 2016), but Peñas et al. (2010) observed slightly increasing numbers up to three months of storage. The pH of the product stays stable (Pundir & Jain, 2010). Sauerkraut is often pasteurized to reduce its microbial load and to avoid excessive acidification (Holzapfel et al., 2003; Belitz et al., 2004). Spoilage of sauerkraut appears as soft, pink, slimy and rotten kraut (Jay et al., 2005; Sperber & Doyle, 2009). Soft kraut results when the normal sequence of bacterial growth is disturbed. *Lactobacillus* spp., that normally appear in the late stages of fermentation, grow earlier up to the initiation. These *Lactobacillus* spp. seem to have a greater ability to break down cabbage tissues. Poor salting procedure and varying temperatures increase the risk for soft kraut (Battcock & Azam-Ali, 1998; Jay et al., 2005). Formation of a pink color is another frequent spoilage problem. It is attributed to the surface growth of yeasts such as *Rhodotorula* spp. and *Torula* spp., especially *Torula gulinis*. It is caused by an excessive concentration or uneven distribution of salt (Battcock & Azam-Ali, 1998; Jay et al., 2005; Sperber & Doyle, 2009). The rapid growth of *Lactobacillus cucumeris* and *Lactobacillus plantarum*, especially at higher temperatures, is responsible for slimy kraut (Jay et al., 2005). Another type of spoilage, namely rotted sauerkraut, is caused by spoilage organisms. These organisms could be favored by an uneven distribution of salt, a high fermentation temperature and exposure to air. Generally, sauerkraut is subjected to spoilage of molds at its surface when it comes in contact with air (Battcock & Azam-Ali, 1998; Jay et al., 2005; Pundir & Jain, 2010). The last spoilage problem that can be encountered, is the excess accumulation of lactic acid, although some people prefer a more acidic product (Sperber & Doyle, 2009).

3 ENSET AS A SOURCE OF STAPLE FOOD IN ETHIOPIA

3.1 Botanical information and distribution

Enset is a perennial, monocarpic herbaceous plant. It belongs to the order Zingiberales, family *Musaceae* and genus *Ensete*. Enset is often called 'false banana' because of its resemblance with banana. However, banana is situated in the same family, but in the genus *Musa* (Baker & Simmonds, 1953; Brandt et al., 1997). The genus *Ensete* comprises eight species and is distributed in Madagascar, tropical Africa and Asia (Champion, 1967). Enset is only domesticated in Ethiopia. It is classified taxonomically as *Ensete ventricosum*. The distribution of enset is concentrated in the Southern and Central Ethiopia (Baker & Simmonds, 1953; Brandt et al., 1997; Väre & Häkkinen, 2011).

The height of an enset tree ranges from 4 to 11 m and the circumference of the pseudostem from 1,5 to 3,0 m. The length of the pseudostem varies from 2 to 5 m. The pseudostem is made of a system of tightly clasping leaf sheaths. Figure 3.1 shows matured enset plants, of which the plant shown in Figure 3.1a at flowering stage. Enset growing areas are located at altitudes ranging from 1200 to 3200 m above sea level, but it grows best at elevations between 2000 and 2750 m. These areas have an average temperature between 10 and 21 °C and the relative humidity is 63 to 80%. Most enset growing areas receive annual rainfall of about 1100 to 1500 mm (Smeds, 1955; Brandt et al., 1997; Tsegaye & Struik, 2002; Negash & Niehof, 2004). Enset is able to withstand dry periods up to six months (Huffnagel, 1961; Westphal, 1975; Negash, 2001). Diseases, especially *Xanthomonas campestris* pathovar *musacearum*, are the most important threat for enset (Brandt et al., 1997; Hunduma et al., 2015; Yemataw et al., 2016; Yemataw et al., 2017).

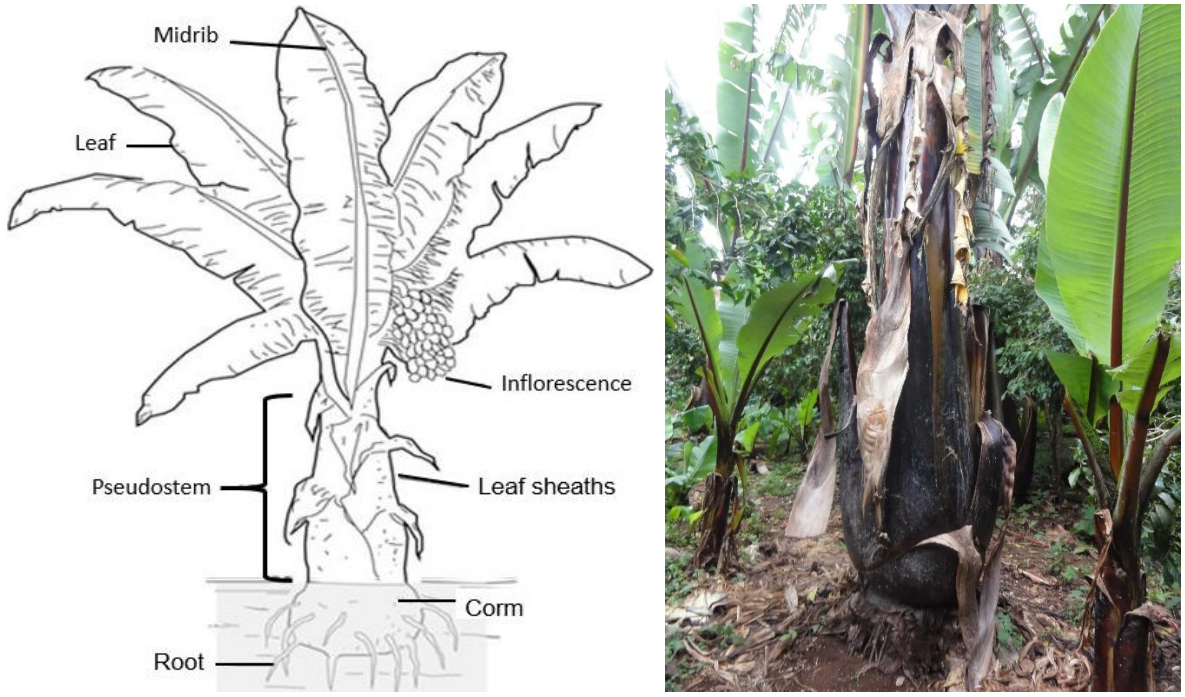

Figure 3.1 (a): Matured enset at flowering stage (adapted from Brandt et al., 1997) (b): Young enset plants with a matured one in the middle (own photograph)

The edible parts are not the seeded fruits that enset produces, but the single corm underground and the pseudostem above the ground. When enset is cultivated, the plant is usually not allowed to flower, but it is reproduced vegetative from suckers. These suckers are usually produced from two to three years old corms (Simmonds, 1963; Olango et al., 2014; Garedew et al., 2017). In contrast to banana, these suckers need to be induced by harvesting enset plants, removing the pseudostem and roots and cutting out the apical bud. By destroying the apical bud, the apical dominance is arrested and thereby allowing dormant lateral buds to grow into usually 20 to 100 suckers (Brandt et al., 1997; Yemataw et al., 2014; Garedew et al., 2017). Nevertheless, seeds are sometimes used to create genetic diversity (Zippel, 2005).

In addition, farmers distinguish 'male' from 'female' clones. The distinction has nothing to do with the biological reproduction of the plant, but is based on qualities and characteristics. The so-called male clones have qualities, such as disease and drought resistance, hardness and later maturing. These properties are preferred by men, because they cultivate. The female clones, for instance, have higher yield of fiber and food content and are softer. These characteristics are desired by women (Brandt et al., 1997; Negash & Niehof, 2004; Zippel, 2005).

3.2 Importance of enset

Enset (*Ensete ventricosum* (Welw.) Cheesman, Musaceae) is an indigenous, multipurpose plant on which a large part of the Ethiopian population, more than 15 million people, rely for subsistence (Brandt et al., 1997; Olango et al., 2014; Yemataw et al., 2014). It provides food and fodder, fibers, traditional medicine (Mohammed et al., 2013) and it can be used as a partial substitution of barley malt in beer production (Temesgen & Getasew, 2015). Enset, often referred as the 'tree against hunger', can be used as a buffer to overcome droughts as it is a drought tolerant crop. It can be harvested at any time in case of food shortage. Thus, it can improve food security for millions of people from drought prone areas in Ethiopia (Brandt et al., 1997; Mohammed et al., 2013). That is crucial for Ethiopia where 25 to 35% of the population is undernourished (The FAO Statistics Division, 2015). Nevertheless, during Ethiopia's tragic drought and famine prone decades of the 1970s and 1980s, population dependent upon enset survived famine thanks to the cultivation of enset (Brandt et al., 1997). Moreover, famine is rare in enset growing regions (Tsegaye, 2002). Farmers even stated that "enset is the enemy of hunger, and human and livestock life is impossible without it' (Tsegaye & Struik, 2002). In Sidama Zone, enset is considered as the single most important root crop (Regassa & Stoecker, 2012).

According to Westphal (1975), one of the four agricultural systems that can be distinguished in Ethiopia is the enset-based farming. Still, the cultivation of enset and its use as a staple food are almost restricted to the people in south-west Ethiopia (Figure 3.2). Vavilov (1951) states that the densely populated highlands of southern Ethiopia form the geographical center of enset cultivation. Nowadays, enset covers more than 300 000 ha of land in Ethiopia (CSA, 2011).

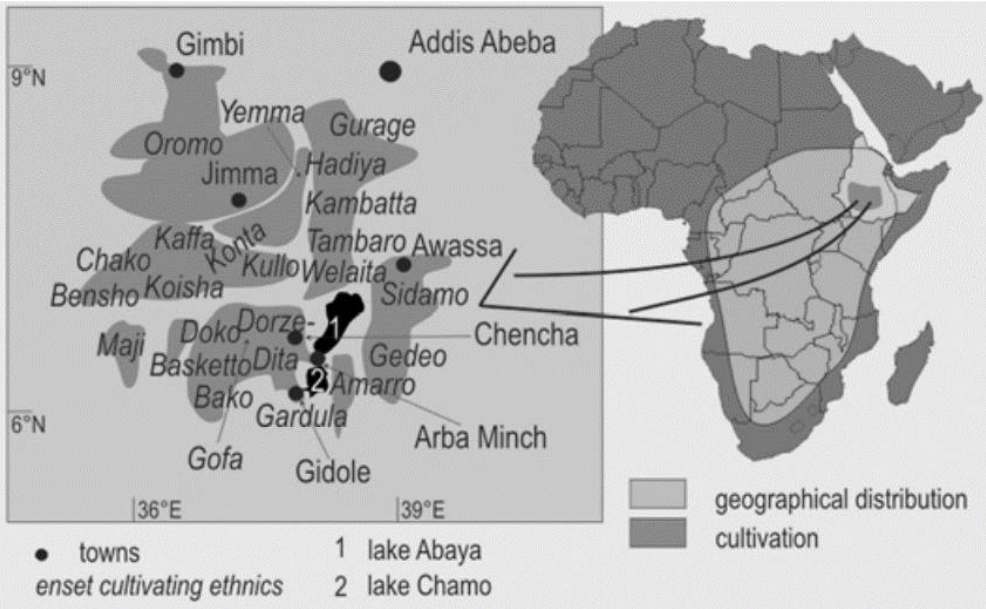

Figure 3.2 Geographical distribution of the genus *Ensete* (light grey) in Africa and the major growing areas (grey) of cultivated *Ensete ventricosum*, including the study area Dorze (Zippel, 2005; changed from Westphal, 1975)

Three main food products are derived from enset, namely kocho, bulla and amicho. Kocho is the fermented mixture of scraped pulp of the pseudostem and pulverized underground corm. After fermentation, bread and other local dishes are prepared from kocho. The preparation method of kocho will be discussed in 4.1. Bulla is the small amount of starchy liquid that is separated from kocho during processing. Amicho is the cooked inner part of the enset corm and it tastes like potatoes (Tsegaye & Struik, 2002; Negash & Niehof, 2004; Karssa et al., 2014). The kocho yield of enset per unit space and time, in terms of edible dry weight and energy, is the highest compared to the yields of other main starch crops cultivated in Ethiopia, such as teff, barley, cassava and maize (Tsegaye & Struik, 2001).

4 TRADITIONAL FERMENTATION OF ENSET

4.1 Traditional processing procedures

The local processing of enset to ferment it, is based on traditional knowledge of the people and varies among different enset growing regions (Hunduma & Ashenafi, 2011b). Still, the basic traditional processing steps are the same, but some steps differ among individual villages.

4.1.1 Maturity indicators

Farmers prefer to harvest matured enset plants before flowering, because it is thought to have high storage of food in the pseudostem and corm and besides that, the seeds of enset are not necessary for propagation (Hunduma, 2010). Enset needs three to more than ten years to mature (Brandt et al., 1997). The maturity indicators depend on the region. In West Shewa and Gedeo Zone, the age of the plant, the emerging of the central folded leaf shoot, appearance of inflorescence and exposed junction of pseudostem and corm are used as indicators (Hunduma & Ashenafi, 2011b; Tsegaye & Gizaw, 2015). In Sheka, the farmers determine the maturity state by the size of the plant, exposition of the corm and appearance of inflorescence (Garedew et al., 2017), while farmers in Masha Woreda base their decision on the drying leaves and outer leaf sheaths and appearance of inflorescence (Million et al., 2003). If inflorescence appears, harvesting is ideal done as soon as possible since most of the plant's carbohydrates stored in the corm and pseudostem are consumed for flower development. Thereby the nutritional value of kocho decreases (Taye & Asrat, 1996; Steinkraus, 1996; Urga et al., 1997; Hunduma & Ashenafi, 2011b).

4.1.2 Harvesting

The processing starts with harvesting (preferably matured) enset plant(s). The leaves and older leaf sheaths are first removed from the plant. Then the inner leaf sheaths are separated. The concave side of the leaf sheaths are peeled and cut into pieces of about half meter length in order to have a workable size. Finally, the ground around the corm is loosened and the corm is excavated (Brandt et al., 1997; Yirmaga, 2013). Both the pseudostem as the corm will be treated and mixed together in order to ferment it, as will be discussed in the following paragraphs.

4.1.3 Decortication of the pseudostem

After harvesting, the decortication of the pseudostem starts. Therefore, the pseudostem is either attached with fibers on a wooden pole or hold in place on a wooden plank by the raised heel of a processer. In the first case women stand to decorticate, whereas in the second case women sit on the ground or on enset leaves. The used flat wooden plank is placed obliquely against a standing enset tree. The woman scrapes the fleshy part of the leaf sheaths using a scraper (Brandt et al., 1997; Ashenafi, 2006; Hunduma & Ashenafi, 2011b; Tsegaye & Gizaw, 2015). A bamboo scraper around 50 cm long is a common tool. It is handled at both ends.

Nowadays, also metal scrapers are used. Figure 4.1 shows the process. Thereby fibers are produced as a useful by-product (Brandt et al., 1997; Urga et al., 1997; Hunduma & Ashenafi, 2011b; Tsegaye & Gizaw, 2015; Bosha et al., 2016). These fibers are strong enough to make high quality ropes (Smeds, 1955; Assefa & Fitamo, 2016; Garedew et al., 2017).

Figure 4.1 A local woman processes enset using a wooden plank and a bamboo scraper (own photograph)

4.1.4 Pulverization of the corm

The corm can be pulverized by two techniques. One method is to grate the corm from the inside out while the corm is still in the ground (Brandt et al., 1997). Another way is to remove the soil and other unwanted parts of the corm with a knife and then the corm is pulverized. The pulverizing is shown in Figure 4.2. Different traditional tools, such as a serrated animal shoulder bone and a large bamboo pole, are used to pulverize (Brandt et al., 1997; Ashenafi, 2006; Yirmaga, 2013; Karssa et al., 2014). In some areas, the corm is pulverized with a wooden, locally made tool which has a sharp serrated edge (Brandt et al., 1997; Urga et al., 1997; Karssa et al., 2014). In West Shewa, the local tool called ‘Javga’ has two different shaped ends. Each end fulfills a different function. One end is serrated to pulverize the corm while the other end is flat to smash the lower piece of the pseudostem. The tool is also used in the preparation of the local starter called ‘Gamma’ (Hunduma & Ashenafi, 2011b). In Gedeo Zone, a similar tool, locally called ‘Cheko’, is used for the same purposes as ‘Javga’, only the starter there is ‘Gamama’ (Tsegaye & Gizaw, 2015). Whether and how these tools are cleaned before reusing them, is not mentioned.

Figure 4.2 Two women pulverizing corms of enset (own photograph)

4.1.5 Bulla preparation

The mass from the scraped leaf sheaths (and possibly grated corm) is squeezed to a liquid containing starch. Thereafter the liquid is decanted in order to obtain bulla. In West Shewa Zone, that is done by putting the decorticated pseudostem mass into a cloth and squeezing by feet and knees (Figure 4.3). Alternatively, some processers use a plastic sieve in a bucket or squeeze manually. More modern squeezing equipment is also used. After allowing the filtrate some time to rest, precipitation is formed. The filtrate is decanted and only the sediment, called bulla, is kept. Bulla is left to dehydrate into a powder form (Brandt et al., 1997; Amede et al., 2004; Tsegaye & Gizaw, 2015). To consume bulla, water is added and a porridge is prepared (Negash & Niehof, 2004; Ashenafi, 2006).

Figure 4.3 A local woman squeezes the decorticated pseudostem using a sack to obtain bulla (own photograph)

4.1.6 Preparation of a starter

Some enset growing areas use a starter, mainly via inoculation of already fermented enset (called backslopping (Holzapfel, 2002)) to initiate the fermentation process and suppress spoilage. The preparation of the starter differs among the regions. Sometimes various ingredients, such as herbs and aromatic plants, are added to the starter (Sandford & Kasa, 1993; Brandt et al., 1997; Tsegaye & Struik, 2000; Ashenafi, 2006; Hunduma & Ashenafi, 2011b; Bosha, et al., 2016).

In Sidama Zone, a small part of the pulverized corm is fermented separately. First it is rubbed with decomposed enset leaves which are recovered from the enset farm, and wrapped with fresh enset leaves. It is left for five to eight days at ambient temperature. In some villages, they expose the fermenting part of the corm to sunlight for some hours after five days of fermentation. Afterwards it is wrapped again and allowed to ferment further. Eventually, the processed mixture of the fine pieces of the corm and the scraped pseudostem sheath is inoculated with the prepared starter (Urga et al., 1997; Karssa et al., 2014). The name of the starter in Sidama Zone is called 'Gamancho' (Karssa et al., 2014). In some parts of the West Shewa Zone, another starter, namely 'Gamma', is prepared. Therefore a part of the corm which is still in the ground is pulverized. That mass is put into a cavity opened at the center of the same corm. Different ingredients, such as herbs, tree leaves, aromatic plants, rotten enset leaf sheaths and the supernatant from the bulla preparation, can be added. Everything is packed into enset leaves and leaf sheaths and a heavy load is placed on it. The starter and the mixture of corm and pseudostem are both allowed to ferment separately for one month before mixing it together (Hunduma & Ashenafi, 2011b).

These examples are just a small fraction of possibilities to prepare a starter in Ethiopia. The preparation of these starters can even differ on a household level (Brandt et al., 1997; Hunduma & Ashenafi, 2011b). In some areas (e.g. the Gamo Highlands) no starter is used at all (Brandt et al., 1997; Andeta et al., 2017; Andeta et al., 2018).

4.1.7 Storage and fermentation

The decorticated pieces of the corm and the scraped pseudostem are mixed together and kneaded. If no starter is applied, the mixed mass is wrapped in enset leaves and left at ambient temperature for about two to more than five days. The local farmers believe this phase (hereinafter referred to as "pre-fermentation phase") helps initiating the fermentation process. Afterwards, the mass is placed in an earthen pit and is then pressed by hands or feet (Gashe, 1987a; Nigatu, 1992; Brandt et al., 1997). A bamboo basket, called 'erosa', is also used as a fermentation system in the Gamo Highlands. Moreover, no starter is used there and the mixture is left for 15 days at ambient temperature (Andeta et al., 2017; Andeta et al., 2018).

In the mid altitude area (2252 m above sea level) of West Shewa Zone, where no starter is added, the processed enset also has a pre-fermentation phase for about two weeks before putting in a pit. Though, in the high altitude area (2908 m above sea level) the pulverized mass is immediately put in an earthen pit and the starter 'Gamma' is added one month later (Hunduma & Ashenafi, 2011a; Hunduma & Ashenafi, 2011b).

The inner part of the pit is lined with enset leaves. The mass is pressed down by feet and hands before the pit is covered and heavy materials, such as stones and logs, are put on top to create airtight conditions to facilitate the fermentation process (Gashe, 1987a; Ashenafi,

2006; Tsegaye & Gizaw, 2015; Bosha et al., 2016). The depth and diameter of the pit depends on the mass of the processed enset and family size (Hunduma & Ashenafi, 2011b), but standard dimensions are around 1 x 1 x 1 m (Smeds, 1955; Gashe, 1987a; Bosha et al., 2016).

4.1.8 Mixing and check-up

During both the pre-fermentation phase and the fermentation, the fermenting mass is regularly mixed and checked for any undesirable signs. Also, the leaves lining the pit are changed at varying time intervals in different regions. For instance, in Northwest Wolaita the fermenting mass is transferred every 15 days to a newly lined pit (Sandford & Kasa, 1993), while in West Shewa Zone remixing and sealing with new leaves is also done when signs of spoilage appear. Checking the fermenting mass to detect spoilage (e.g. blackening, undesirable odor and slime formation) is usually done by making a small narrow opening in the fermenting mass (Gashe, 1987a; Hunduma, 2010; Hunduma & Ashenafi, 2011a).

4.1.9 Ending the fermentation

The fermentation is the slowest step of the process. The length of fermentation time depends on several factors, such as altitude, incubation temperature and storage condition (Hunduma & Ashenafi, 2011a; Andeta et al., 2017). It varies from a few weeks to several months or years. In colder areas, kocho is kept in a pit for years. The fermentation in warmer regions is more rapid and can be terminated within two to six months. There is a consensus that the quality of kocho gets better with increasing fermentation time (Gashe, 1987a; Ashenafi, 2006; Hunduma & Ashenafi, 2011b; Karssa et al., 2014; Assefa & Fitamo, 2016). At the same time, that consensus needs further clarification as a lot of the nutritional contents decrease during the fermentation time (Urga et al., 1997; Hunduma & Ashenafi, 2011b).

4.1.10 Baking the kocho

When the fermentation is considered finished or in case of food shortage, (a part of) the fermenting mass, called kocho, is removed from the pit to bake it. Prior to baking, the kocho is squeezed using a few long fibers as a net, kneaded and shredded on a ground stone to remove remaining fibers. Afterwards it is placed in a folded enset leaf, which serves as a frying pan, to bake a thick pancake (Tedla & Abebe, 1994; Steinkraus, 1996; Negash & Niehof, 2004; Ashenafi, 2006; Yirmaga, 2013; Arthur, 2014; Olango et al., 2014; Assefa & Fitamo, 2016). At a later stage, there is direct contact between the kocho pancake and the baking plate in the Gamo Highlands (personal observation). These actions are shown in Figure 4.4. The baking takes more than 10 minutes and the temperature is at least 100 °C (Nigatu & Gashe, 1998). Baked kocho is called ‘aradisame’ (Agren & Gibbson, 1969; Nigatu & Gashe, 1998).

Figure 4.4 (a): Shredding kocho on a ground stone; (b): Baking kocho between a folded enset leaf; (c): Baking kocho with direct contact to the baking plate (own photographs)

4.1.11 Flow chart of processing

The general process is shown in Figure 4.5. The solid lines indicate the key steps, while the dotted lines are steps that are followed depending on the region. Steinkraus (1996) questions more than 20 years ago whether enset would stay a staple food in the future due to the tedious and hard way of processing. However, the age-old process is still used with little modification, but innovation of the various traditional processing tools that can reduce the workload and improve sanitary conditions during processing, is necessary (Ashenafi, 2006; Hunduma & Ashenafi, 2011b).

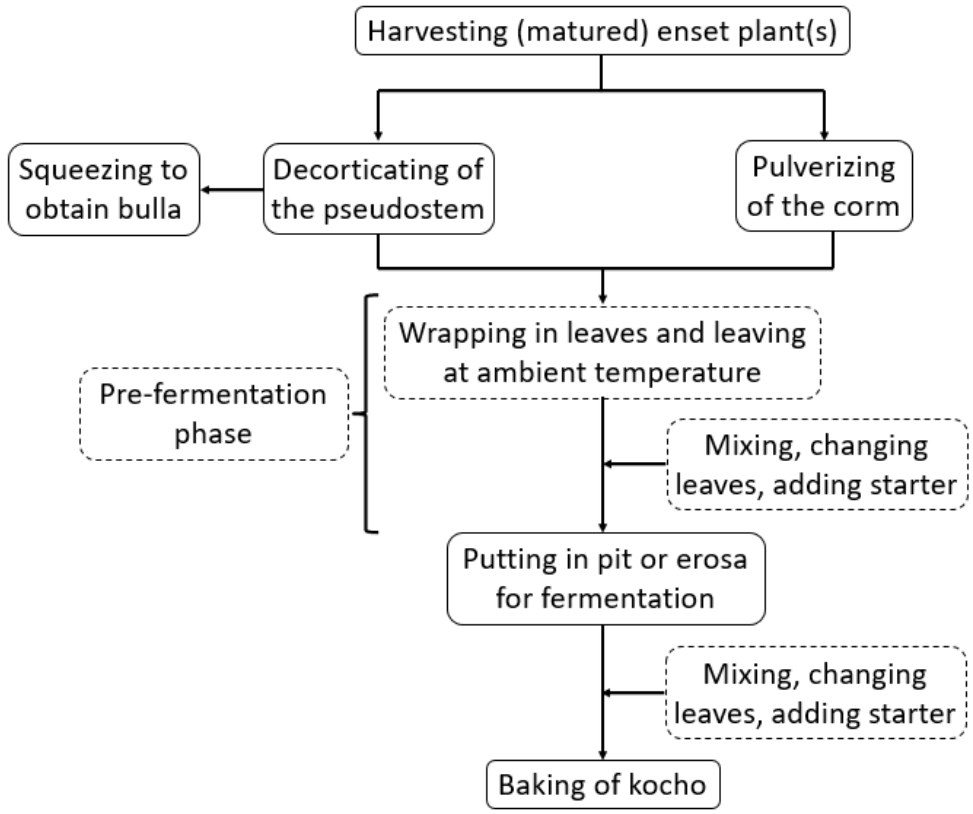

Figure 4.5 Flowchart of the general processing of enset to produce kocho. The dotted lines implicate optional process steps, implemented in some regions (adapted from Karssa et al., 2014 and Andeta et al., 2018)

4.2 Gender related work

The labor related with enset production is divided according to gender. In general, men are responsible for preparing the soil and doing the planting and transplanting. Women are involved in processing and baking of enset, but in Gurage Zone men also participate in the processing of enset to kocho (Brandt et al., 1997; Negash & Niehof, 2004). As the processing is labor and time intensive, women often work together in small groups (Tsegaye & Struik, 2002; Negash & Niehof, 2004; Hunduma & Ashenafi, 2011b). If households have enough financial capacity, they hire local processors. Women also sell kocho, bulla and amicho on the local market to earn money for household necessaries (Brandt et al., 1997; Negash & Niehof, 2004). Several authors (Sandford & Kasa, 1993; Brandt et al., 1997; Tsegaye & Struik, 2002) report that women also participate in production activities, such as choosing landraces, manuring and hand weeding, which indicates that the role of women in enset-based households is more than only processing and marketing the enset products.

4.3 Microbial features of kocho

This section deals with the microbial features of (baked) kocho. Only a few microbiological studies have reported actual microbial numbers present in fermenting kocho (Gashe, 1987a; Gashe, 1987b; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Bosha et al., 2016). These studies indicate that kocho is a lactic-fermented enset product, but yeasts (and molds) are also present in high numbers. Due to the different processing methods and fermentation conditions, the results vary between studies.

4.3.1 Microbial features during fermentation

Table 4.1 gives an overview of microbial counts expressed as log cfu/g per process step. The numbers in italics between brackets refer to the fermentation day. Each study will be discussed below.

Table 4.1 Overview of microbial counts (expressed as log cfu/g) during the fermentation of enset

	Gashe (1987a)			Karssa et al. (2014)				Bosha et al. (2016)			
Process step	TVC	Y&M	Spore-formers	TVC	LAB	Entero	Y	TVC	LAB	Y&M	Coli-forms
Pre-fermentation phase	1 (*0*)	1 (*0*)	ND (*0*)	4,5 (*0*)	2,3 (*0*)	3,7 (*0*)	2,5 (*0*)				
				6,0 (*2*)	4,2 (*2*)	6,0 (*2*)	3,8 (*2*)				
				7,3 (*4*)	5,2 (*4*)	7,2 (*4*)	4,5 (*4*)				
	3 (*2*)	2 (*2*)	ND (*2*)	8,4 (*6*)	6,8 (*6*)	7,3 (*6*)	5,8 (*6*)				
				9,2 (*8*)	7,9 (*8*)	5,1 (*8*)	6,3 (*8*)				
				8,5 (*12*)	8,9 (*12*)	3,0 (*12*)	8,3 (*12*)				
				7,6 (*15*)	9,3 (*15*)	2,1 (*15*)	8,2 (*15*)				
Fermentation	4 (*4*)	2 (*4*)	ND (*4*)	7,5 (*16*)	9,3 (*16*)	2,1 (*16*)	8,2 (*16*)	-	-	-	-
	7 (*6*)	2 (*6*)	2 (*6*)	6,6 (*18*)	7,5 (*18*)	<1 (*18*)	6,3 (*18*)	-	-	-	-
	8 (*8*)	3 (*8*)	3 (*8*)	5,7 (*20*)	6,4 (*20*)	<1 (*20*)	5,0 (*20*)	-	-	-	-
	10 (*15*)	3 (*15*)	3 (*15*)	4,8 (*22*)	5,6 (*22*)	<1 (*22*)	4,2 (*22*)	-	-	-	-
	10 (*22*)	3 (*22*)	2 (*22*)	3,7 (*24*)	5,0 (*24*)	ND (*24*)	3,5 (*24*)	-	-	-	-
	9 (*29*)	3 (*29*)	3 (*29*)	2,7 (*28*)	4,2 (*28*)	ND (*28*)	2,8 (*28*)	-	-	-	-
	8 (*36*)	3 (*36*)	2 (*36*)	2,2 (*31*)	4,0 (*31*)	ND (*31*)	2,2 (*31*)	5-7 (*30*)	± 5 (*30*)	± 5 (*30*)	3-4 (*30*)
	7 (*43*)	4 (*43*)	1 (*43*)					-	-	-	-
	6 (*50*)	2 (*50*)	3 (*50*)					-	-	-	-
	5 (*64*)	2 (*64*)	2 (*64*)					-	-	-	-
	4 (*79*)	1 (*79*)	2 (*79*)					>8 (*90*)	>8 (*90*)	± 3 (*90*)	<3 (*90*)

*TVC = total viable count, Y&M = yeasts and molds, LAB = lactic acid bacteria, Entero = Enterobacteriaceae, Y = yeasts
The fermentation day is presented in italics in parentheses

Gashe (1987a) examined the microbiology of kocho fermentation in Sebeta. The enset was processed following traditional methods (as discussed in 4.1) using a bamboo splinter and a shoulder long wooden pestle. Thereafter the kocho, kneaded into a mass, was covered in enset leaves and was left for three days at ambient temperature. At the end of the third day, the kocho was buried in a pit. Gashe (1987a) reported that the fermentation initiation starts with *Leuconostoc mesenteroides*. That was later confirmed by Andeta et al. (2018) via Illumnia MiSeq platform. *Leuconostoc mesenteroides* is associated with other fermentation processes (Johanningsmeier et al., 2007; Jung et al., 2012; Nyambane et al., 2014). *Enterococcus faecalis* was the second most abundant species at the beginning of the fermentation. After a week of fermentation, *Leuconostoc mesenteroides* and *Lactobacillus* spp. reached similar counts (between 6,5 and 7,5 log cfu/g). They are considered as responsible for reducing the pH and contributing to desirables changes. After 15 days, the counts for *Leuconostoc mesenteroides* rapidly declined. *Enterococcus faecalis* was no longer observed after 22 days. That is probably due to the lower pH. *Lactobacillus* spp. however remained the most predominant group until the end of the fermentation. The same pattern for *Leuconostoc* and *Lactobacillus* spp. is observed in other fermentations, such as sauerkraut and kimchi, both

fermented cabbage products (Belitz et al., 2004; Tamminen et al., 2004; Cho et al., 2009). Yeasts and molds were present in kocho at levels between 1 log cfu/g and more than 3 log cfu/g. Whether yeasts were involved in bringing desirable changes or were spoilage organisms is not clear. Spore forming bacteria reached high counts (up to 3 log cfu/g) during the fermentation. Both the population of *Clostridium* spp. and *Bacillus* spp. were abundant.

Karssa et al. (2014) determined the microbial changes in a pre-fermentation phase and fermentation in a pit with starter 'Gamancho'. During a 15 day long pre-fermentation phase, the counts of aerobic mesophilic bacteria increased from 4,5 log cfu/g to 9,2 log cfu/g in eight days, but declined afterwards. A similar pattern was observed for Enterobacteriaceae. A gradual increment from 3,7 log cfu/g to 7,3 log cfu/g in six days was noticed and then the counts decreased to 2,1 log cfu/g at day 15. LAB and yeasts counts increased from 2,3 log cfu/g to 9,3 log cfu/g and 2,5 log cfu/g to 8,2 log cfu/g, respectively. Afterwards, the fermentation in the pit was observed for 15 days. All the counts showed a decreasing pattern. The counts of aerobic mesophilic bacteria further declined to 2,2 log cfu/g. The counts of LAB and yeasts ended at 4,0 log cfu/g and at 2,2 log cfu/g, respectively. Enterobacteriaceae were not detected after eight days. Karssa et al (2014) concluded that the pre-fermentation phase supports the growth of the dominant LAB and yeasts. The co-occurrence of yeasts and LAB is reported in different spontaneous fermented foods (Narvhus & Gadaga, 2003; Lacerda et al., 2005; Moroni et al., 2011; Carvalho et al., 2017). Yeasts may be responsible for the hydrolysis of carbohydrates to sugars, which LAB can use in their metabolism (Oyewole, 2001). Nevertheless, a number of amylolytic LAB strains have been isolated, mostly belonging to the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus* and *Weissella* (Petrova et al., 2013). On top of that, amylolytic *Bacillus* spp. have been isolated in the beginning stage of kocho fermentation, but later in the fermentation the breakdown of starch was overtaken by *Lactobacillus* spp. (Nigatu et al., 1997; Hunduma & Ashenafi, 2011a).

A different pattern was observed by Bosha et al. (2016). They took samples on day 30 and 90 of the fermentation of different varieties. The researchers used three common used cultivars and three cultivars propagated from seeds. After 90 days, there was an increased amount of aerobic mesophilic bacteria and LAB compared to the 30 days record. The counts of aerobic mesophilic bacteria increased from 5 à 7 log cfu/g to more than 8 log cfu/g. Bosha et al. (2016) concluded that due to the regular mixing (four times every four days, and thereafter every 15 days), oxygen is still available for aerobic bacteria. Thus, they were able to grow and even increase. For LAB, an increment from around 5 log cfu/g to more than 8 log cfu/g was noticed. Yeasts and molds on the other hand decreased from circa 5 log cfu/g to around 3 log cfu/g. Coliform bacteria were present (< 3 log cfu/g) after even 90 days of fermentation. The difference in variety had a significant impact ($p < 0,05$) on the counts of aerobic mesophilic bacteria and LAB, but not on the presence of coliforms and yeasts and molds.

The effect of a pre-fermentation phase and starter 'Gamma' on the fermentation of enset in West Shewa Zone is reported by Hunduma and Ashenafi (2011a). As mentioned earlier, the high altitude and mid altitude region have notable differences in processing techniques. In the high altitude region, starter 'Gamma' is mixed with enset that has already been fermenting for one month. Fermentation continues for two to five months. In the mid altitude region, the processed enset undergoes a pre-fermentation phase of two weeks. The fermentation takes two to four months. In this experiment, the researchers considered putting the processed enset in a pit as the first day of the fermentation at the high altitude area, while the start of the pre-fermentation phase was considered as the first day at the mid altitude area. In the fermenting kocho of the mid altitude, significantly higher counts of coliforms and other Enterobacteriaceae

(up to almost 7 log cfu/g) were observed until 36 days of fermentation. Later, after more than 60 days, these microorganisms were not detected anymore. LAB counts increased during fermentation time from more than 3 log cfu/g to approximately 8 log cfu/g in an analogous way for both areas. The counts of yeasts and molds started for both study sites at more than 3 log cfu/g and reached counts of more than 5 log cfu/g. However, a decrease was noted after more than 80 days of fermenting. Thus, the fermentation occurred at both sites in a similar way, with exception of the high count of Enterobacteriaceae at the mid altitude area in the beginning of the fermentation.

4.3.2 Microbial features of the finished product

Ashenafi and Abebe (1996) described the microbial load of 30 samples kocho obtained from Hawassa open market. Counts of aerobic mesophilic bacteria and yeasts ranged between 6 and 7 log cfu/g. Coliforms were present at levels of 2,93 ± 1,84 log cfu/g. Kocho had high counts of *Enterococcus*, namely 4,99 ± 0,98 log cfu/g. The pH values were around neutral (6,9 ± 0,1). They isolated 327 strains of the 30 samples of market kocho. These isolates were dominated by *Micrococcus* spp., *Bacillus* spp. and *Staphylococcus* spp., especially *Staphylococcus aureus*. Among the yeasts species, the undesirable *Rhodotorula glutinis*, *Kluyveromyces marxianus* and *Pichia membranifaciens* were dominant. They concluded that the kocho brought to the market was not fermented yet or did not undergo appropriate fermentation, which can explain the high pH values and microbial load. Market kocho can cause health problems and is likely to spoil easily if the kocho is not fermented properly.

Kocho can be stored for a long period of time, even up to years in a pit without spoilage as long as the supposed anaerobic conditions are maintained (Gashe, 1987b; Pijls et al., 1995; Brandt et al., 1997; Karssa et al., 2014). When kocho is removed from the pit, it is exposed to the air and it becomes easily contaminated. Indications of spoilage are softness, discoloration and sliminess. Gashe (1987b) analyzed 100 samples of assumed unspoiled kocho, 100 moldy and 35 slimy kocho purchased at the market of Addis Ababa. The major spoilage fungi belonged to *Penicillium* spp., *Botrytis* spp., *Trichoderma* spp. and *Chaetomium* spp. The low pH and the considerable amount of moisture (around 50%) favored the presence of fungi. The bacterial genera causing softness and sliminess were *Erwinia* spp., *Bacillus* spp., *Pseudomonas* spp. and *Leuconostoc* spp. Aflatoxins B_1 and G_1 were present in kocho, up to a concentration of 40 mg aflatoxins per kg kocho. That is far above the legal maximum content of the sum of aflatoxins B_1, B_2, G_1 and G_2 in groundnuts for human consumption in Europe, namely 15,0 µg per kg (Commision Regulation (Ec) No 1881/2006).

4.3.3 Microbial features of baked kocho

As kocho is first baked before consumption, it is necessary to check whether the baking process is sufficient enough to eliminate pathogens. Therefore, Nigatu and Gashe (1998) inoculated different test organisms (*Bacillus cereus*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* spp. and *Shigella* spp.) on kocho. That was baked at 100 °C for 5 minutes. The heat treatment killed most of the test organisms, except for the spores of *Bacillus cereus*, and yeasts and molds naturally present on kocho. Due to the actual baking time of kocho (more than 10 minutes) and temperature (≥ 100 °C) and the low pH of kocho, they consider fresh aradisame as safe with respect to asporogenous bacterial

pathogens if post-baking contamination is minimized or prevented. Still, caution should be taken for possible present spore-formers, yeasts and molds and heat stable mycotoxins.

The processing of kocho happens in poor hygienic conditions. Important sources of contamination are hand contact, utensils and soil (Gashe, 1987b; Ashenafi & Abebe, 1996). Nevertheless, the fermentation itself seems to reduce foodborne pathogens. In combination with the baking process, kocho is generally considered as safe (Hunduma & Ashenafi, 2011a).

4.4 Physicochemical dynamics

Different factors influence the microbial activity during the fermentation. Some of these factors will be discussed below.

4.4.1 pH

During the fermentation, the pH decreases due to the activities of fermenting microorganisms, especially LAB. The pH of the end product lies between 4,0 and 4,3 (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014). It is therefore not surprising that LAB and yeasts are the predominant species during the fermentation as they are more acid tolerant. They can survive a pH around 3 to 4 (Wareing et al., 2011).

The pH values on the first day of the fermentation of the high and mid altitude area in West Shewa zone were almost neutral, namely 6,11 and 6,05 respectively. There was a sharp decline in pH for both study areas. After two weeks of pre-fermentation phase, the pH of the mid altitude region was significantly lower (p-value $< 0,05$) than the pH of kocho of the high altitude that was fermenting for two weeks. After 97 days of fermentation, the pH of kocho of the high altitude reached its lowest point at 4,30 and that remained so till the end of the fermentation at day 142. A similar end pH value was reached after almost 100 days at the mid altitude region, as the fermentation was finished there. Hunduma and Ashenafi (2011a) concluded that both the pre-fermenting phase and the starter ‘Gamma’ seemed to help to get a faster rate of pH fall. In another study, a different starter, namely ‘Gamancho’, also seemed to reduce the pH faster than without a starter. The pH declined from 6,12 to 4,50 during a 15-day pre-fermentation phase. After 15 days of fermentation in a pit, the pH decreased further to 4,0 (Karssa et al., 2014). In the study of Gashe (1987a), no starter was applied. The initial pH value was 6,5. After three days of pre-fermenting phase, the pH decreased to 6,3. The pH was further reduced to 4,2 after 80 days.

4.4.2 Titratable acidity

The increase of titratable acidity (TA) of fermenting enset (expressed as percent lactic acid) is attributed to the growth and activities of LAB (Hunduma & Ashenafi, 2011a; Karssa et al., 2014). The TA of fermenting enset increased to more than 0,3% (Hunduma & Ashenafi, 2011a). During a pre-fermentation phase of 15 days, the TA gradually increased from 0,01% to 0,41%. This sharp increment was continued during the 15-day fermentation in the pit. The TA ended at 0,61% (Karssa et al., 2014). In another study, the TA increased from 0,27 ± 0,02% to 1,54 ± 0,40% in seven weeks. That was accompanied with a sharp decrease in pH (Urga et al., 1997).

4.4.3 Moisture content

The moisture content of fermenting enset was followed up in different studies. The water activity (a_w) is a more important parameter on a microbiological level than the moisture content, but no data about the water activity of kocho are available. If the moisture content drops, the nutrients (discussed in 4.6) are more concentrated and the water activity is expected to be lower.

The moisture content of the fermenting enset decreases during fermentation time. The results between studies are very similar. For both study areas in West Shewa zone, the initial moisture content of the fermenting mass was around 86% and dropped to about 60% at the end of the fermentation for both regions (Hunduma & Ashenafi, 2011a). According to Urga et al. (1997), the moisture content declined especially during the first week of fermentation (from 84 ± 3% to 66 ± 4%). After seven weeks of fermentation, the moisture content was 60 ± 4%. During a pre-fermentation phase of three days, the moisture content decreased from 84% to 75%. At the end of the fermentation (day 80), the moisture content was diminished to 60% (Gashe, 1987a). A same pattern was observed by Karssa et al. (2014). The moisture content went from 84,4% to 68,6% during a pre-fermentation phase of 15 days. After a 15-day fermentation in a pit, the moisture content ended at 59,2%. Permeation is considered as the major reason of moisture removal (Karssa et al., 2014; Bosha et al., 2016).

4.4.4 Inside temperature

The inside temperature of kocho during a two-week pre-fermentation phase was higher than 20 °C and even reached temperatures above 25 °C. That was significantly higher than the temperature of kocho which was immediately put in a pit. The temperature both started around 17 °C. That pattern remained during the fermentation. The inside temperature of kocho which did not have a pre-fermentation phase, remained beneath 20 °C, while the temperature of kocho which underwent a pre-fermentation phase ranged from 19 °C to 23 °C (Hunduma & Ashenafi, 2011a). A similar trend was noticed by Karssa et al. (2014). During a pre-fermentation phase of 15 days, the inside temperature increased from 19,0 °C to 24,0 °C. Afterwards during the fermentation in pit, the inside temperature of the mass varied between 20,5 °C and 26,1 °C. In another study, the inside temperature of the fermenting enset ranged between 14 °C and 18 °C (Gashe, 1987a). The inside temperature of kocho is similar to that of cabbage during sauerkraut fermentation. There, the temperature ranges from 15 °C to 24 °C (Holzapfel et al., 2003; Belitz et al., 2004).

4.5 Sensory quality

Kocho is reported to have a sour taste (Urga et al., 1997; Negash & Niehof, 2004; Hunduma, 2010) and a “characteristic penetrating butyrous smell” (Urga et al., 1997). Not many studies are performed on the sensory characteristics of kocho (Yirmaga, 2013; Bosha et al., 2016).

Kocho bread (the so-called aradisame) made from three different cultivars, three wild types and two different fermentation times (namely 30 and 90 days) was evaluated by 25 panelists regarding color, taste, texture and overall acceptance on a 7-point hedonic scale. All the panelists had at least five years of experience with consuming kocho. The genotype was significant for all the evaluated features. The fermentation time had only significant effect on

the color. The color of aradisame was more appreciated after 30 days than after 90 days when the color was darker (Bosha et al., 2016). That is in agreement with the fact that kocho should have a light color (Smeds, 1955; Steinkraus, 1996; Daba & Shigeta, 2016).

Two different varieties (Kinnare and Astare), two different processing methods and three fermentation times were tested in Gurage Zone by Yirmaga (2013). One processing method was the traditional method of the Gurage people, including a bamboo scraper to decorticate the pseudostem, a serrated animal bone to grate the corm and no starter. The other processing method was modified by adding boiled (at 72 °C for 20 minutes) decorticated enset pulp to the decorticated pulp ready for fermentation. Sensory analysis was performed after 10, 20 and 30 days of fermentation with 30 panelists using a rating scale. Within the short fermentation times, it appears that a longer fermentation time of 30 days had a positive impact on all the sensory characterizations (namely color, texture, flavor and overall acceptance). It seems that a minimum of 30 days of fermentation is at least required. The flavor acceptance was evaluated as poor (ranging between 1,6 and 2,8 on 10). It is not mentioned whether the panelists were familiar with consuming kocho. These low scores could be explained if it was (one of) the first time(s) for the panelists to taste kocho as the taste of it is quite sour. The variety and processing method had effect on the color, texture and overall acceptance. The modified method scored higher on texture acceptance and overall acceptance.

4.6 Nutritional values of kocho

4.6.1 Energy value

Kocho is known for its high energy values. For fresh kocho, Pijls et al. (1995) reported an energy value of 646 kJ per 100 g, while Tsegaye and Struik (2001) determined 833 kJ per 100 g edible yield. On a dry weight basis, the energy value is reported to range between 1410 kJ to 1950 kJ per 100 g (Agren & Gibbson, 1969). The energy value of potatoes on dry weight falls into that range (Mu et al., 2017).

4.6.2 Moisture content

The crop enset holds a high amount of water, between 85% and more than 90% (Nurfeta et al., 2008; Mohammed et al., 2013). It is therefore not surprising that fermented kocho itself has a water content around 47% to 63% (Agren & Gibbson, 1969; Gashe, 1987b; Pijls et al., 1995; Yirmaga, 2013; Bosha et al., 2016). That broad range is attributed to the variety and fermentation time. Both have a significant impact on the moisture content. The water content decreases when the kocho is fermented longer, probably due to excessive permeation during pit fermentation (Karssa et al., 2014; Bosha et al., 2016). Nevertheless, Yirmaga (2013) did not measure loss of moisture during pit fermentation, but during mixing they added a little amount of water. That amount could compensate the water loss due to seepage. The moisture content of aradisame is observed to be around 33,7% to 43,7% (Agren & Gibbson, 1969; EHNRI, 1997). Hence, there is a lot of moisture loss in the different steps in the preparation of kocho (e.g. squeezing, fermentation and baking).

4.6.3 Carbohydrates

Kocho has nutritional values similar to potatoes (Mohammed et al., 2013). It is rich in carbohydrates, but only a small fraction are sugars. Table 4.2 shows the average amount of carbohydrates in kocho (n=12), which was fermented for 30 or 90 days. No separate data were given for each fermentation time. The genotype and fermentation time both had a significant effect on the average carbohydrate content, except the fermentation time did not have a significant effect for non-sugar carbohydrates (Bosha et al., 2016). Agren and Gibbson (1969) reported values between 95 g and 98 g carbohydrates per 100 g kocho DW and for fibers between 2,3 and 6,2 g per 100 g kocho DW. The initial crude fiber content of the variety Astare was 4,42% on dry weight, while for another variety Kinnare it was 3,37%. The crude fiber content decreased during fermentation time. After ten days of fermentation, the crude fiber was 2,67% and decreased further to 1,89% after 30 days of fermentation (Yirmaga, 2013).

Table 4.2 Average carbohydrate content of unbaked kocho (n=12), which was fermented for 30 or 90 days (Bosha et al., 2016)

Carbohydrates	Amount (g per 100 g dry weight)
Non-sugar carbohydrates	81,68 ± 3,92
Sugar	9,06 ± 2,94
Fiber	3,44 ± 0,58

4.6.4 Proteins

The amount of protein in kocho is low. The protein content varies between studies, ranging between 1,1 g and 2,8 g protein per 100 g kocho on dry weight (Agren & Gibbson, 1969; Shank & Ertiro, 1996; Abebe et al., 2006; Bosha et al., 2016). Table 4.3 shows the amino acid content of kocho and the human requirements (Abebe et al., 2006; WHO/FAO/UNU Expert Consultation, 2007).

Table 4.3 Amino acid content of kocho (mean of duplicates) (Abebe et al., 2006) and human requirements (WHO/FAO/UNU Expert Consultation, 2007)

Amino acid	Amount in kocho (g/100 g protein)	Amount of proteins in kocho (mg/100 g kocho)°	Requirements (mg/kg body weight per day)
Histidine	2,06	41,2	10
Isoleucine	4,12	82,4	20
Leucine	7,56	151	39
Lysine	5,50	110	30
Methionine + cysteine*	3,44	68,8	15
Methionine	-	-	10
Cysteine*	-	-	4
Phenylalanine + tyrosine*	6,87	137	25
Threonine	2,75	55,0	15
Tryptophan	2,75	55,0	4
Valine	5,50	110	26

* Semi-essential amino acid (Bender, 2014)

° Based on the assumption kocho contains 2,00 g protein per 100 g

4.6.5 Fat

The fat content of kocho is low. On dry weight basis, the crude fat content varies between 0,20 and 1,60 g fat per 100 g kocho (Agren & Gibbson, 1969; EHNRI, 1997; Urga et al., 1997; Yirmaga, 2013; Bosha et al., 2016). Pijls et al. (1995) found 0,2 g fat per 100 g edible kocho.

4.6.6 Micronutrients

Micronutrients, such as minerals and vitamins, are essential components in the human food. They are required in relatively small quantities measured in micrograms or milligrams per day. Inadequate micronutrient intake causes deficiencies and insufficiencies (WHO & FAO, 2004; Shergill-Bonner, 2013).

4.6.6.1 Minerals

The mineral content of kocho varies considerably between studies (Table 4.4), which can be due to variation in genotypes (Bosha et al., 2016), properties of the soil, application of manure, storage and processing of kocho and other factors (Atlabachew & Chandravanshi, 2008). As kocho ferments longer, the nutritional value decreases, probably due to leaching of the water-soluble nutrients (Gashe, 1987b; Steinkraus, 1996; Karssa et al., 2014). Kocho contains sufficient concentration of different minerals for human requirement compared to cereal flours (Atlabachew & Chandravanshi, 2008).

Table 4.4 Mineral content of different studies

Mineral (per 100 g kocho DW)	Requirement (mg/day) (WHO & FAO, 2004)	Abebe et al. (2007)°	Bosha et al. (2016)	Atlabachew & Chandravanshi (2008)	
				Woliso kocho	Welkite kocho
Calcium (mg)*	1000	270	180,4 ± 57,73	58,4 ± 0,6	49,8 ± 1,1
Cobalt (mg)	-	-	-	0,61 ± 0,03	0,55 ± 0,01
Copper (mg)*	-	-	-	0,43 ± 0,04	0,34 ± 0,03
Chromium (µg)*	-	-	-	642 ± 34	596 ± 30
Iron (mg)*	9,1 - 58,8	10	5,24 ± 3,30	13,5 ± 0,9	9,25 ± 0,41
Magnesium (mg)*	220 - 260	-	1,83 ± 0,54	29,0 ± 1,4	18,0 ± 1,1
Manganese (µg)*	-	-	-	858 ± 35	101 ± 40
Potassium (mg)*	-	-	65,29 ± 7,34	438,0 ± 2,0	275,3 ± 2,2
Sodium (mg)*	-	-	3,58 ± 0,49	46,2 ± 0,3	68,8 ± 0,5
Zinc (mg)*	3,0 - 14,0	0,87	2,63 ± 1,16	2,7 ± 0,09	31,0 ± 0,08

* Essential mineral (Shergill-Bonner, 2013)
° Based on aradisame and the assumption that it contains 40% water

Kocho provides a relatively good source for calcium (Abebe et al., 2007; Atlabachew & Chandravanshi, 2008; Bosha et al., 2016). In comparison, milk contains 120 mg calcium per 100 ml (Belitz et al., 2004). The daily recommended intake for adults (19 years until 65 years or menopause), as can been seen in Table 4.4, is 1000 mg calcium (WHO & FAO, 2004). That might be an explanation why kocho is considered as a medicine to heal bone fractures (Tsehaye & Kebebew, 2006; Giday et al., 2010; Tsegaye & Gizaw, 2015; Assefa & Fitamo,

2016). Atlabachew & Chandravanshi (2008) did not find detectable amounts of the toxic heavy metals cadmium and lead in kocho.

4.6.6.2 Vitamins

Kocho is poor in terms of vitamins. The vitamin A content in kocho is low or even absent (Pijls et al., 1995; Shank & Ertiro, 1996; Abebe et al., 2006). EHNRI (1997) reported 0,2 µg vitamin A per kg kocho. That might be a concern for Ethiopia where vitamin A deficiency is a major health problem, especially for children (Demissie et al., 2010; Amede et al., 2004; Busse et al. 2017). Vitamin A intake needs to come from other sources than enset, such as cabbage, pumpkin, beans and cheese (Pijls et al., 1995; Shank & Ertiro, 1996; Abebe et al., 2006).

For other vitamins, there is not much research done yet. Agren and Gibbson (1969) examined different vitamin contents in kocho. For vitamin B_1, they reported 0,06 mg per 100 g kocho DW, while EHNRI (1997) found 0,03 mg per 100 kg kocho. Kocho contains 0,08 mg vitamin B_2 and 0,6 mg vitamin B_3 per 100 g on dry weight basis (Agren & Gibbson, 1969). No vitamin C was present (EHNRI, 1997).

4.6.7 Dietary consequences

In enset growing regions, households including children (Abebe et al., 2006), consume kocho as the most frequently served main meal. In Kaffa-Shake zone, kocho is consumed at least twice a day (Negash & Niehof, 2004). The average consumption by one person is estimated to be between 430 g and 700 g a day (Steinkraus, 1996) or 10 to 20 plants a year (Smeds, 1955; Nigatu, 1992; Negash, 2001). As kocho has low protein and vitamin content, but high energy values and carbohydrates, poor households will not become hungry, but their monotonous kocho-based diet will be lacking essential nutrients (Negash & Niehof, 2004). Supplementation with a protein and vitamin source is recommended (Abebe et al., 2006; Daba & Shigeta, 2016). However, it should be mentioned that enset is cultivated together with cereals, pulses and root crops (Tsehaye & Kebebew, 2006), which are used to supplement the diet (Tsegaye & Struik, 2001). Wealthy households also consume meat and dairy products (Assefa & Fitamo, 2016) and dishes made from enset are traditionally served with other protein and vitamin sources (Ashenafi, 2006). Nevertheless, there is a shortage of protein-supplying crops in poor households (Abebe et al., 2010). Cultivation of other crops than enset should thus be encouraged (Nigatu & Gashe, 1998; Abebe et al., 2010; Mohammed et al., 2013).

Part 2: Practical work

5 COMPARISON OF JAR, EROSA AND PIT

5.1 Aim

The aim of this experiment is twofold. The first objective is to gain more insight of the microbiota involved in the fermentation process. Only a few microbiological studies on the fermentation of enset have been carried out hitherto (Gashe, 1987a; Gashe, 1987b; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Bosha et al., 2016; Andeta et al., 2018), but none of them was carried out in the Gamo Highlands. Due to the differences in processing techniques (discussed in 4.1), fermentation might proceed differently depending on the region. It is therefore useful to characterize spontaneous enset fermentations with the help of culture dependent analyses. The second objective is to introduce sauerkraut jars as a fermentation system. As already indicated in the literature study, the sauerkraut and enset fermentation have a lot of similarities. The use of sauerkraut jars might be a good alternative for the common pits and erosas. It has a lot of advantages (it is cleaner, no problems with insects, more controllable,…), but the feasibility of the jars still needs to be tested. Therefore, this experiment was set up to compare fermentation in the pits, erosas and sauerkraut jars on a physicochemical and microbial level.

5.2 Material and methods

5.2.1 Trial design

Nine enset plants of Gena variety were traditionally processed by local women and put immediately in three jars, three erosas and three pits. All the fermentation systems were placed in the same room where the temperature and relative humidity were monitored. On day 1,7, 15, 31, 60 and 90, three samples from each fermentation system were taken. The pH, titratable acidity, moisture content and inside temperature of the fermenting mass were determined. On the same days, different bacterial counts were achieved via culture based methods, namely total viable count (both aerobic and anaerobic), lactic acid bacteria (LAB), yeasts and molds, Enterobacteriaceae and *Clostridium* endospores.

5.2.2 Study site

The experiment took place in Dorze in the Gamo highlands of Ethiopia. It is one of the enset growing areas in the Southern Nations, Nationalities and Peoples' Regional State (SNNPR) of Ethiopia. Dorze has an altitude around 2600 m above sea level (Sperber, 1974). The mean annual rainfall for Chencha (close to Dorze) is about 1392 mm, based on meteorological data measured from 1970 and 2008 on 2700 m above sea level (Assefa, 2002). The study area has a bimodal rainfall distribution. The highest rainfall occurs in the months of April and July. Normally, the first rainy season extends from February to May. The second season occurs from June to October. The temperature ranges from 10 °C to 25 °C (Assefa, 2002; United States Agency International Development, 2005).

5.2.3 Processing of enset

Nine enset plants of Gena variety were purchased. The Gena variety was chosen because it is preferred for preparing kocho (Daba & Shigeta, 2016). A survey conducted in the Gamo Highlands, including the study area Dorze, pointed out that three varieties of enset, namely Gena, Maze and Ketishe, are preferred by over 90% of the respondents and Gena accounts for the highest preference for kocho and bulla preparation (Andeta et al., 2018). Local, knowledgeable women scraped the leaf sheaths of the pseudostem with metal and bamboo scrapers and pulverized the corms of the plants with bamboo scrapers. The process is described in 4.1.3 and 4.1.4 and was executed in a backyard of a farmer. To obtain bulla, a new squeezing equipment, bought from Wolaita Rural Technology Transfer center (Figure 5.1), was used. The mass of the scraped pseudostem and corm was put in the cylindrical shaped container. With a lever system, the mass was squeezed. The liquid was obtained in a plastic basin. After a rest period, the liquid was decanted and the remaining residuals were left to dehydrate in order to become bulla.

Figure 5.1 Squeezing equipment from Wolaita Technology Transfer center to obtain bulla (own photograph)

The squeezed mass of the pseudostem and the mass of grated corm from the nine plants, were carefully mixed. That was done manually. The mixture was put on a plastic sheet and chopped with knives. Thereafter equal masses (45 kg) of the mixture were put in each fermentation system (three pits, three erosas and three jars). The pits and erosas were compacted using enset leaves, plastic bags and large stones according to traditional practices, so anaerobic conditions could be created. Underground pits are commonly used in Ethiopia to ferment enset (Gashe, 1987a; Nigatu, 1992; Brandt et al., 1997; Hunduma & Ashenafi, 2011a; Karssa et al., 2014). However, in the study area, bamboo baskets, locally called erosas, are the most common fermentation practice (Andeta et al., 2018). Since enset fermentation resembles the sauerkraut fermentation, sauerkraut jars were tested as well. No starter or backslopping were applied to aid the fermentation. In other areas in Ethiopia, a starter, mainly consisting of already fermented kocho (called backslopping), is added to aid the fermentation, as explained in 4.1.6.

Figure 5.2 shows the three fermentation systems. The sauerkraut jar made of ceramics had a volume of 10 liter and the height was 33,5 cm, excluding the lid. Each jar had two heavy plates

of 15 cm intended for compaction and a water lock was applied to create anaerobic conditions. The in and outside of the jar was first glazed (lead-free) and then the jars were baked at 1185 °C. The glazed layer made the jars waterproof.

Figure 5.2 From left to right: pit, erosa and sauerkraut jar (own photographs)

5.2.4 Sampling

At the already mentioned sampling times, three samples (about 150 g) from each fermentation system were taken aseptically using spoons and pre-sterilized beakers. The beakers covered with aluminum foil were sterilized by putting them on 270 °C for 2 hours. The three fermentation systems were placed in the same room of a house in Dorze (6°11'N, 37°34'E, Southern Ethiopia) to ensure identical environmental conditions. There, the room temperature and humidity were monitored (HTC-1). To take samples, the spoons were sterilized with burning ethanol 96%. After taking the samples, the fermenting enset was pressed so the anaerobic conditions could be restored. The samples were taken to the laboratory in an ice box. Styrofoam was placed between the samples and the cooling elements so the samples would not have direct contact with the cooling elements. Analysis was done as quick as possible. The samples were kept in the refrigerator until analysis.

5.2.5 Microbial analysis

Each sample was subjected to microbial plating. The total viable mesophilic count (both aerobic and anaerobic), yeasts and molds, LAB, Enterobacteriaceae and *Clostridium* endospores were determined. The performed classical plate counts (culture dependent analysis) were carried out according to the ISO standards for microbial analysis of food (Dijk et al., 2015). First, all the necessary media were prepared. The total viable count was determined on Plate Count Agar (PCA, Biokar Diagnostics), Enterobacteriaceae on Violet Red Bile Glucose (VRBG, Biokar Diagnostics) and yeast and molds on Oxytetracycline Glucose Agar (OGA, Biokar Diagnostics) supplemented with Oxytetracycline (10 ml per 1,1 liter OGA). LAB were enumerated on pour plates of de Man Rogosa Sharpe agar (MRS, Biokar Diagnostics). Reinforced Clostridium Agar (RCA, Biokar Diagnostics) was used to count *Clostridium* endospores. The composition and method of preparation of the used media can be found in **annex A**. For the decimal dilutions, peptone physiological salt solution was prepared. It consisted of 1,0 g/l peptone from casein (Biokar Diagnostics) and 8,5 g/l sodium

chloride (Neolab). Thereafter, the named media and the peptone physiological salt solution were autoclaved for 15 minutes at 121 °C by 15 pounds per square inch (psi). When autoclaving was done, the growth media were placed in a water bath (Bluefic Industrial and Scientific Technologies) at 50 °C, so the agar would not solidify. The peptone physiological salt solution was refrigerated until needed. The media and peptone physiological salt solution were prepared one day before the microbiological analysis.

Subsequently, about 5 g of fermenting enset was taken aseptically into a sterile Falcon tube for possible future metagenomics analyses. These samples were stored in the freezer at -18 °C. Further, another 5 g was transferred aseptically into a sterile stomacher bag (Whirl-pak®) and weighed. A nine-fold amount of sterile peptone physiological salt solution was added. The mixture was homogenized for 60 s in a stomacher (StarBlender™ LB 400). In the stomacher bag, the primary decimal dilution (10^{-1}) was present. Starting from that dilution, a ten-fold serial dilution was prepared. However, to count *Clostridium* endospores, a heat shock treatment prior the decimal dilution series was necessary. Endospores can survive the heat treatment, but vegetative cells are killed. Therefore about 6 ml of the primary dilution was pipetted from the stomacher bag in a sterile test tube. Together with a blank test tube filled with around 6 ml deionized water, it was placed in a water bath at 75 °C. The temperature of the blank test tube was measured. When the temperature reached 75 °C, both test tubes were held for 15 minutes in the water bath. After the heat treatment, the test tube which contained the primary dilution was cooled by putting it in a beaker of cold water in the refrigerator. From that primary dilution, the serial dilution to determine *Clostridium* spores was made.

Afterwards, the pour plate method was used. From the appropriate dilution, 1 ml was taken using a micropipette and added to an empty sterile plate. Then around 20 ml of the growth medium, which was cooled to 50 °C in the water bath, was poured. The plates were well mixed. Each appropriate dilution was plated in duplicate. On the already solidified media, a double layer of the same media (around 5 ml) was poured for the determination of LAB and Enterobacteriaceae. That created a semi-anaerobic environment. Finally, the plates where the agar was solidified, were finally placed inverted in an incubator (Coslab, Bluefic Industrial and Scientific Technologies for yeasts and molds). The petri dishes for the determination of total viable count and LAB were incubated at 30 °C for three days. The plates for yeasts and molds were incubated at 25 °C for 24 h. Enterobacteriaceae and *Clostridium* endospores were incubated at 37 °C for 24 h. The plates for the anaerobic total viable count and *Clostridium* endospores were first put in jars containing gas generating kits (IVD, Microbiology Anaerocult®A) and an anaerobic indicator (IVD, Microbiology Anaerotest®) for anaerobic incubation. During sample handling, the worktops were regularly disinfected with Dettol® and the work was carried out near Bunsen burners.

After the mentioned incubation time, the number of colonies on each plate was counted. Only pink to red colonies surrounded with bile precipitation were counted for Enterobacteriaceae. Only countable plates were considered. For the total viable count, LAB and *Clostridium* endospores a plate was considered as countable if the plate contained at least 10 and maximum 300 colonies. For Enterobacteriaceae and yeasts and molds, a countable plate contained 10 to 150 colonies. Using formulas 5-1 and 5-2, the colony forming units (cfu) were calculated. All microbial counts are expressed as log cfu/g. Afterwards, the mean log cfu/g value and the standard deviation were calculated.

$$N = \frac{S * BF}{(n_1 + 0{,}1 * n_2 + 0{,}01 * n_3) * VF} \quad (5\text{-}1)$$

With

N = bacterial count, expressed as colony forming units (cfu) per gram

S = the sum of the counted colonies

BF = confirmatory factor, if no confirmatory tests have been carried out, the confirmatory factor is equal to 1

n_1 = the number of petri dishes with the lowest dilution

n_2 = the number of petri dishes with the subsequent tenfold dilution

n_3 = the number of petri dishes with the subsequent tenfold dilution

VF = dilution factor, the volume in ml or mass in g of the undiluted sample that was present in the petri dish with the lowest dilution

$$\log \frac{\text{cfu}}{\text{g}} = \log(N) \quad (5\text{-}2)$$

5.2.6 Physicochemical dynamics

5.2.6.1 pH determination

After the plates were poured, a certain mass (around 10 g) of each sample was taken and put in a blender bag (VWR®). Due to its dry character, a ninefold amount of deionized water was added and the bag was homogenized for 60 s in a stomacher (StarBlender™ LB 400). Thereafter the mass was put in a beaker. The pH was measured three times with a pH meter (pH 1100 H). The pH meter was first calibrated using standard buffer solutions (pH 4 and 7). To show the results of the pH values, the average of the three measurements was calculated and rounded to two decimal places.

5.2.6.2 Titratable acidity

After measuring the pH, the titratable acidity (TA) was analyzed by titrating the sample with NaOH to the phenolphthalein endpoint. That was according to the method used by Lefebvre et al. (2002), Oguntoyinbo & Dodd (2010) and Karssa et al. (2014). Therefore, approximately 10 g kocho sample was blended with 90 ml distilled water using a stomacher (StarBlender™ LB 400). Three drops of phenolphthalein indicator were added to an aliquot of 10 ml filtrate. Then it was titrated with 0,1 M NaOH. As the color of the sample changed from clear to pink, the volume of NaOH consumed until the endpoint reached, was used to calculate the TA using formula 5-3. The TA is expressed as percent of lactic acid. For each fermentation system, the TA was measured in triplicate. Just as for the pH, results are expressed as averages and standard deviations of the three repetitions and rounded to two decimal places.

$$\text{titratable acidity } (\%) = \frac{V * Na * 0{,}009}{W} * 100 \quad (5\text{-}3)$$

With

V = Volume consumed of 0,1 M NaOH (ml)

Na = Normality of NaOH

W = weight of the sample (g)

5.2.6.3 Moisture content

In previous studies on enset fermentation, the moisture content was followed up. The water activity a_w is a more important microbial parameter, but no device to measure the a_w was available. Therefore, only the moisture content of kocho was determined by the oven drying method (AOAC, 1990). From each fermentation system, three samples of a known quantity (around 10 g) were placed on pre-dried watch glasses. These watch glasses were first weighed on a balance (Sartorius). The watch glasses with kocho were allowed to dry at 105 °C for 24 h. Due to the absence of a desiccator, the oven was first put off in order to cool down the watch glasses. Subsequently, they were weighed again. The moisture content was calculated from the weight loss using formula 5-4. The moisture content of kocho was determined in triplicate per fermentation system, so nine monsters were analyzed per sampling day. The average moisture content of the three samples was calculated and rounded to one decimal place.

$$\text{moisture content } (\%) = [1 - \frac{(W_3 - W_2)}{W_1}] * 100 \qquad (5\text{-}4)$$

With

W_1 = weight of the fresh sample of kocho (g)

W_2 = weight of an empty watch glass (g)

W_3 = weight of the watch glass with the dried sample of kocho (g)

5.2.6.4 Inside temperature

The inside temperature of the fermenting mass was measured before sample taking. A digital thermometer (Traceable® Long-Stem Thermometers) was first disinfected with 96% ethanol. Then it was inserted at three different depths. The average and standard deviation of the three temperatures were calculated and rounded to two decimal places. Weather data were obtained from the Gamo Highlands Ethiopian Meteorological Stations located in Tegecha and Chencha, both close to Dorze. The altitude of the station in Tegecha is 2140 m above sea level and of the station in Chencha is 2738 m above sea level. Dorze its elevation is around 2600 m above sea level (Sperber, 1974), so it is located between the two stations.

5.2.7 Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 22. For the analysis of the pH, TA, moisture content, inside temperature and the different microbial counts, significant differences between means were determined using One-Way ANOVA. Per sampling day, it was determined whether the parameters were different between the three fermentation systems. Subsequently, the post-hoc Tukey honestly significant different (HSD) was used the identify

these differences. Before One-Way ANOVA was performed, the Levene's test was used to check homoscedasticity. Normality was assumed since all parameters were measured in triplicate. If the Levene's test showed there was no homoskedasticity, the non-parametric Kruskal-Wallis test was used. In all cases, a significance level of 5% was considered.

5.3 Results and discussion

5.3.1 Physicochemical dynamics

This paragraph discusses the pH, TA, moisture content and inside temperature during fermentation. These properties are useful to estimate the possibility of growth of bacteria, yeasts and molds during fermentation time.

5.3.1.1 pH

The obtained results for the pH are presented in Figure 5.3. On the first day of the fermentation, the pH values were 5,13 ± 0,00 for the pits, 5,17 ± 0,01 for the erosas and 5,02 ± 0,12 for the jars. As expected, no significant differences were observed on the first day. In the first 15 days, there was a sharp decline in pH due to the activities of fermenting microorganisms, especially LAB. The pH values dropped to 4,14 ± 0,03 for the pits, 4,14 ± 0,02 for the erosas and 4,39 ± 0,01 for the jars on day 15. That sharp decline was already noticed in previous studies (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Andeta et al., 2018). The pH on day 7 in the jars was significantly higher than the pH in the pits and erosas, and the pH of the erosas was significantly the lowest (p-value = 0,000). The pH in the jars stayed significantly the highest at day 15, with a value of 4,39 ± 0,01 (p-value = 0,000), so its drop in pH was the slowest. Thereafter, the pH of the kocho in the pits and erosas started to increase over fermentation time, as can be seen in Figure 5.3. That was not the case for the jars. The increment in pH was not expected since no study hitherto mentioned an increase (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Andeta et al., 2018). When the pH increases, the environment becomes more favorable for clostridia and the germination of spores is possible (Jay et al., 2005; Wheeldon et al., 2008). If saccharolytic clostridia, such as *Clostridium tyrobutyricum*, were present, they could convert two molecules of lactic acid into one molecule of the weaker butyric acid. In that reaction, hydrogen gas and carbon dioxide are produced and the pH increases. That process is undesirable in silage processes (Johnsson, 1991; Pahlow et al., 2003; Weinberg, 2008). A "characteristic penetrating butyrous smell" and an increasing amount of butyric acid from kocho throughout the fermentation have been detected (Urga et al., 1997). A lack of fermentable carbohydrates is a major cause for the clostridial fermentation. Since the pH eventually started to decrease between day 60 and 90 and the LAB counts (discussed in 5.3.2.2) were still high at the end of the fermentation, it is expected that there was no lack of fermentable carbohydrates. Moreover, proteolytic clostridia can break down amino acids in ammonia and thereby increasing the pH, but as the protein content in kocho is very small (see also 4.6.4), that process would probably be negligible (Johnsson, 1991; Pahlow et al., 2003; Weinberg, 2008). All that does not explain why the pH started to increase. Besides that, the pieces of plant material may interfere with the pH measurement. It can also be noticed that an increase in pH only occurred when the fermenting enset came in direct contact with the enset leaves, as is the case for the pits and erosas. Possibly, a substance in the leaves can cause a pH increment. The minimum pH values for the most known foodborne clostridia are

reported to be 4,6 for *Clostridium botulinum* and 5,0 for *Clostridium perfringens* (Pahlow et al., 2003; FDA, 2015). Furthermore, *Clostridium* spp. can grow under a pH of 4 when the moisture content is high (McDonald et al., 1991). A rapid decline in pH inhibits the growth of undesirable microorganisms, such as Enterobacteriaceae and *Clostridium* spp. (Kung, 2001; Pahlow et al., 2003; Weinberg, 2008). The pH in the pits and erosas continued to increase until at least day 60. On that day, the pH in the jars was 4,32 ± 0,02. That was significantly lower (p-value = 0,001) than these of the pits (4,35 ± 0,02) and erosas (4,55 ± 0,05).

The end pH on day 90 was 4,35 ± 0,02 for the pits, 4,55 ± 0,05 for the erosas and 4,29 ± 0,02 for the jars. That is similar to the end pH values of other reported enset fermentations, namely between 4,0 and 4,3 (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Andeta et al., 2018). The jars obtained the lowest pH on day 90, but that did not differ significantly from the pH of the pits. The pH in the erosas on the other hand was significantly higher than in the pits and jars (p-value = 0,000). Presumably, these end values remained stable as was previously observed in enset fermentation (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014), sauerkraut fermentation (Pundir & Jain, 2010) and silage fermentation (Weinberg, 2008).

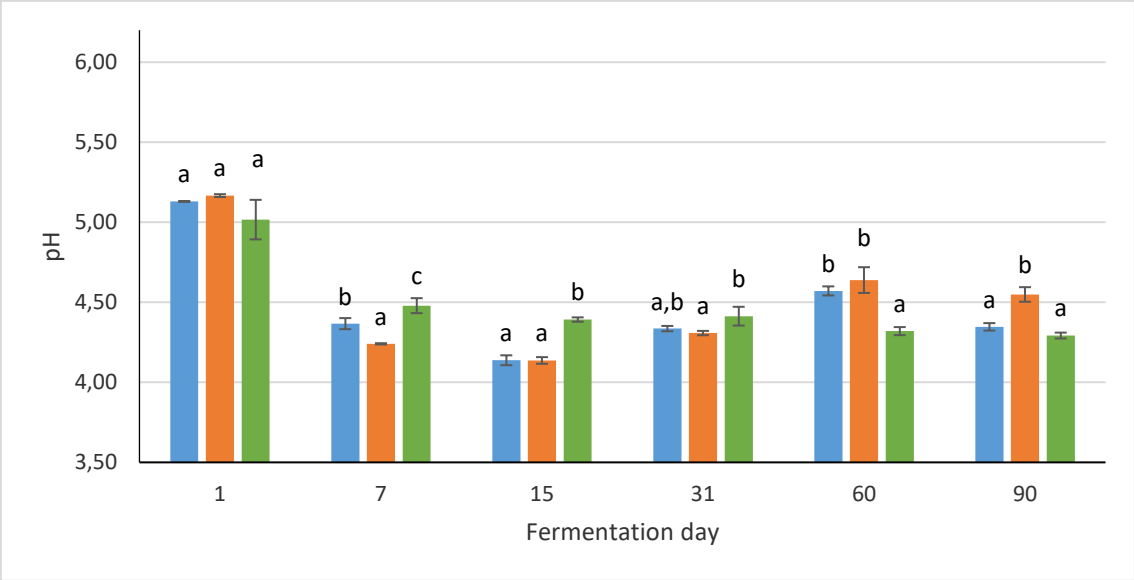

Figure 5.3 pH of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b,c]

5.3.1.2 Titratable acidity

The results of the TA (expressed as percent lactic acid) are presented in Figure 5.4. In the first 15 days, an increase from 0,30 ± 0,00% for the pits, 0,32 ± 0,02% for the erosas and 0,29 ± 0,00% for the jars to 0,36 ± 0,01%, 0,38 ± 0,01% and 0,34 ± 0,01% respectively, was recorded. That is attributed to the microbiota, especially LAB, which produce organic acids (Hunduma & Ashenafi, 2011a; Karssa et al., 2014). The increase of TA was accompanied with the fall of the pH in the first 15 days. Thereafter, there was a decrease in TA in all fermentation systems, including the jars. That pattern was also noticed when investigating the pH (5.3.1.1), except for the jars, where the pH remained constant. The TA started to increase again and went to

0,34 ± 0,01%, 0,33 ± 0,01% and 0,43 ± 0,01% in the pits, erosas and jars, respectively on day 60. The TA in the jars was significantly higher (p-value = 0,000) than in the other fermentation systems. That pattern continued until day 90, when the highest TA was achieved, namely 0,48 ± 0,02% in the pits, 0,50 ± 0,01% in the erosas and 0,56 ± 0,01% in the jars. The TA in the jars was again significantly higher (p-value = 0,000). That is consistent with the lowest pH noticed for the jars (discussed in 5.3.1.1).

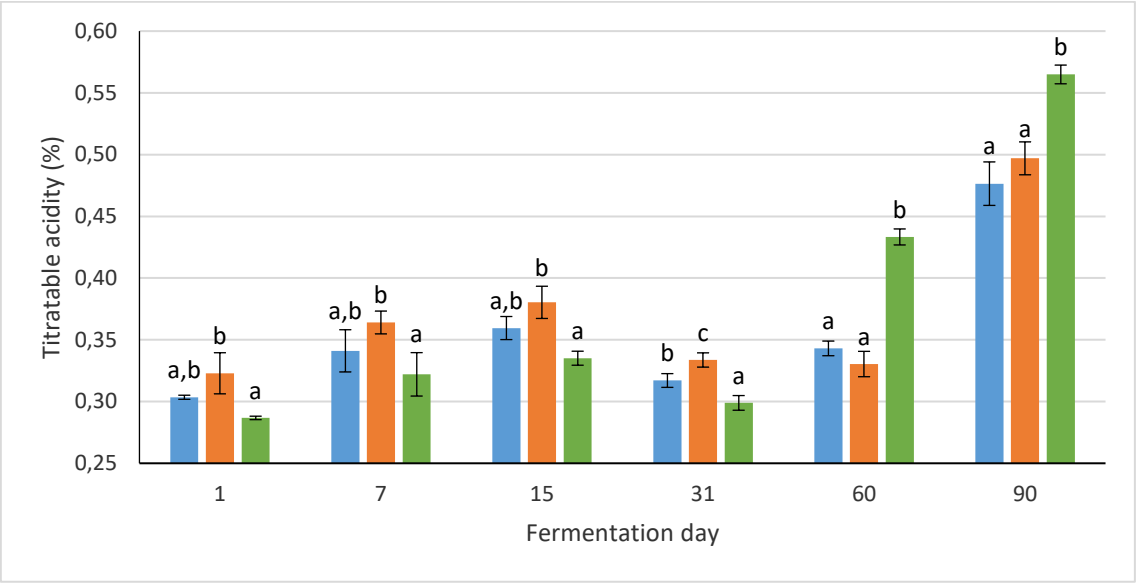

Figure 5.4 Titratable acidity of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. The titratable acidity is expressed as percent lactic acid. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b,c]

5.3.1.3 Moisture content

The moisture content during fermentation is shown in Figure 5.5. On the first day, the moisture content in the jars was 84,8 ± 0,12%, while in the pits and erosas, the moisture contents were 73,9 ± 0,88% and 73,4 ± 0,74%, respectively. In this experiment, the enset was pressed mechanically with a new squeezing equipment bought from Wolaita Rural Technology Transfer center (explained in 5.2.3) instead of by hand, which is the common practice (explained in 4.1.5), so lower initial moisture contents were expected. Initial values of the moisture content between 78% and 86% have been reported, including from scraped and pulverized materials of the Gena variety. These moisture contents were all achieved manually (Gashe, 1987a; Urga et al., 1997; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Andeta et al., 2018). So, the initial moisture content in the jars was higher than expected. The samples of day 1 were taken approximately 12 hours after putting the processed mass in each system. Since the starting material of all the three fermentation systems was equal, the moisture loss of the pits and erosas should have occurred during the fermentation itself, instead of prior to fermentation. So, there was a huge change in moisture content for the pits and erosas in only 12 hours, while the moisture content in the jars did not change much. An explanation might be that the pits and erosas were not completely closed and to some extent permeable for water, while evaporation in the jars was improbable due to the glazed in and outside material and the water lock. Permeation is considered as the major reason of moisture removal (Karssa et al., 2014; Bosha et al., 2016). Around 4 liter water had seeped through the pits and erosas in circa 12 hours.

Over fermentation time, a decrease in moisture content was noticed. The moisture contents in the jars were continuously and significantly higher than in the pits and erosas throughout the whole experiment. As can be seen in Figure 5.5, the moisture contents especially dropped during the first 15 days of fermentation, which has been noticed earlier (Gashe, 1987a; Urga et al., 1997; Karssa et al., 2014; Andeta et al., 2018). Thereafter, they remained quite constant in each fermentation system. The moisture contents in the pits, erosas and jars were 69,4 ± 0,53%, 70,4 ± 0,25% and 74,5 ± 0,70%, respectively on day 15, while the moisture contents at day 90 were just a little lower, being 68,0 ± 1,81%, 69,3 ± 0,25% and 73,6 ± 0,89%. These end levels were relatively high compared to earlier studies, where the moisture contents dropped to approximately 60%, as discussed in 4.4.3 (Gashe, 1987a; Urga et al., 1997; Hunduma & Ashenafi, 2011a; Karssa et al., 2014). Nevertheless, Andeta et al. (2018) reported a moisture content of 69,18 ± 0,78% for kocho from the Gena variety after 60 days of fermentation, including a pre-fermentation phase of 15 days. In the jars, the biggest decline was noticed, namely from 84,8 ± 0,12% to 74,5 ± 0,70%. That was unexpected, but it is possible that the water locks were not always applied and evaporation could be possible due to the high environmental temperatures. Two guard had to make sure that the water locks were always filled with water, but that was impossible to check.

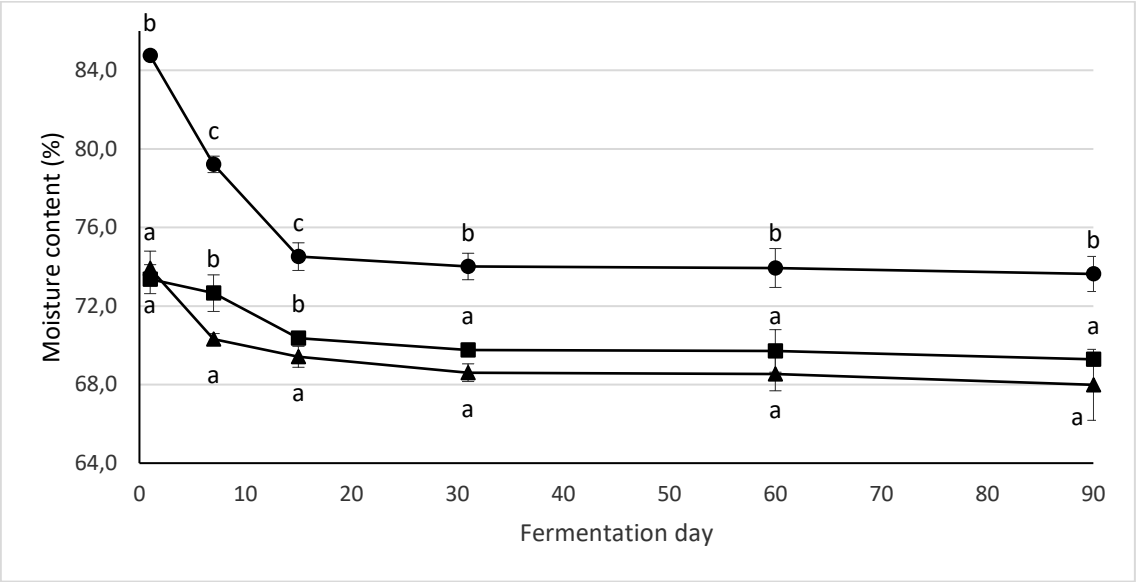

Figure 5.5 Moisture content of kocho in pits (▲), erosas (■) and jars (●) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b,c]

In Western silage processes, the moisture content of materials is an essential factor. Preferably, it is not too high to minimize the likelihood of growth of undesirable microorganisms, such as Enterobacteriaceae and clostridia. *Clostridium* spp. need a high moisture content for their development. Germination of *Clostridium* endospores is inhibited by a water activity lower than 0,95 when NaCl was used, but vegetative *Clostridium perfringens* has a minimum a_w of 0,93 (Kung, 2001; Jay et al., 2005; Weinberg, 2008; Zheng et al., 2011; FDA, 2015). The higher moisture contents in the jars compared to the pits and erosas are probably related to the process. In sauerkraut fermentations, the moisture content needs to be sufficiently high so anaerobic conditions can be created (Arthey & Dennis, 1991; Holzapfel et al., 2003).

Furthermore, the moisture in the jars could not seep through as in the pits and erosas, since the jars were completely closed. As fermentation at high moisture contents seems to proceed well in the jars, it could perhaps be possible to omit the manual or mechanical squeezing or pressing. That would mean a major optimization since the squeezing takes long and it is tedious work. The consequence of less squeezing, is that less bulla is obtained, but more carbohydrates remain in the processed mass, resulting possibly in a sharper pH drop.

5.3.1.4 Inside temperature

The recorded inside temperatures during fermentation are shown in Figure 5.6. The maximum inside temperature of the fermenting mass was observed on the first day, being 22,5 ± 0,2 °C for the pits, 22,7 ± 0,2 °C for the erosas and 19,3 ± 0,1 °C for the jars. On that day, the monitored temperature in the room was also the highest, being 16,9 ± 1,9 °C. The temperature of kocho during fermentation dropped to a final value of 16,3 ± 0,2 °C for the pits, 15,6 ± 0,3 °C for the erosas and 14,7 ± 0,1 °C for the jars on day 90. The results followed the course of the monitored maximum values of the Gamo Highlands Ethiopian Meteorological Stations at Chencha and Tegecha. The inside temperatures were thus mainly affected by the environmental temperature. An effect of the metabolic activity could not be observed.

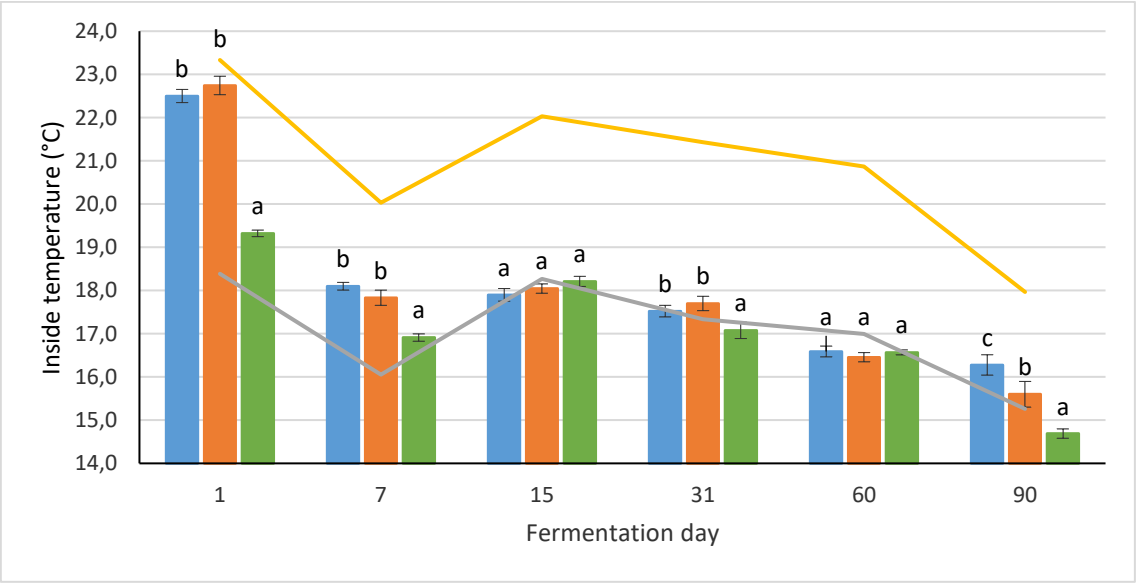

Figure 5.6 Inside temperature of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b,c]. Maximum temperatures of each day at the Gamo Highlands Ethiopian Meteorological Stations of Chencha (grey line) and Tegecha (yellow line) are presented as well.

5.3.2 Microbial dynamics

5.3.2.1 Total viable counts

Figure 5.7 shows the total aerobic viable counts of the fermenting mass in the different systems. The total viable count includes all vegetative cells, yeasts and molds, bacterial endospores, that can grow aerobically on PCA on a temperature of 30 °C. It is an estimation of the total amount of microorganisms present in kocho. On the first day, the total viable counts were 9,02 ± 0 ,06 log cfu/g for the pits, 9,21 ± 0,31 log cfu/g for the erosas and 8,93 ± 0,35 log

cfu/g for the jars. No significant differences were observed, which was expected since the starting material was the same for each fermentation system. The initial values were higher than in previous studies (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Andeta et al., 2018). The initial load of the processed enset is dependent on many factors, such as the properties of the soil, utensils and hand contact (Ashenafi & Abebe, 1996). Differences in these factors can lead to differences in the initial microbial load. The maximum total viable counts were recorded on day 7 for all the systems. They were 9,31 ± 0,01 log cfu/g for the pits, 9,43 ± 0,35 log cfu/g for the erosas and 9,48 ± 0,45 log cfu/g for the jars. On day 15, the counts were 9,28 ± 0,20 log cfu/g, 8,62 ± 0,04 log cfu/g and 8,84 ± 0,15 log cfu/g for the pits, erosas and jars respectively, which was significantly different (p-value = 0,004). However, from a microbial point of view, these differences were too small to be meaningful. A meaningful difference is only considered when the counts differ at least one log unit from each other. Taken that into account, no meaningful differences were noticed between the fermentation systems and fermentation days. The values all remained quite constant around 9 log cfu/g during the fermentation. That was in contrast with other studies where at first an increase and later a decrease in total viable counts were recorded (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Andeta et al., 2018). The increases in total viable count of each system between the first and the seventh day were negligible. The high initial values may explain why an increase not observed. In previous studies, the observed decreases in total viable counts were attributed to the lowering pH and nutrient depletion (Karssa et al., 2014; Andeta et al., 2018). However, no decreases were observed in this experiment, which might be an indication that the fermentation was not completely finished yet. Nevertheless, the low inside temperature (discussed in 5.3.1.4) can indicate low metabolic activity. In the Gamo Highlands, a pre-fermentation phase of 15 days is normally carried out. In this experiment, the kocho had been fermenting for 90 days. That should be sufficient enough to complete the fermentation, since a minimum of two months is generally required in the Gamo Highlands (Andeta et al., 2018). The effect of the pre-fermentation phase still needs to be examined.

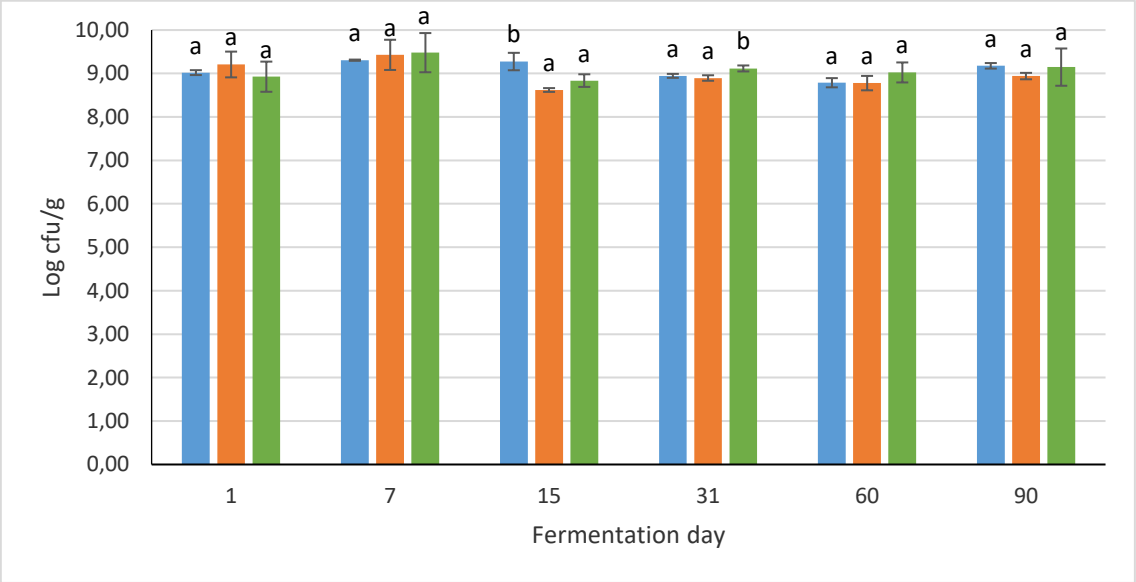

Figure 5.7 Total aerobic viable counts of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b]

Figure 5.8 presents the total anaerobic viable counts, which are an estimation of the total amount of anaerobic microorganisms present in kocho. On the first day, the values were 8,77 ± 0,02 log cfu/g for the pits, 8,94 ± 0,06 log cfu/g for the erosas and 8,89 ± 0,27 log cfu/g for the jars. Even though significant differences were observed on day 31 and 60 (p-value = 0,000; 0,040), these differences were less than one log value and thus not taken into account. Between the aerobic and anaerobic counts, no differences of more than one log value have been observed, indicating that most species were probably facultative anaerobic. The end values were almost identical to the initial values, being 8,55 ± 0,33 log cfu/g for the pits, 8,52 ± 0,16 log cfu/g for the erosas and 8,91 ± 0,20 log cfu/g for the jars.

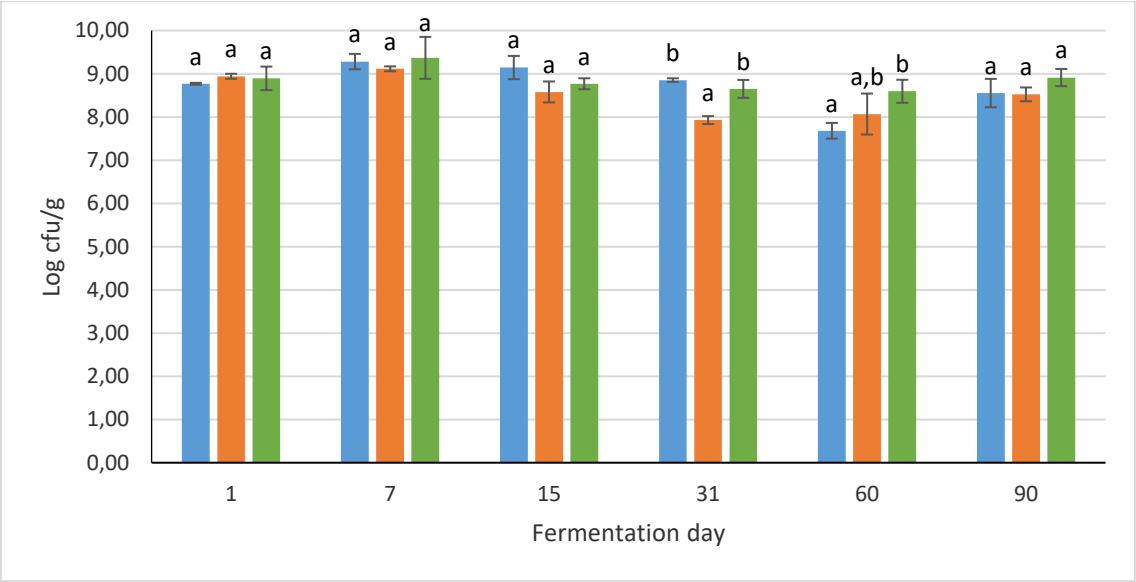

Figure 5.8 Total anaerobic viable counts of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b]

5.3.2.2 Lactic acid bacteria

LAB were present in high numbers during fermentation time, as shown in Figure 5.9. None of the differences exceeded one log value, so no meaningful differences were observed. The counts of LAB were comparable to these of total aerobic viable counts, indicating the high dominance of LAB. On the first day, the counts were 8,73 ± 0,05 log cfu/g for the pits, 8,86 ± 0,08 log cfu/g for the erosas and 8,68 ± 0,19 log cfu/g for the jars. On day 7, little increments of LAB for all the fermentation systems were noticed. The counts increased to the maximum values, being 9,26 ± 0,28 log cfu/g for the pits, 9,04 ± 0,03 log cfu/g for the erosas and 9,35 ± 0,46 log cfu/g for the jars. Thereafter, little increments and decrements were noticed, but no cohesive pattern was observed. The end values on day 90 were very similar to the starting values, namely 8,98 ± 0,05 log cfu/g for the pits, 8,47 ± 0,03 log cfu/g for the erosas and 8,94 ± 0,17 log cfu/g for the jars. High counts of LAB at the end of the fermentation of enset have been observed earlier. In the study of Bosha et al. (2016), LAB were present at a level of more than 8 log cfu/g after 90 days, Hunduma and Ashenafi (2011a) reported around 8 log cfu/g after more than 100 days and Andeta et al. (2018) more than 7,5 log cfu/g after 60 days. Only Karssa et al. (2014) noticed end values of 4,0 log cfu/g after 31 days of fermentation.

The high counts indicate that LAB were the predominant species during fermentation. Towards the end of the fermentation, both declining (Karssa et al., 2014; Andeta et al., 2018) and increasing (Hunduma & Ashenafi, 2011a; Bosha et al., 2016) counts have been reported. LAB are held responsible for pH reduction, mainly by producing lactic acid. LAB can grow down to pH 3 (Wareing et al., 2011). As discussed in 2.2, LAB can inhibit the growth of other micro-organisms.

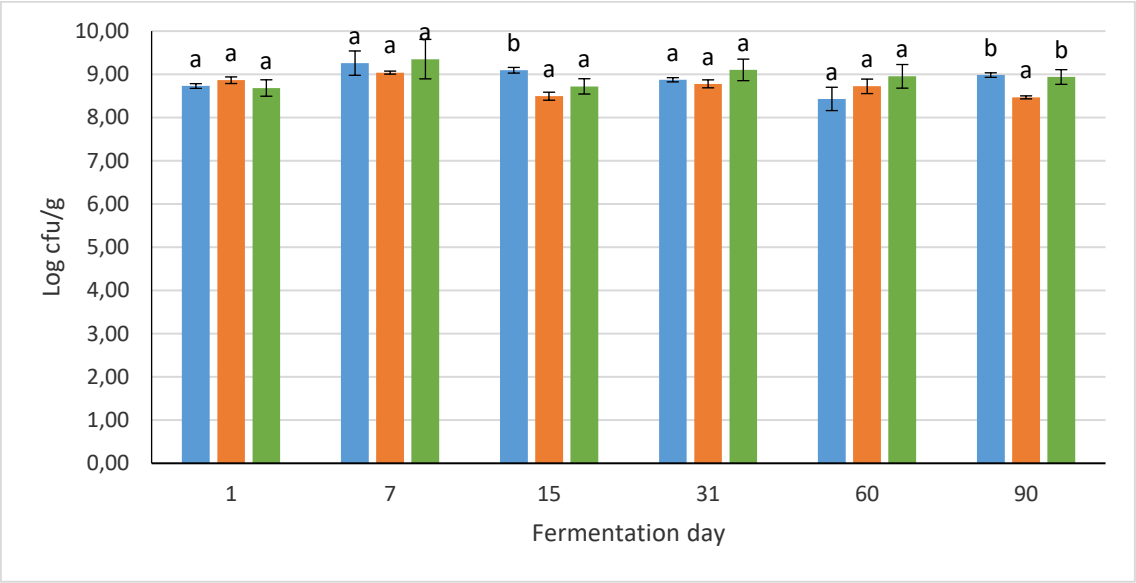

Figure 5.9 Lactic acid bacteria counts of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b]

5.3.2.3 Yeasts and molds

Figure 5.10 shows the counts of yeasts and molds of each fermentation system in function of fermentation time. The initial counts were 5,44 ± 0,13 log cfu/g, 5,83 ± 0,22 log cfu/g and 5,45 ± 0,89 log cfu/g for the pits, erosas and jars, respectively. A declining pattern was observed for each system. The end values on day 90 were 3,40 ± 0,23 log cfu/g for the pits, 3,60 ± 0,23 log cfu/g for the erosas and 3,79 ± 0,15 log cfu/g for the jars. Previous studies also indicated that the end values of yeasts and molds were lower than the initial values (Gashe, 1987a; Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Bosha et al., 2016; Andeta et al., 2018).

There is no consensus yet over the function of yeasts in the fermentation of kocho. Many yeasts species are considered as spoilage organisms (Jay et al., 2005), but Karssa et al. (2014) and Andeta et al. (2018) stated that yeasts may be responsible for breaking down starch into simple sugars. After hydrolysis, LAB can convert these simple sugars into organic acids. Karssa et al. (2014) already isolated starch hydrolyzing yeasts genera from kocho, especially deriving from the pre-fermentation phase. That hypothesis was already discussed for cassava fermentation (Oyewole, 2001), but Lacerda et al. (2005) did not identify any yeasts species able to degrade starch during cassava fermentation.

Mold growth is especially unwanted, since they have the ability to produce mycotoxins. The toxic effects of mycotoxins are divers and dependent on the mycotoxin itself and the host. Mycotoxins can cause for instance cancer, vomiting, liver diseases to both animals and

humans (Bräse et al., 2013; Landbouwcentrum voor voedergewassen, 2015). Gashe (1987b) already identified aflatoxins B_1 and G_1 up to a concentration of 40 mg aflatoxins per kg kocho. Mycotoxins are known as heat resistant (Bullerman & Bianchini, 2007), so they will probably not be inactivated by the baking process. Since mycotoxins are secondary products, the growth of molds is not directly related to the formation of mycotoxins. Still, the prevention and reduction of the amount of molds are important measures (Makkink, 2004; Bräse et al., 2013). Molds and yeasts can tolerate lower a_w-values than bacteria. If the water activity gets lower then 0,60, no growth is possible (Weinberg, 2008; Ergun et al., 2010). Generally, they also can tolerate more acidic conditions than bacteria. Since most molds are strict aerobes, the development of anaerobic conditions can inhibit the growth of molds (Weinberg, 2008). Both yeasts and molds were counted together, so no distinction could be made between them.

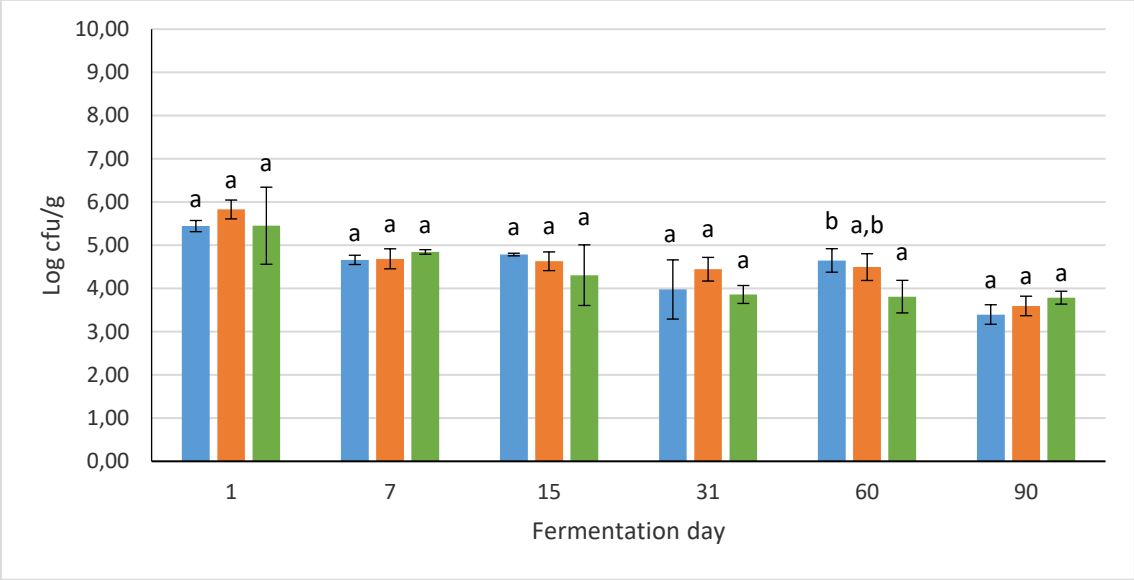

Figure 5.10 Yeasts and molds counts of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b]

5.3.2.4 Enterobacteriaceae

Enterobacteriaceae are a big family wherein different pathogens, such as *Salmonella* spp. and *Escherichia coli*, belong to. Many Enterobacteriaceae are present in the normal intestinal flora of humans and animals. In the food industry, they are used as indicator organisms for the detection of (fecal) contamination (Commission Regulation (EC) No 2073/2005; Jay et al., 2005). Their presence in kocho is therefore undesirable. Enterobacteriaceae counts were the highest in the beginning of the fermentation, namely 6,92 ± 0,10 log cfu/g for the pits, 7,98 ± 0,51 log cfu/g for the erosas and 6,73 ± 0,05 log cfu/g for the jars. Andeta et al. (2018) also observed high starting counts of Enterobacteriaceae, ranging between 7,91 log cfu/g and 8,09 log cfu/g. The Enterobacteriaceae originated most likely from the epiphytic microflora (which proved to be the case for silage (Pahlow et al., 2003)) and from fecal contamination since most Enterobacteriaceae are adapted to the gastrointestinal tract of humans and animals (Jay et al., 2005). Enterobacteriaceae could have attributed to the initial pH decrease. After the seventh day, the counts decreased below the detectable level (< 1,00 log cfu/g) for pits and jars and after fifteen days for erosas. The reduction in pH over fermentation time and possibly

antimicrobial substances, such as bacteriocins, produced by LAB created an unfavorable environment for Enterobacteriaceae. Different authors (Hunduma & Ashenafi, 2011a; Karssa et al., 2014; Andeta et al., 2018) reported a similar pattern and did not observe Enterobacteriaceae at the end of the fermentation, only Bosha et al. (2016) reported coliform bacteria (< 3 log cfu/g) after 90 days of fermentation. *Salmonella* spp. have an optimum pH for growth of 7,0 to 7,5 and the minimum pH is 4,2 (FDA, 2015). That pH value was just not reached after 90 days (discussed in 5.3.1.1), so if *Salmonella* was present, it could still survive. Even though the Enterobacteriaceae were under the detection level from day 7 or 15, still some caution is advised because there is no guaranty that kocho is free from pathogens, as stated by Andeta et al. (2018).

5.3.2.5 Clostridium endospores

Figure 5.11 shows the counts of *Clostridium* endospores. *Clostridium* is a genus of anaerobic spore formers. They are widely distributed in nature. In the genus *Clostridium*, many pathogens are present, such as the toxin producers *Clostridium botulinum* and *C. perfringens* (Gibbs, 2002; Jay et al., 2005). Their presence in kocho is obviously undesirable. High counts of *Clostridium* spores were observed during the whole fermentation time. The starting values were 3,28 ± 0,08 log cfu/g for the pits, 3,77 ± 0,09 log cfu/g for the erosas and 3,29 ± 0,04 log cfu/g for the jars. The counts for the pits and erosas increased until day 31, ending at maximum values of 4,66 ± 0,16 log cfu/g and 4,55 ± 0,02 log cfu/g for the pits and erosas, respectively. Between day 15 and 60 of the fermentation, a pH increase was noticed for the kocho in the pits and erosas (discussed in 5.3.1.1). It is possible that due to the increase in pH, the environment became more conducive for *Clostridium*. Thereby, their amount could have increased, and thus more spores could have been identified. On day 15 and 31, the counts for the jars were significantly lower (p-value = 0,001 and 0,000 respectively) than for the other fermentation systems. Furthermore, the counts of *Clostridium* spores were consistently lower for the jars than for the other fermentation systems. On day 60, the counts for pits and erosas decreased to similar values for all fermentation systems, being 3,13 ± 0,44 log cfu/g for the pits, 3,37 ± 0,20 log cfu/g for the erosas and 2,99 ± 0,04 log cfu/g for the jars. On day 90, the counts of *Clostridium* spores remained quite constant compared to day 60. Even though the counts at day 90 were similar, the lower counts in the jars during fermentation should be considered as an advantage since in case of food shortage, kocho that is not fully fermented, for instance after 30 days of fermentation, is consumed (Ashenafi, 2006; Olango et al., 2014).

The high counts are evoking questions on food safety. As stated before, the genus *Clostridium* contains different pathogens. Gashe (1987a) and Andeta et al. (2018) already reported high abundance of anaerobic *Clostridium* spore counts. Since kocho is baked before consumption, *Clostridium* spores may survive the baking process. It has been proven that the spores of *Bacillus cereus* can survive the heat treatment (Nigatu & Gashe, 1998). If the spores germinate post baking, problems related to food safety may arise since there is no additional treatment. *Clostridium* spores are unharmful, but when they germinate and thus become vegetative cells, they can cause problems (Gibbs, 2002; Jay et al., 2005). Furthermore, saccharolytic clostridia, such as *Clostridium tyrobutyricum*, one of the important species in silage fermentation, could be present, as explained in 5.3.1.1.

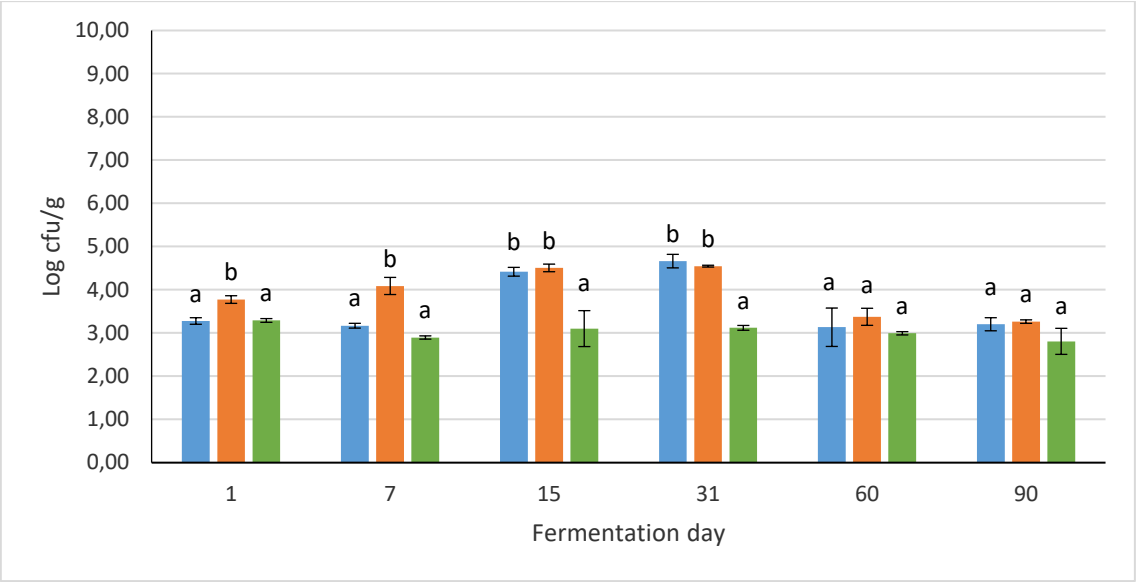

Figure 5.11 *Clostridium* spore counts of kocho in pits (■), erosas (■) and jars (■) in function of fermentation time. For values on the same sampling day, significant differences (p-value < 0,05) received different suffices[a,b]

5.3.3 Financial and practical aspects

The implementation of sauerkraut jars could be financially heavy for a lot of families. Locally made jars (Figure 5.12) costs around 5 euro, while the monthly income is generally around 50 euros (converted from Ethiopian birr). That means that around 10% of the monthly wage needs to be invested to buy one jar. The used sauerkraut jars costs 40 euros, so import is impossible. A family owns two to three pits or erosas in general, so at least two sauerkraut jars are needed for one family (written communication with Addisu Fekadu Andeta on 21-04-2018). Since pits and erosas are not expensive, the advantages of the sauerkraut jars need to be big enough to even consider the investment. Because enset is mainly processed in group (discussed in 4.2), the establishment of a co-operative could be a possible solution to overcome the financial impact.

Figure 5.12 Locally produced sauerkraut jars (own photograph)

Besides that, the feasibility of the locally made jars still needs to be examined. That could not be carried out yet because the locally produced jars were not finished yet when the research for this mater thesis was performed. The glazed layer is the major difference between the imported sauerkraut jars used in this experiment and the locally made jars. The unglazed locally made jars will probably absorb more moisture and odor. Furthermore, if the fermentation proceeds wrong, the unwanted bacteria could probably stay easier in the pores of the unglazed jars.

5.3.4 Conclusion

The results showed that a sauerkraut jar is a good, alternative system for enset fermentation. The jars were the best in terms of pH reduction, however the rate was slower compared to the pits and erosas. There, a pH increase was noticed. Saccharolytic clostridia, such as *Clostridium tyrobutyricum*, might be involved. The moisture content showed a declining pattern. In the jars, it was consistently and significantly higher than in the other systems. The pits and erosas were not completely closed and to some extent permeable for water, while evaporation in the jars was improbable due to the glazed material and water lock. The higher moisture content in the jars did not seem to cause any problems. That may create opportunities to omit the manual or mechanical squeezing in the future. The total viable counts were around 9 log cfu/g during the whole fermentation in all systems. LAB were present in high numbers throughout the fermentation, indicating their dominant role. They are held responsible for reducing the pH. The counts of yeasts and molds started above 5 log cfu/g, but decreased under 4 log cfu/g. Yeasts may break down starch, providing sugars for LAB. At the start of the fermentation, high counts of Enterobacteriaceae were observed, but they decreased below the detectable level after 15 days. *Clostridium* endospores were present in high amounts, bringing up questions about food safety. The counts for the jars were consistently lower than for the other systems, which indicates that the jars provide a safer alternative even in case of consumption of not fully fermented kocho. Further research should clear out if that is also the case in the locally produced unglazed jars. The financial impact also needs to be examined.

6 SENSORY ANALYSIS

6.1 Aim

In addition to the characterization of the microbial dynamics in the three different fermentation systems (discussed in 5), it is useful to perform sensory analysis on the unbaked and baked kocho. The locals of Dorze will only accept the use of jars if the sensory properties of kocho will stay the same or will improve. Furthermore, no research has been performed yet whether there are correlations between the sensory characteristics of the unbaked and baked kocho. That is an important question since local women first touch, smell and taste the unbaked kocho on the local market and then negotiate the price. The generally poor and varying sensory quality makes that kocho is sold for a lower price compared to other crops in Ethiopia (Brandt et al., 1997; Ashenafi, 2006). The introduction of sauerkraut jars may lead to a more controlled, standardized process with less variation in the fermentation and thus in sensory quality.

6.2 Material and methods

6.2.1 Trial design

Sensory analysis of kocho obtained by the three different fermentation systems (pit, erosa and sauerkraut jar) was conducted with a 10 cm line scale. Different characteristics of kocho were evaluated by a consumer panel. Fifty persons were selected to perform the sensory evaluation, 23 being women and 27 men. The ages were ranking between 14 and 70 years old. At the college of Natural Sciences, Abaya Campus of the Arba Minch University (AMU) in Ethiopia, 28 of them were surveyed. The others (22 persons) were locals from Dorze (Southern-Ethiopia). As the locals speak the local language Gamo and a lot of the people are illiterate, two translators were needed. They explained the line scale and the questions to the locals.

6.2.2 Test validation

Different people, mainly women, were asked informal questions about (un)baked kocho on the market of Dorze before the sensory analysis was executed. Based on their answers, a provisional version of the questionnaire was made. The color, smell, texture and taste were pointed out as important features.

Two translators were hired. They were informed about the aim of the sensory analysis, the procedure and the questionnaire. Thereafter, a preliminary test was conducted in Dorze to check whether the local people understood the questions and the principle of a 10 cm line scale (Brinkman, 2006; Lawless & Heymann, 2010). To test the questionnaire, kocho bread from a household fermentation was made. The locals were divided in two groups. The translators explained the questionnaire with them and let them try to work with the 10 cm line scale while tasting the kocho bread. Figure 6.1 shows some locals of Dorze with a translator during the preliminary test. Based on their remarks, the questionnaire was changed to the definitive version, which can be found in **annex B**. The final questionnaire contained questions

about details of the respondents, such as gender and age. Then there were questions about several characteristics of kocho, namely color, smell, hardness, taste and overall acceptance. First, the color was questioned before the consumer had touched or tasted the kocho. Then, the next characteristics were the smell and hardness, so the panelists did not taste the kocho yet. Finally, the taste and overall acceptance were questioned. After each sample, the panelists had the opportunity to write or say to the translators their remarks on the monsters.

Figure 6.1 Locals of Dorze with a translator during the preliminary test (own photograph)

6.2.3 Baking of kocho

From each fermentation system, kocho bread (also known as aradisame) was baked. First, the content of three pits (or three erosas or three jars, respectively) were carefully mixed. That is shown in Figure 6.2.

Figure 6.2 Mixing the content of three jars into a homogeneous mass to bake aradisame from it (own photograph)

Aradisame was prepared following the traditional procedure explained in 4.1.10. A network of long fibers was used to squeeze the kocho. Afterwards, the kocho was repeatedly cut in a series of diagonal lines with a sharp knife on a ground stone. The baking process was carried out by experienced women. Therefore, the kocho was placed in a folded enset leaf and placed on a baking plate. After a while, the enset leaf was removed so there was direct contact

between the kocho and the baking plate. The baking took between 10 and 20 minutes. No additional ingredients were added. The aradisame was first cooled down so there would be no influence of the serving temperature. Then each baked bread was divided in eight similar pieces, as can be seen in Figure 6.3.

Figure 6.3 Baked kocho (aradisame) divided in eight similar pieces ready for sensory analysis (own photograph)

6.2.4 Sensory analysis

In Dorze, a consumer panel consisting of 22 persons was questioned. More details about the consumer panel in Dorze is given in 6.3.1.1. Two locals could be surveyed at the same moment since two translators were available. An interrogation lasted maximum 45 minutes, so the panelist could stay focused. Between the two translators, a cloth was hung so the panelists could not see and thus influence each other (Brinkman, 2006). Through a hatch of an open window (one at each side of the cloth), an external person gave one sample to the translator. When the panelist finished judging the kocho, the remainders were given back to the same place and the next sample was transferred. Between each tasting, the panelists took a little water. Figure 6.4 shows a translator who questions a local person with the cloth behind them. The open window from where the samples were transferred, is also visible.

Figure 6.4 A translator questions a local person during the sensory analysis performed in Dorze; a cloth was hang up between the two translators (own photograph)

The samples, namely unbaked kocho (referred to as UP deriving from the pits, UE from erosas or UJ from jars, see abbreviations) and aradisame (referred to as BP, BE or BJ depending on the fermentation system, see abbreviations), were stored in a local building. When a new consumer was surveyed, the translator was given a questionnaire with a different order of samples, so that the panelists got a random sequence of samples (Brinkman, 2006; Lawless & Heymann, 2010). In the middle of the sensory analysis, which took a few hours during which the structure of the unbaked and baked kocho started to alter, new unbaked kocho was taken from each fermentation system and mixed in order to have fresh unbaked samples and to bake new aradisame.

The next day, sensory analysis was performed in Arba Minch. First, new aradisame from each system was baked in Dorze. After cooling it down, the aradisame was packed in plastic and the unbaked kocho was packed in three boxes. Both were transported to AMU. The sensory test was conducted at the college of Natural Sciences, Abaya campus of AMU. A panel of 28 persons participated. More information about the panel in Arba Minch is discussed in 6.3.1.2. The participants were students or staff at AMU. The questionnaires were prepared in English as it is the official language at the universities in Ethiopia. Before the start of the sensory analysis, an explanation and translation of the questions were given in Amharic. No additional information about the aim of the experiment was mentioned. Two translators were present in case someone needed a translation to Gamo. All the panelists answered their questionnaire simultaneously. No talking was allowed during the test, unless a translation or explanation was needed. The consumers were randomly divided into three groups. Each group was given a different order of samples. Each questionnaire received a random three-digit code to define which order of samples should be been given to the consumer (Brinkman, 2006; Lawless & Heymann, 2010).

6.2.5 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 22. To check whether the two panels scored the characteristics of kocho differently, the t-test was used. The non-parametric Kolmogorov-Smirnov test and the Levene's test were first performed to test the normality and homoscedasticity, respectively. When at least one of the conditions (normality and/or homoscedasticity) was not met, the non-parametric Mann-Whitney test was used. A significance level of 5% was considered. The following null hypothesis (H_0) and alternative hypothesis (H_1) were tested:

- H_0 = the two panels gave the same scores to the kocho for color, smell, hardness, taste and overall acceptance, respectively
- H_1 = the two panels gave different scores to the kocho for color, smell, hardness, taste and overall acceptance, respectively

A significance level of 5% means that there is a 5% chance to reject H_0 incorrectly. That is referred as type I error. Since 30 similar tests were performed (15 on unbaked kocho and 15 on baked kocho), there is a chance to reject H_0 incorrectly in 1,5 tests. Two significant differences between the panels were observed (discussed in 6.3.3). Due to the chance on a type I error, the observed differences between the two panels were not taken into account for determining differences between the fermentation systems. That means that the scores obtained in Dorze and in AMU were added together for each questioned characteristic.

Subsequently, to reveal significant differences in the questioned features between the three fermentation systems, homoscedasticity and normality were first tested with the Levene's test and the non-parametric Kolmogorov-Smirnov test, respectively. If both conditions (homoscedasticity and normality) were met, One-way ANOVA was performed to reveal significant differences of the questioned features between the three fermentation systems. Thereafter, the post hoc Tukey honestly significant different (HSD) was used. In case at least one condition was not met, the non-parametric Kruskal-Wallis test was performed. Since Kruskal-Wallis does not have a post hoc, the independent samples Kruskal-Wallis was used, followed by pairwise comparison to identify these differences. A significance level of 5% was considered.

To correlate the scores of the unbaked kocho with the scores of the baked kocho, a linear trend line was plotted using Excel 2016 and the correlation coefficient R^2 of the linear trend line was calculated as well.

6.3 Results and discussion

6.3.1 Consumer panel

In total, 50 consumers participated in the sensory analysis. Consumer panels normally need to consist of minimum 100 persons, but that is often not reached due to budgetary and practical reasons (Brinkman, 2006).

6.3.1.1 Panel in Dorze

In Dorze, 22 persons were questioned of which five men and 17 women, with ages between 14 and 70. The average age was 33 ± 18. All the women declared to have experience with processing enset, but only one of the five men stated he had experience. That is in agreement with the discussion in 4.2. Of the 17 women, seven had at least 35 years of experience. One woman of 19 years old stated that she eats kocho at least twelve times a month, five other persons at least 15 times a month and one person 18 times a month. All the others, thus 15 persons, consume kocho on a daily basis. That shows the importance of enset in Dorze.

6.3.1.2 Panel in Arba Minch

28 persons participated at AMU, six being women and 22 men. The minimum age was 22 and the maximum 45. The average age was 30 ± 7,2. Only nine of the 28 persons (of which five women) stated to have experience with processing. One men of 42 years old had 17 years of experience, what was the longest time of the panel. Six persons stated they generally do not consume kocho. 20 persons consume kocho maximum 13 times a month and only one man consumes kocho daily. Compared to the panel in Dorze, this panel had less experience with kocho.

6.3.2 Unbaked kocho

The scores of the different characterizations of the unbaked kocho are presented in Figure 6.5. In Dorze, the average scores of the color for the unbaked kocho were 7,4 ± 1,9 for the pits, 3,8 ± 1,3 for the erosas and 1,9 ± 1,4 for the jars. In Arba Minch, the average scores of the color were 7,0 ± 1,9 for UP, 3,9 ± 2,3 for UE and 3,1 ± 2,2 for UJ. A score of 10 means it was

considered much too dark and a score of 0 much too light. The scores for color did not differ significantly between the panel in Dorze and in Arba Minch. Between the fermentation systems, the scores of the color from the jars were significantly lower than these of the pits and erosas (p-value = 0,000; 0,046). It was not expected that the color of UJ would be scored too light since the whitest kocho is normally considered as the best quality (Smeds, 1955; Steinkraus, 1996; Daba & Shigeta, 2016). Six persons remarked that the color of the kocho deriving from pits needed improvement and only one person for the kocho coming from the erosas. Since no one commented on the color of the kocho from the jars, a hypothesis for the low scores could be a wrong interpretation of the translation. As the inhabitants of Dorze are used to the fermentation in pits or erosas, and thus to a darker color, it is plausible they compared the color of the kocho from the jars to the kocho they generally buy and bake, making the scores for UJ very low. Since the question about the color was the first question and the following questions had a different scale, it is plausible that only the question of the color was interpreted wrong.

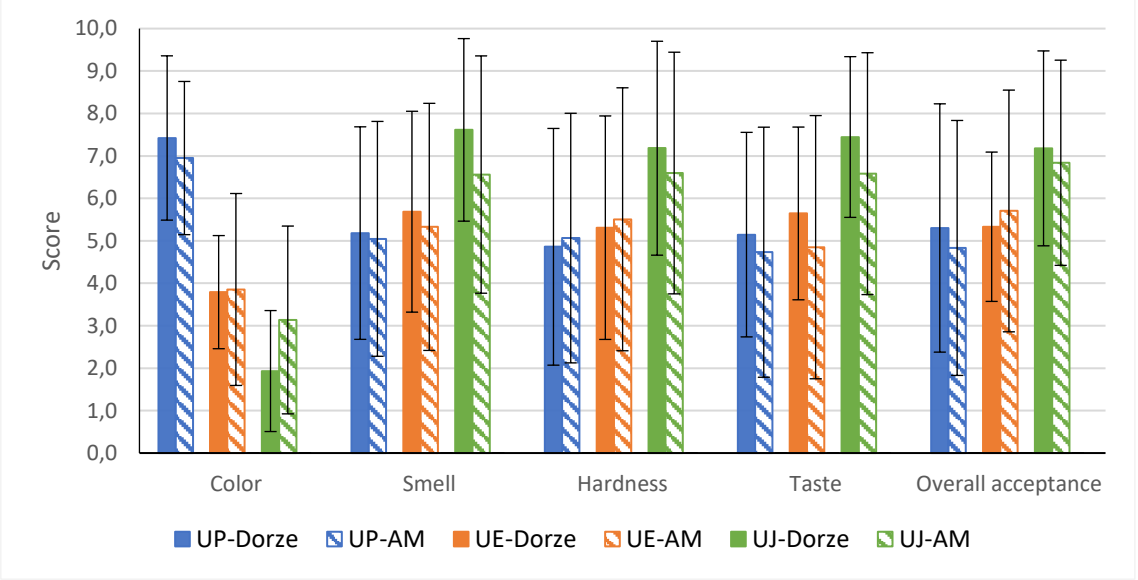

Figure 6.5 Results of the sensory analysis with standard errors (for color: 0 = much too light, 10 = much too dark, for the other characteristics: 0 = disliked very much, 10 = liked very much) performed on unbaked kocho made in pits (UP), erosas (UE) and jars (UJ). The bars with solid fill represents the results from the panel in Dorze, and the bars with lines from the panel in Arba Minch (AM).

For the other characteristics, no significant differences between the panels were observed as well. So from here on, only the total scores (so the scores from the panel in Dorze and Arba Minch together) will be discussed. The higher the scores for smell, hardness, taste and overall acceptance, the better the questioned features were assessed. In all cases, the unbaked kocho from the jars received the highest average scores. The scores for the smell were 5,1 ± 2,6 for UP, 5,5 ± 2,7 for UE and 7,0 ± 2,6 for UJ. The score of UJ was significantly higher than these of UP and UE (p-value = 0,000; 0,005). That pattern was identical for the hardness. The panels gave the hardness a score of 5,0 ± 2,8; 5,4 ± 2,9 and 6,9 ± 2,7 for UP, UE and UJ, respectively. Again, the score for UJ was significantly higher than these of UP and UE (p-value = 0,002; 0,023). Considering the taste, UP was given a score of 4,9 ± 2,7; UE 5;2 ± 2,7 and

UJ 7,0 ± 2,5. The scores for UP and UE were significantly lower than the score for UJ (p-value = 0,000; 0,002). Finally, the scores for the overall acceptance were 5,0 ± 2,9 for UP, 5,5 ± 2,4 for UE and 7,0 ± 2,3 for UJ. Here again, the score for UJ was significantly the highest compared to the scores for UP and UE (p-value = 0,001; 0,006).

The high scores of UJ for smell, hardness, taste and overall acceptance indicate that the unbaked kocho from the jars was rated as the best compared to the kocho from the pits and erosas. Only the color was scored too light, but as discussed, a wrong interpretation of the translation could be the cause for the low scores.

The panelists got the opportunity to tell the translators or write down some remarks on each given sample. Two persons of the panel in Dorze made a remark that the UP still needed more fermentation. UE and UJ got the same remarks, each from seven or eight persons, respectively. The high counts of total viable count and LAB (discussed in 5.3.2.1 and 5.3.2.2) might be an indication that the fermentation was not completely finished yet. The panel in Arba Minch did not make any remarks about the fermentation time. That once again shows that the panel in Dorze was more experienced and familiar with kocho fermentation than the panel in Arba Minch.

6.3.3 Baked kocho

The results of the sensory analysis performed on baked kocho are shown in Figure 6.6.

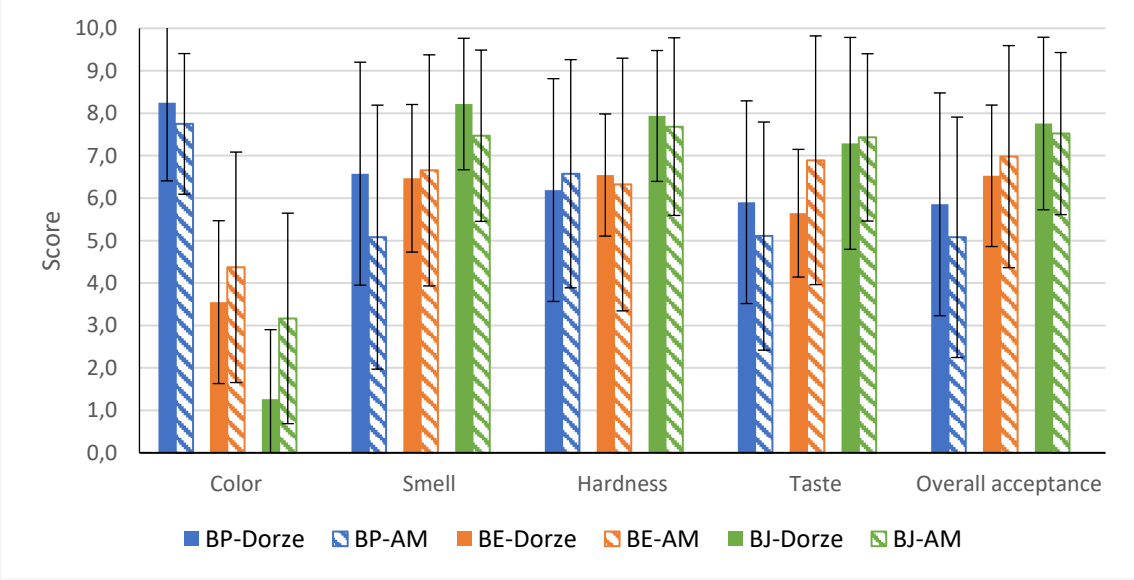

Figure 6.6 Results of the sensory analysis standard errors (for color: 0 = much too light, 10 = much too dark, for the other characteristics: 0 = disliked very much, 10 = liked very much) performed on baked kocho (aradisame) made in pits (BP), erosas (BE) and jars (BJ). The bars with solid fill represents the results from the panel in Dorze, and the bars with lines from the panel in Arba Minch (AM).

There were significant differences between the two panels for the color of BJ (p-value = 0,006) and for the taste of BE (p-value = 0,025). The panel in Dorze scored the color of BJ 1,3 ± 1,6 while the panel in Arba Minch rated it 3,2 ± 2,5. Considering the taste of BE, the panel in Dorze

scored it 5,5 ± 1,5 and the panel in Arba Minch 6,9 ± 2,9. As explained in 6.2.5, these differences between the two panels were not taken in account to determine differences in fermentation system. So from here on, only the scores from the panel in Dorze and Arba Minch together will be mentioned.

For the color, a score of 10 means that the aradisame was rated much too light, 5 just about right and 10 much too dark. The color of BJ was scored significantly lighter than BP and BE (p-value = 0,000; 0,025). The scores for the color were 8,3 ± 1,8 for BP; 3,6 ± 1,9 for BE and 1,3 ± 1,6 for BJ. In a previous sensory analysis, 25 panelists appreciated the color of aradisame more when prepared with kocho from a fermentation of 30 days compared to kocho from a fermentation of 90 days when the color was darker (Bosha et al., 2016). Furthermore, as stated before, the color of kocho is preferably light (Smeds, 1955; Steinkraus, 1996; Daba & Shigeta, 2016). As discussed in 6.3.2, a wrong interpretation of the translation could have caused these low scores. The hypothesis of the wrong interpretation is supported again by the fact that five persons in Dorze and two persons in Arba Minch commented that the color of BP needed improvement, but no one commented on the color of BE and BJ.

For the other questioned characteristics, a score of 10 means that that feature was liked very much. The mean scores of BJ were always the highest. The panels scored the smell of aradisame 5,7 ± 3,0 for BP; 6,6 ± 2,3 for BE and 7,8 ± 1,8 for BJ. Between the scores of BP and BE, no significant differences were observed, but BJ was scored significantly higher than BP and BE (p-value = 0,000; 0,019). The scores for the hardness were 6,4 ± 2,6 for BP; 6,4 ± 2,4 for BE and 7,8 ± 1,9 for BJ. Again, the hardness of BJ was scored significantly higher than these of BP and BE (p-value = 0,013; 0,006). The panels gave a score of 5,5 ± 2,6 for BP; 6,3 ± 2,5 for BE and 7,4 ± 2,2 for BJ regarding the taste. The taste of BJ was scored significantly higher than the taste of BP (p-value = 0,000), but not than BE. There was no significant difference between the scores of BP and BE. Considering the overall acceptance, the scores were 5,4 ± 2,7; 6,8 ± 2,2 and 7,6 ± 1,9 for BP, BE and BJ, respectively. The scores of BP were significantly lower than the scores of BE and BJ (p-value = 0,045; 0,000). Between the scores of BE and BJ, no significant difference was observed.

No one of the 28 panelists in Arba Minch commented on the fermentation time of BP and BE, but only one person stated that BJ was not fermented enough. The panel of Dorze did not comment on the fermentation time of BP, but six persons did on BE and three on BJ. These remarks were in line with the remarks on the unbaked kocho, discussed in 6.3.2. Three of the 22 persons of Dorze remarked that BJ was more acidic than usually, one person even found it too acidic. The panel of Arba Minch did not comment on the acidity of BJ. The remarks on the acidity of BJ can be linked to the end pH of the kocho, which was the lowest for the jars, and the end TA, which was the highest for the jars compared to the other fermentation systems (discussed in 5.3.1.1 and 5.3.1.2, respectively).

6.3.4 Correlations

Women sell kocho on the local market, providing a primary source of income (Shank & Ertiro, 1996; Assefa & Fitamo, 2016). The price of kocho is rather low compared to other crops sold in Ethiopia due to the generally poor and varying sensory quality (Brandt et al., 1997; Ashenafi, 2006). Before negotiating about the price, potential buyers first touch, smell and taste the unbaked kocho. When they like the characteristics of the kocho, the price will be higher. No research hitherto has been performed whether these assumptions (such as the unbaked kocho

tastes well so the aradisame from it will also tastes good) are correct. For each questioned characteristic (color, smell, hardness, taste and overall acceptance), a linear trend line was plotted and R^2 was calculated.

Figure 6.7 shows the plotted trend line for the color. In many sensory analyses, the data do not correlate well with one another (Lawless & Heymann, 2010), but in this case, the correlation coefficient R^2 was 0,4409. So, a reasonable correlation was observed despite that the panel only consisted of 50 persons. The differences between the fermentation systems are clearly visible in Figure 6.7. The scores from the jars are centered in the bottom left corner, while these from the pits in the top right corner.

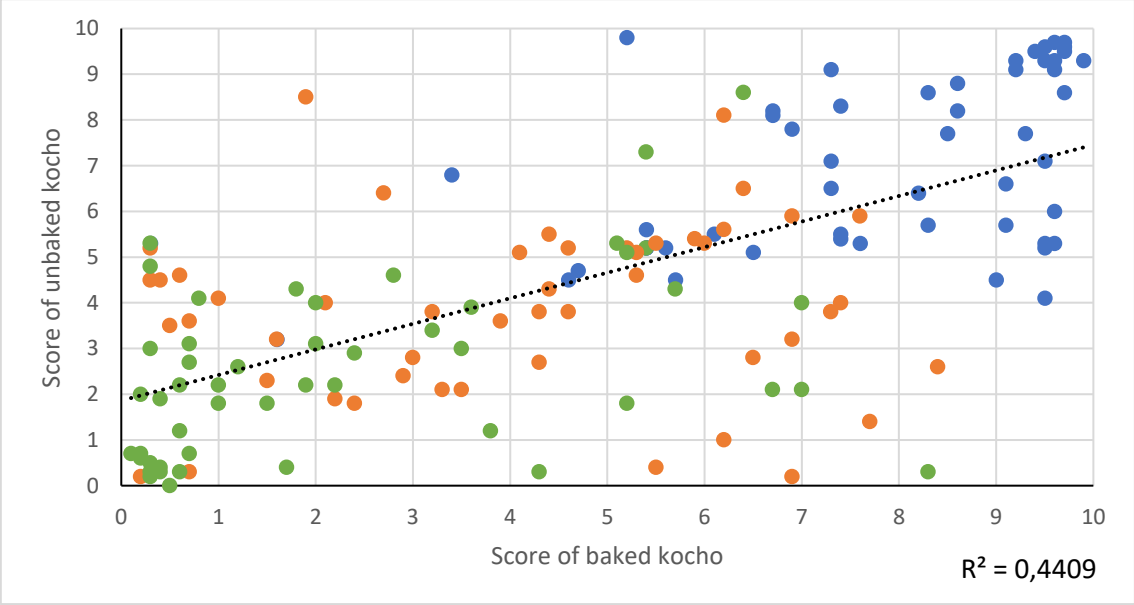

Figure 6.7 Correlation between the scores for color of the unbaked kocho and baked kocho in pits (■), erosas (■) and jars (■). Each dot represents the scores of an individual panelist. The correlation coefficient R^2 is displayed in the figure.

The plotted trend line for the overall acceptance is presented in Figure 6.8. The correlation coefficient R^2 was 0,1703. That means that only a weak, positive correlation has been noticed. As can be seen in Figure 6.8, the scores obtained from the jars are quite concentrated in the top right corner, while the scores from the pits and erosas are more scattered.

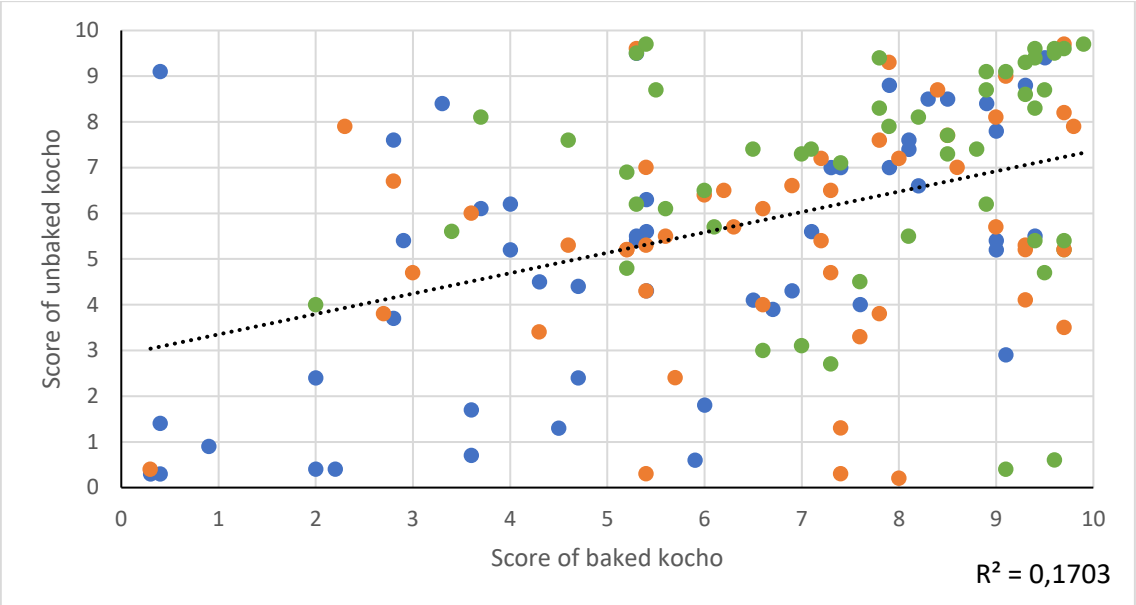

Figure 6.8 Correlation between the scores for overall acceptance of the unbaked kocho and baked kocho in pits (■), erosas (■) and jars (■). Each dot represents the scores of an individual panelist. The correlation coefficient R^2 is displayed in the figure.

For the other features, the correlation coefficients were 0,0906 for smell, 0,0907 for hardness and 0,1121 for taste. The corresponding figures can be found in **annex C**. All the correlation coefficients were positive, generally meaning that when the score for the unbaked kocho was higher, so did the score for the baked kocho. Even though the observed correlation coefficients were quite small (between 0,0906 and 0,4409), these indicate that there are positive correlations between the unbaked and baked kocho for color, smell, hardness, taste and overall acceptance.

6.3.5 Conclusion

The sensory analysis showed promising results for the introduction of sauerkraut jars. The panels gave kocho derived from the jars the highest scores for smell, hardness, taste and overall acceptance, both for unbaked and baked kocho. Only the color of kocho from the jars was considered too light. Since there were negative comments on the color of the kocho and aradisame derived from the pits and erosas, but no panelist commented on the color of the kocho from the jar, a possible hypothesis for the low scores is a wrong interpretation of the translations. Since the inhabitants of Dorze are used to fermentation in pits and erosas and thus to a darker color, they may have compared the color of kocho from the jars to the kocho they normally obtain. Small, positive correlations between the scores of the unbaked and baked kocho were observed for all questioned features. The highest correlation coefficient was identified for the color, being 0,4409. Since the fermentation of enset is spontaneous and thus variations could occur, it could be useful to repeat sensory analysis of another fermentation.

Conclusions

Enset (*Ensete ventricosum* (Welw.) Cheesman, *Musaceae*), which is fermented to kocho, is a major food security crop in Ethiopia, but different fermentation practices are in use and the quality of kocho is variable. The aim of this master thesis was to examine the impact of the different fermentation systems and to introduce the use of sauerkraut jars. The characterization of spontaneous enset fermentations in pits, erosas and sauerkraut jars in the Gamo Highlands showed there was an initial, sharp decline in pH for the three systems, but the rate in the jars was slower. After 15 days, the pH in the pits and erosas started to increase until at least day 60. Saccharolytic clostridia, such as *Clostridium tyrobutyricum*, might be involved. Possibly, interference of pieces of plant material during pH measurement may have occurred. The pH ended at 4,35 ± 0,02 for pits, 4,55 ± 0,05 for erosas and 4,29 ± 0,02 for jars after 90 days. The moisture content dropped during fermentation in all systems. In the first 12 hours, there was a major decline in moisture content in the pits and erosas. The difference between the systems might be that the pits and erosas were not completely closed and to some extent permeable for water, while evaporation in the jars was improbable due to the glazed in and outside material and the water lock. The moisture contents in the jars were continuously and significantly higher than in the other systems throughout fermentation, but that did not seem to cause a problem. In fact, in sauerkraut fermentations, the moisture content needs to be sufficiently high to create anaerobic conditions, so that may create opportunities to omit in the future the manual or mechanical squeezing prior fermentation. In all systems, the total viable counts were around 9 log cfu/g during the whole fermentation. Throughout the fermentation, lactic acid bacteria were present in high numbers, indicating their dominant role. They are held responsible for reducing the pH. The counts of yeasts and molds started above 5 log cfu/g, but decreased under 4 log cfu/g after 90 days. The role of yeasts is not clear yet. They may be responsible for breaking down starch, providing fermentable sugars for lactic acid bacteria. Enterobacteriaceae were initially present in high amounts, but all counts decreased below the detectable level after 15 days due to the lowering pH and possibly antimicrobial substances. Still, there is no guaranty that kocho is free from pathogens. *Clostridium* endospores were also present in high amounts, bringing up questions about food safety. The counts of the jars were consistently lower than in the other systems which indicates that the jars provide a safer alternative even in case of consumption of not fully fermented kocho. The sensory analysis also showed promising results for the introduction of sauerkraut jars. The kocho derived from the jars received the highest mean scores for smell, hardness, taste and overall acceptance, both for unbaked and baked kocho. Only the color from the jars was considered too light. Since there were negative remarks on the color of the kocho and aradisame derived from the pits and erosas, but not from the jars, a possible hypothesis for the low scores is a wrong interpretation of the translation. Since the locals of Dorze are used to fermentation in pits and erosas and thus to a darker color, they may have compared the color of kocho from the jars to the kocho they normally obtain. Small, positive correlations between the scores of the unbaked and baked kocho have been observed for all questioned features. Sauerkraut jars are thus a good alternative for pits and erosas. They were the best in terms of pH reduction, counts of *Clostridium* endospores and sensory properties. It seems that the fermentation of enset can be better understood and the quality of kocho can be better controlled. Therefore, this research can be a step in the right direction to make a difference for the inhabitants of Ethiopia. Still, the financial impact and the feasibility of the locally made jars without glazed layer need to be examined.

Future research needs

Spontaneous fermentations, as is the case now in the Gamo Highlands, lead to variation in quality. The development of a starter culture could be a good way to control the fermentation. The starter culture needs to be easily cultivated, to outcompete other microbes and to produce consistently high quality kocho. Possibly, the starter culture needs to consist of a mix of LAB and yeasts, but therefore, the role of yeasts needs to be clarified. A preliminary study to develop a starter culture has been performed during this research thesis.

As discussed in 5, the use of Western sauerkraut jars with glazed layer is feasible for enset fermentations. The jars made by locals from the Gamo Highlands, do not contain a glazed layer. That can influence the process of the fermentation, as stated in 5.3.3. The characterization of spontaneous enset fermentation in these locally produced jars is therefore necessary. Analogous to sauerkraut fermentation, enset fermentation could be tested with the addition of salt.

Furthermore, there was a big difference in moisture content between the pits and erosas on the one hand and the jars on the other hand, discussed in 5.3.1.3 The jars showed higher moisture contents. In sauerkraut fermentations, the moisture content needs to be sufficiently high so anaerobic conditions can be created. With that in mind, it might be possible to omit the manual or mechanical squeezing or pressing during processing of enset. That would mean a major optimization if a tedious and unstandardized process step could be skipped, but further research is needed.

High numbers of *Clostridium* endospores have been observed during the enset fermentation (discussed in 5.3.2.5). As the genus *Clostridium* contains pathogens (Gibbs, 2002; Jay et al., 2005), the high counts are raising questions about food safety. Gashe (1987a) already reported that *Clostridium* showed active growth in the fermenting mass. Identifying the *Clostridium* species present in the fermentation would be a key factor in elucidating the food safety. Furthermore, the presence of saccharolytic clostridia, such as *Clostridium tyrobutyricium*, can be investigated.

A preliminary study on the pre-fermentation phase has been performed during this thesis research. Still, further research is needed to examine the necessity of it.

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Annexes

Annex A Composition and preparation of the used media

Annex B Questionnaire of sensory analysis

Annex C Correlations between unbaked and baked kocho

Annex A Composition and preparation of the used media

Each media was immediately prepared in a Duran bottle.

Composition of dehydrated Plate Count Agar (PCA) for 1 liter medium (Biokar Diagnostics)

Component	Amount (g)
Bacteriological agar	12,0
Tryptone	5,0
Yeast extract	2,5
Glucose	1,0

The media was prepared by dissolving 20,5 g PCA powder in 1 l deionized water.

Composition of dehydrated Violet Red Bile Glucose Agar (VRBG) for 1 liter medium (Biokar Diagnostics)

Component	Amount (g)
Bacteriological agar	13,0
Glucose	10,0
Enzymatic digest of animal tissues	7,0
Sodium chloride	5,0
Yeast extract	3,0
Bile salts	1,5
Neutral red	0,03
Crystal violet	0,002

39,5 g of VRBG powder was suspended in 1 l deionized water.

Composition of dehydrated Oxytetracycline Glucose Agar (OGA) for 1 liter medium (Biokar Diagnostics)

Component	Amount (g)
Glucose	20,0
Bacteriological agar	15,0
Yeast extract	5,0

The medium was prepared by dissolving 40 g in 1,1 l deionized water. After autoclaving, it was supplemented with Oxytetracycline (10 ml per 1,1 liter OGA).

Composition of dehydrated De Man, Rogosa, Sharp Agar (MRS) for 1 liter medium (Biokar Diagnostics)

Component	Amount (g)
Glucose	20,0
Bacteriological agar	16,0
Enzymatic digest of casein	10,0
Meat extract	10,0
Sodium acetate	5,0
Yeast extract	4,0
Dipotassium phosphate	2,00
Ammonium citrate	2,00
Tween 80	1,10
Magnesium sulfate	0,20
Manganese sulfate	0,050

The medium was prepared by dissolving 70,3 g dehydrated MRS powder in 1 l deionized water.

Composition of dehydrated Reinforced Clostridial Agar (RCA) for 1 liter medium (Biokar Diagnostics)

Component	Amount (g)
Bacteriological agar	15,0
Tryptone	10,0
Meat extract	10,0
Glucose	5,0
Sodium chloride	5,0
Yeast extract	3,0
Sodium acetate	3,0
Soluble starch	1,0
Cysteine hydrochloride	0,5

52,5 g of RCA powder was suspended in 1 l of deionized water.

Annex B Questionnaire of sensory analysis

This example is only for one sample. In total, there were six samples.

1. **Details of respondent**

Gender: _____ Age:_____ Village/ Got _____

How many times a month do you eat in general kocho? _____

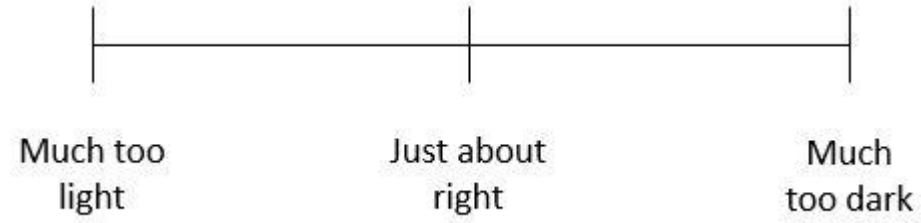
Do you process Enset? YES-NO

If yes: how many years of experience do you have on fermenting Enset? _____

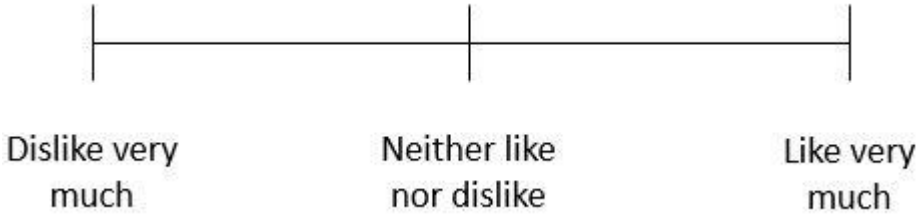
Section 1: Unbaked kocho

Sample A

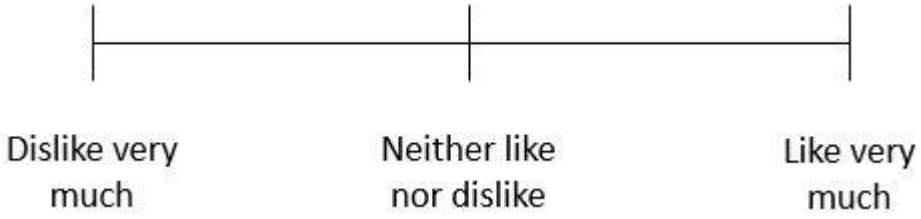
1. What do you find of the color of the sample?



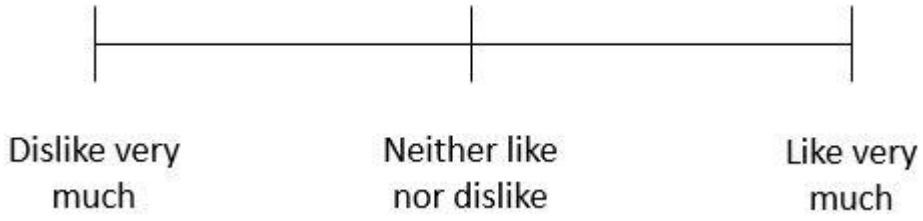
2. How much do you like the smell of the sample?



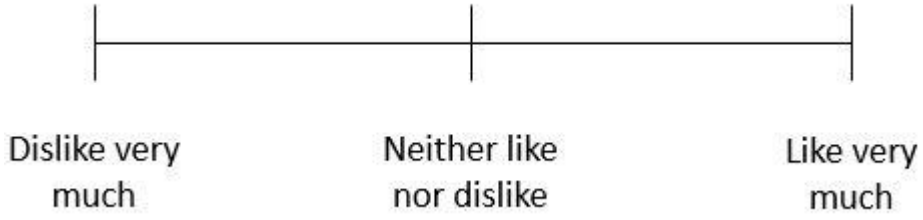
3. How much do you like the hardness of the sample?



4. How much do you like the taste of the sample?



5. How much do you like the sample overall?



6. Would you buy this sample? YES-NO
    a. If yes, how many birr would you pay for one kilo? _____
7. Do you have any remarks on the sample?

Annex C Correlations between unbaked and baked kocho

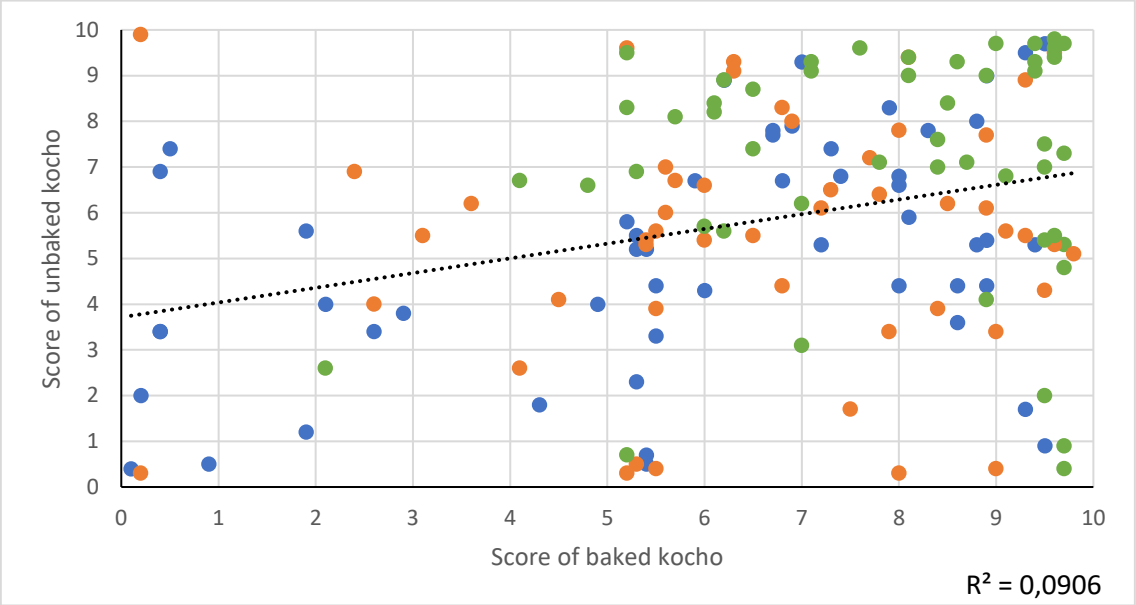


Figure 1 Correlation between the scores for smell of the unbaked kocho and baked kocho in pits (■), erosas (■) and jars (■). Each dot represents the scores of an individual panelist. The correlation coefficient R^2 is displayed in the figure.

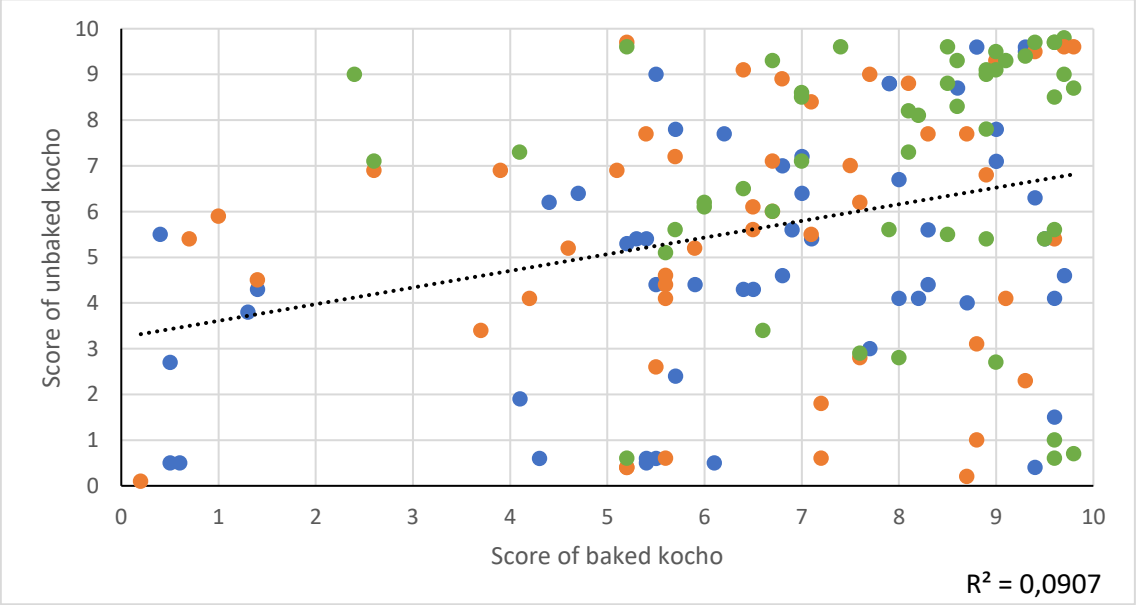


Figure 2 Correlation between the scores for hardness of the unbaked kocho and baked kocho in pits (■), erosas (■) and jars (■). Each dot represents the scores of an individual panelist. The correlation coefficient R^2 is displayed in the figure.

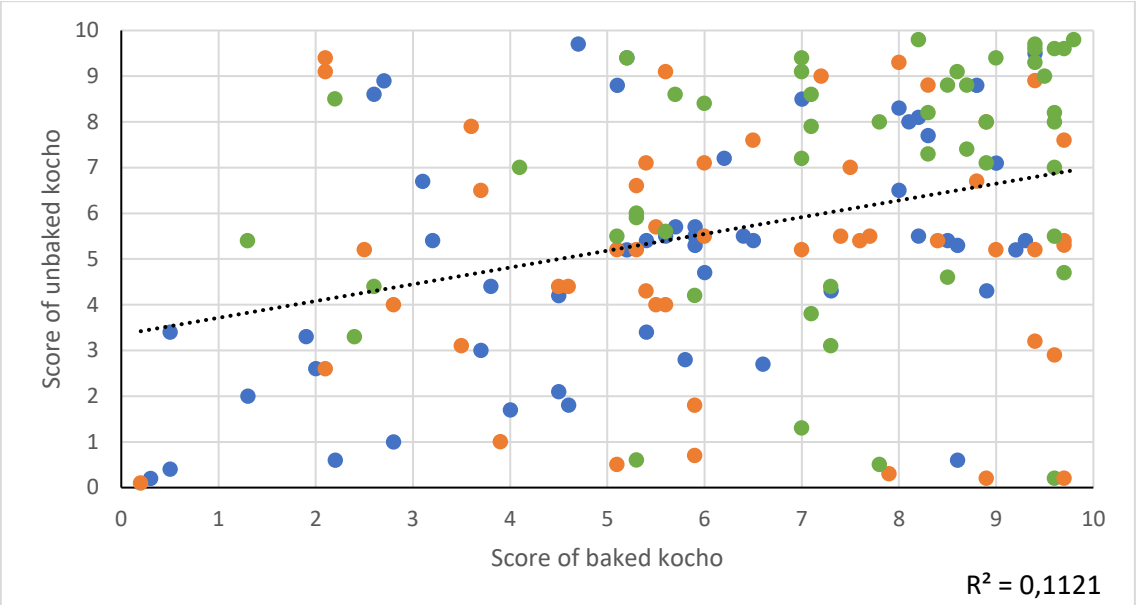


Figure 3 Correlation between the scores for taste of the unbaked kocho and baked kocho in pits (■), erosas (■) and jars (■). Each dot represents the scores of an individual panelist. The correlation coefficient R^2 is displayed in the figure.

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