

Review Article

Microbial and Physicochemical Dynamics of Kocho, Fermented Food from Enset

Dereba Workineh Seboka ¹, Abay Tabor Bejiga ¹, Debela Jufar Turunesh ²,
Andualem Arimo Turito ¹ and Abayeneh Girma ³

¹Department of Biology, College of Natural and Computational Science, Mizan-Tepi University, P.O. Box. 121, Tepi, Ethiopia

²Department of Chemistry, College of Natural and Computational Science, Mizan-Tepi University, P.O. Box. 121, Tepi, Ethiopia

³Department of Biology, College of Natural and Computational Science, Mekdela Amba University, P.O. Box. 32, Tuluawlia, Ethiopia

Correspondence should be addressed to Dereba Workineh Seboka; derebaworkineh19@gmail.com

Received 24 May 2023; Revised 16 September 2023; Accepted 11 October 2023; Published 19 October 2023

Academic Editor: Giuseppe Comi

Copyright © 2023 Dereba Workineh Seboka et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Over 20 million Ethiopians depend on enset (*Ensete ventricosum*) as a staple or costaple food. “Kocho,” “Bulla,” and “Amicho” are the three main food types obtained from enset. This review aimed to summarize the physicochemical and microbial dynamics of kocho. It is the most common food obtained from the scraped pseudostem and decorticated corm of enset after a long period of fermentation. The quality of kocho depends on the maturity of the enset plant, the enset processing method, the fermentation period, and the dynamics of microorganisms during the fermentation process. Microorganisms play a significant role in kocho fermentation to enhance its nutritional quality, improve sensory properties, and reduce spoilage and disease-causing agents. The populations of microbes available in kocho fermentation include lactic acid bacteria (LAB), Enterobacteriaceae, acetic acid bacteria (AAB), yeasts and molds, and *Clostridium* spp., which have both positive and negative impacts on kocho quality. There is a visible variation in microbial dynamics during kocho fermentation caused by the fermentation period. As the fermentation day increases, species of LAB also increase, whereas counts of Enterobacteriaceae decrease. This is due to a decrease in pH, which leads to an increase in titratable acidity. Moisture content also slightly decreases as fermentation progresses. Dynamics in the microbial population and physicochemical parameters ensure the development of desirable qualities in kocho and enhance the acceptability of the final product. Organic acids (such as lactic acid, acetic acid, and propionic acid), bacteriocins, phenolic compounds, flavonoids, and tannins are bioactive compounds produced by microorganisms during Kocho fermentation. Further research is needed on the molecular identification of microorganisms during Kocho fermentation.

1. Introduction

Enset (*Ensete ventricosum*), also referred to as a false banana, is a food security crop that provides staple or costaple food for more than 20 million people in Ethiopia [1, 2]. Enset is a monocarpic, perennial, and monocotyledonous plant that originated and was domesticated in Ethiopia [3]. Geographically distributed as a wild plant species in many parts of Sub-Saharan Africa and Asia, it is commonly cultivated as a food crop only in the Ethiopian highlands [3, 4]. Enset plants are grown in a relatively wide range of environmental

conditions and soil types [3, 5]. The plant possesses deep root systems that enable it to withstand a longer period of drought (about 5 months) than other crops [2]. The plant is accessible throughout the year and has the capability to serve a larger number of people in the future as staple and costaple foods. Recently, the government of Ethiopia started a new project on enset cultivation and adaptation to the Amhara and Tigray regions [6].

Kocho, *Bulla*, and *Amicho* are the main food products obtained from the enset plant after processing. Kocho is a fermented food obtained from the scraped pseudostem and

pulverized corm of the plant [7]. *Kocho* is consumed as a staple or costaple food in central, southern, and southwestern parts of Ethiopia [8]. It is rich in carbohydrates, minerals, phenolics, and fibers [9]. However, all food products prepared from enset are poor in protein, fat, and vitamin content [9, 10]. Enset-based food should be consumed with meat, cheese, peas, or beans to supplement proteins [2, 9, 11].

The variety of enset plants, processing approaches, and fermentation periods influence the quality of kocho [8, 12]. Microbes play a significant role in the fermentation process of traditional foods, which can contribute to improved nutritional quality and organoleptic characteristics, enhance shelf-life, and be used to increase and standardize the fermentation process [13]. The dynamics of microbes are used in the fermentation process for the metabolism of starch, protein, and lipid, which results in an overall change in its nutritional and sensory quality [14]. The proliferation of some microbes under favourable conditions compromises kocho qualities such as taste, color, texture, or aroma [7, 9, 15]. The overall change in nutritional and sensory quality of kocho has resulted from microbial profiles inside the fermenting enset mass [9, 14, 15]. LAB, Enterobacteriaceae, acetic acid bacteria, yeasts, and molds, as well as *Clostridium* spp., are some of the microbial load groups presented in fermenting kocho [7, 8].

Studies by Hunduma [16] and Elifu [17] showed LAB as a dominant microbial group in the entire enset fermentation process. This is due to their tolerance to an acidic environment [18, 19], and hence lactic acid-producing bacteria are suggested to be the potential starter culture organisms for enset fermentation [17, 19, 20]. However, reviews of related literature on physicochemical parameters and microbial dynamics of fermenting enset mass are not analyzed in depth or well organised at all. This review summarizes the microbial and physicochemical dynamics of fermenting enset mass and their roles during fermentation.

2. Enset Plant (*Ensete ventricosum*)

2.1. Brief Description of Enset Plant. Ethiopia is a homeland for many cultivated plant crops such as teff (*Eragrostis tef*), coffee (*Coffea arabica*), and enset (*Ensete ventricosum*) [21]. Different scholars have developed theories that argue for Ethiopia's origin and domestication of the enset plant before 10,000 years ago [4]. Enset, also known as *Ensete ventricosum*, is a common plant that belongs to the root and tuber group. Enset is a member of the genus *Ensete*, the family *Musaceae*, and the order *Scitamineae* [22–24]. Taxonomically, enset plants are categorized under the order *Zingiberales* of the *Musaceae* family and the genus *Ensete* [25, 26]. Morphologically, the plant reaches 4–8 m [26, 27]. It is a tall perennial herbaceous root crop that grows to a height of 4 to 11 metres [13]. It has long (5 m) and broad (0.75 to 1.5 m) spiral leaves and a bulky pseudostem that is 2 to 5 metres long. Adventitious is its root system. A corm, which is 0.70–1.8 metres in length and 1.5–2.5 metres in circumference when fully grown, makes up the plant's underground section [23, 24]. Flowers are unisexual; female flowers grow

closer to the centre of the inflorescence, while male flowers grow farther away. The fruits are fibrous, oblong-obovate, 8–15 cm × 3–4.5 cm, and become orange when fully grown (Figure 1). Enset is distributed as a wild plant species in many parts of Sub-Saharan Africa, central, and eastern Africa and Asia [4, 5, 28] and is only cultivated as a food crop in Ethiopia, mainly in the southern and southwestern parts of the country [4]. Seasonal droughts in the central and northern parts of Ethiopia lead to the expansion of enset cultivations to other parts of Ethiopia [25, 29].

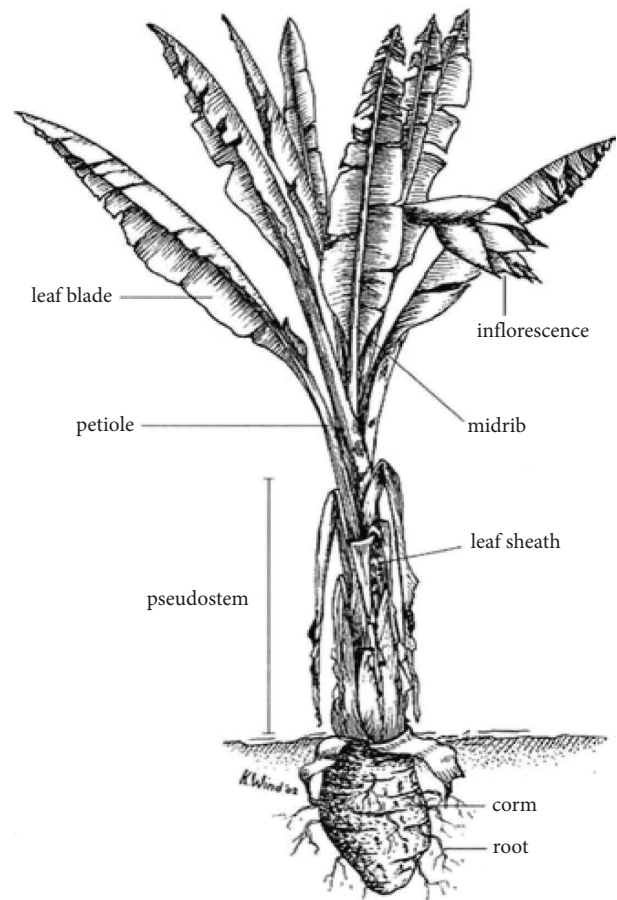
2.2. Utilization of Enset Plant in Ethiopia. In the southern, southwestern, and central parts of the Ethiopian highlands, enset is used as food for humans, fodder for animals, for other uses such as industrial fibre, rob material in fences, and house building, for mattresses and seat making, local packaging material, and a substitute for table plates [4, 6, 30, 31]. It stabilizes soils and microclimates and has valuable cultural importance [3]. Fibre is produced while the enset leaf sheath is decorticated [31, 32]. The fibre in foods also has a prominent role in lowering blood glucose and blood fat levels [9]. The vigorous pseudostem, corm, and inflorescence stalk are used to produce nutritious food for humans and fodder for animals [33]. Foods obtained from enset have divergences in preparation techniques and consumption patterns [10].

Amicho, *Bulla*, and *Kocho* are the main foods prepared from the enset plant [2]. *Amicho* is the fleshy inner portion of the enset corm, which is cooked and eaten separately and has a similar taste to potato [27]. The corm is also used as a source of kocho, and it acts as a fermenting agent or starter called *gamma* or *gamancho* (undefined and locally prepared starter culture from enset corm) [9, 12]. *Bulla* is an enset-based food obtained through the squeezing process of pulverized kocho and consumed as a staple food [30, 34]. *Bulla* food is considered the best quality enset-based food and is mainly produced from a fully matured enset plant [27]. Enset-based food products are rich in carbohydrates and minerals such as calcium and potassium, which is very crucial for consumers, but it lacks many other nutrients such as vitamins and only contain a fewer amount of fat and proteins [10].

2.3. Traditional Kocho Fermentation. Traditional kocho fermentation requires a variety of equipment and ingredients for processing as well as for accelerating its duration time. It is varied from place to place both in equipment used for processing and in ingredients required for facilitating the fermentation process [15]. The first step in the process of kocho fermentation is the collection of matured enset plants carried out by experienced women [13, 35]. After a mature enset plant has been selected, leaves are removed, cleared, and dried leaf sheaths from the plant, and the surrounding parts are prepared for the next steps [15]. The dug-up underground corm is detached and cleaned to isolate the true stem from the root. Then, the inner leaf sheaths are separated from the pseudostem down to the real stem, which is a segment between the corm and the



(a)



(b)

FIGURE 1: Enset plant (a) and its parts (b).

pseudostem [36]. The true stem is isolated from the underground corm [12]. The pseudostem and corm of the enset plant are scraped and pulverized, respectively [36].

Fermentation starts after kocho is stored in an earthen pit, which may range from three months to one year for completion [20, 35, 37]. It depends on the climatic conditions of that environment [15]. In warmer regions, fermentation is rapid and may terminate within 15 days to at least one month [38], while in the cooler regions, it is kept in a pit for years [15]. The traditional processing of enset has two phases (Figure 2): phase one (surface fermentation), the beginning of fermentation which is continued for about 15 days, and phase two (pit fermentation) [35]. Kocho fermentation methods and times are different from location to location [36].

2.4. Fermented Enset. Kocho is a traditionally fermented and indigenous starch-rich food product in Ethiopia that is prepared from enset plants [7, 36]. Next to injera and wheat bread, it is a popular fermented food consumed in Ethiopia [39]. Scraped pseudostem, pulverized corm, and decorticated enset pulp are mixed and fermented to produce carbohydrate-rich kocho [13, 35, 37]. It is widely consumed by millions of Ethiopians [2] and plays a crucial role in

ensuring food security [25]. The bread prepared from fermented enset is known as kocho bread [37]. Bosha et al. [9] reported that kocho is rich in carbohydrates. However, it has low protein content [10, 12]. Kocho production requires a long period of fermentation which is prolonged from three to six months [37, 40].

The quality of kocho food depends on the age of the harvested enset plant, accession type [25], harvesting season, and fermentation period [12]. Moreover, within one plant, the quality is influenced by the part of the leaf sheath and corm processed [27].

2.5. Physicochemical Dynamics during Enset Fermentation. The physicochemical properties investigated during kocho fermentation have great potential to enhance the quality of the final product. Moisture content, titratable acidity, pH, and fermenting temperatures (Table 1) are considered physicochemical parameters, and their dynamics during kocho fermentation play an indispensable role in determining the final quality of kocho.

2.5.1. Moisture Content. Water is essential for the growth and metabolism of fermenting microbes found in kocho dough [35]. Kocho fermented with boiled decorticated enset

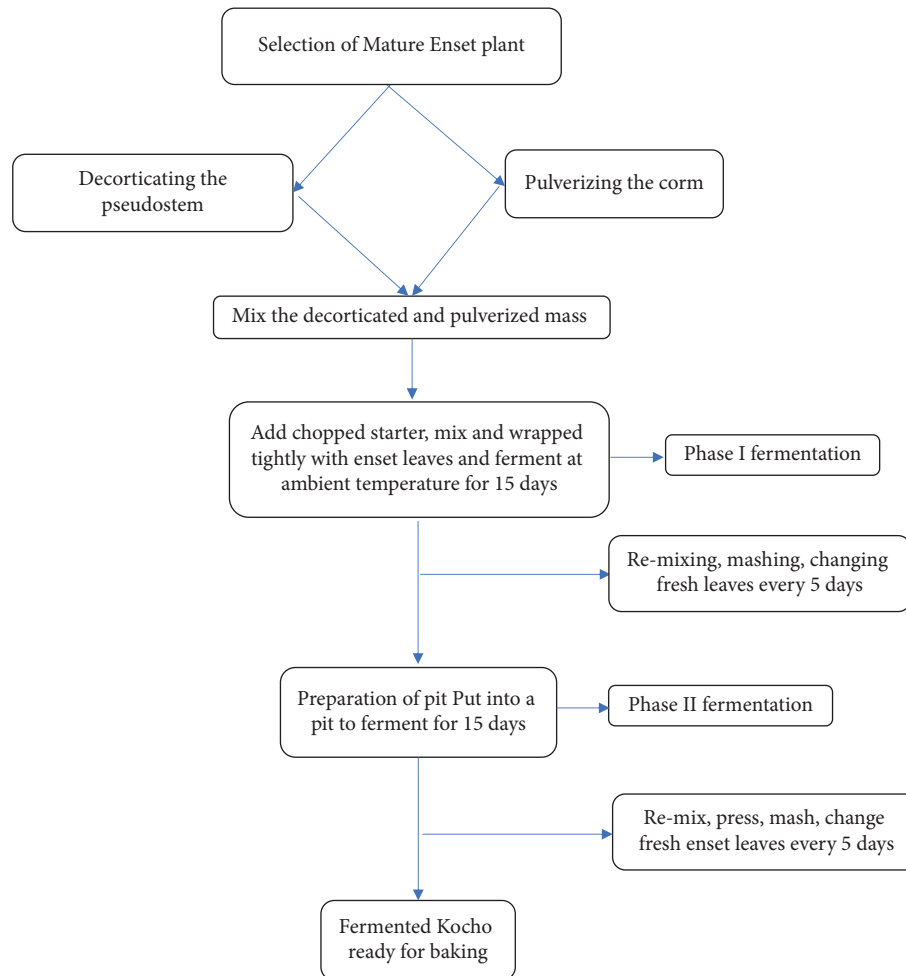


FIGURE 2: A flowchart of traditional kocho fermentation process.

pulp has a higher moisture content but is low in protein, carbohydrate, fibre, and fat content as compared with kocho fermented with nonboiled decorticated enset pulp [12]. Mohammed et al. [11] showed that enset contains a huge amount of water, which ranges between 85 and 90%. As kocho fermentation time is extended, the moisture content decreases, probably due to excessive leaching during pit fermentation and in different steps in the preparation of kocho [9, 35]. The moisture content of kocho generally declined as the time of fermentation increased [8, 13]. These are the characteristics of fermenting microbes that lead to the reduction of moisture within the fermentation time [35]. Enset variety and fermentation periods play a prominent role in determining the amount of moisture in the kocho [12].

2.5.2. Temperature. Hunduma and Mogessie [15] reported that the internal temperature of unfermented kocho remains below 20°C while that of fermented kocho ranges from 19 to 23°C. This report was in line with the findings by Karssa et al. [35], who showed an increment in the internal temperature from 19 to 24°C. Finally, in the pit fermentation, the internal temperature of the kocho varied between 20.5 and 26.1°C.

Andeta et al. [8] reported the internal temperature of fermenting enset biomass as $22.5 \pm 0.2^\circ\text{C}$ for the pit, $22.7 \pm 0.2^\circ\text{C}$ for the erosa (a bamboo basket made from enset leaf sheath), and $19.3 \pm 0.1^\circ\text{C}$ for jar fermentation.

2.5.3. pH. Kocho fermentation within the pit resulted in a decrease in pH and an increase in titratable acidity [8, 35]. Similarly, Andeta et al. [13] reported that the pH value of kocho is influenced by the fermentation methods. A similar finding also reported by Yirmaga [12] showed that as the titratable acidity of kocho increased, the pH value decreased. LAB and yeasts are dominant species during the fermentation of kocho since they are acid-tolerant and can survive at low pH [15]. Karssa et al. [35] reported that the pH of fermented enset inoculated with a traditional starter culture (*gamancho*) is lower than that of treatments fermented without starter culture during fermentation periods. Their difference indicated that treatment with traditional starter cultures accelerates the fermentation time by providing high numbers of fermenting microbes.

2.5.4. Titratable Acidity. According to Andeta et al. [8], titratable acidity was predominantly increased through fermentation periods for pits and jars in different amounts.

TABLE 1: Physicochemical dynamics and their roles in fermenting onset mass.

S. no.	Product	Physicochemical parameter	Their status and roles during fermentation	References
1	Fermenting kocho/onset mass	pH	(i) The pH of fermenting onset mass/kocho decreases as fermentation days increase (ii) It creates an acidic environment that creates unfavorable conditions for pathogenic microorganisms and inhibits them	[8, 15, 35]
		Titrateable acidity	(i) Titrateable acidity increases as fermentation days increase, resulting from a decrease in pH (ii) An increment in titrateable acidity favours the growth of lactic acid bacteria	[12, 35]
		Moisture content	(i) The moisture content of fermenting onset/kocho declined as the fermentation day progressed (ii) A decrease in moisture content allows an increment in the dry matter of kocho	[8, 9, 11, 13]
		Temperature	(i) The internal temperature increases as fermentation day increases, but it depends on the external temperature	[8, 35]

The titratable acidity of jar fermentation was higher than that of pit and erosa fermentations, which is in line with pH evolution. At the start of enset fermentation, the amount of titratable acidity was lower as compared with the later time of fermentation due to the increased population of acid-producing microorganisms [37]. Yirmaga [12] reported that the amount of titratable acidity increased significantly from 10 to 13 days of the fermentation period. Concomitantly, as compared to the 10th and 30th days of fermentation, the amount of titratable acidity is high on the 30th day of fermentation. This reflects that enset fermentation is an acidic-rich environment that allows the reduction of pH and increment of titratable acidity. Karssa et al. [35] reported that the increment in titratable acidity of fermenting kocho dough is favouring the growth and activities of lactic acid bacteria.

2.6. Microbial Dynamics during Enset Fermentation.

Microbial dynamics are the driving force for the traditional fermentation process, which provides the desired quality of fermented food [7]. They play a major role in traditional food fermentation to enhance nutritional quality, extend shelf-life, and contribute to the palatability and wholesomeness of the product [13, 41]. Enterobacteriaceae, lactic acid bacteria, yeasts, and molds are the predominant microbial groups reported during kocho fermentation (Table 2), and some of them are used as starters or initiators of the fermentation process [7, 8].

2.6.1. Acetic Acid Bacteria. Acetic acid bacteria are a group of Gram-negative bacteria with great potential for oxidizing ethanol to acetic acid [46]. Through a long period of fermentation, AAB isolated from the LAB selective media, which may increase the number of metabolites and sensory attributes, maintain hygiene, and promote wholesome kocho fermentation [7, 42]. Concomitantly, higher counts of AAB were isolated from fermenting kocho after inoculating the LAB starter culture at the onset of fermentation [19, 20]. Aeration causes the enhancement of AAB in fermenting kocho mass, which may affect the sensory quality of fermented mass [7, 9, 42]. Organic acids produced during fermentation are used as a preservative for fermented food since they have the potential to produce acetic acid, lactic acid, and bacteriocins [8, 30, 47].

2.6.2. Enterobacteriaceae. The Enterobacteriaceae are microorganisms with great potential for disease-causing activity, encompassing beneficial commensal microbiota, opportunistic pathogens that can inflict considerable morbidity and mortality on compromised hosts, and principal pathogens capable of initiating illness in individuals in perfect health [48]. Enterobacteriaceae are the indicator bacteria for the microbiological quality of food and the hygiene status of a production process and pose a microbiological risk for consumers [49]. Counts of Enterobacteriaceae are relatively higher at the beginning of the fermentation process and reach an undetectable level at the

final stages of Phase II fermentation. This is due to the reduction of pH and an increase in titratable acidity [8, 35]. The growth of Enterobacteriaceae is also inhibited by increasing fermentation periods of kocho, except for *Escherichia coli* (*E. coli*) O157:H7, which can grow in an acidic environment [50]. This reflects the fact that *E. coli* O157:H7 can survive in low PH (around 5.00) medium [50, 51]. The counts of Enterobacteriaceae remained below the detection limit on day 7 and other subsequent sampling days for both uninoculated and inoculated Kocho samples with LAB starter inoculant [52]. This might be due to the acidic environment created by endogenously present or added LAB, which creates unfavorable conditions for the Enterobacteriaceae [9, 13].

The decline in the counts of Enterobacteriaceae plays a profound role in enhancing kocho safety and eliminating pathogenic microbes such as mold [37, 53]. Counts of Enterobacteriaceae were reduced to below the detectable level after day 1 for the pits and jars fermentation and after day 7 for the basket fermentation [8]. Likewise, Karssa et al. [35] and Andeta et al. [13] reported counts of Enterobacteriaceae below the detection limit between 12 and 15 days during the fermentation of kocho due to adverse conditions and a reduction of pH over fermentation periods. This might have resulted from unfavorable conditions occurring during the fermentation process and the production of bacteriocins and other antibacterial substances by some lactic acid bacteria [7, 8]. In the traditional enset fermentation system, counts of Enterobacteriaceae were detected until 30 days of fermentation [35, 37].

2.6.3. Lactic Acid Bacteria (LAB). LAB is a group of heterogeneous bacteria that play a great role in different fermentation processes (Table 2). They can ferment carbohydrate-containing foods and produce lactic acid as a byproduct of fermentation [54]. It constitutes a maximum proportion of total microbial counts and dominates an entire enset fermentation system [7, 8, 35]. To enhance the fermentation process, inoculation of starter culture which is used though the starter should exclude and compete with the natural microbe [55]. The use of starter culture reduces the duration of fermentation and allows its completion in a short period of time [19, 36].

LAB contributes to the stability and safety of kocho by inhibiting pathogenic and spoilage bacteria [17]. They provide metabolites such as lactic acid, acetic acid, carbon dioxide, ethanol, hydrogen peroxide, and antimicrobial peptides [56]. Metabolite compounds produced by LAB have greatly improved the flavour and odour of kocho products [7]. LABs carry out a biochemical conversion of carbohydrates into organic or lactic acid and other metabolites during fermentation [57]. It contributes to the stability and safety of kocho by inhibiting pathogenic and deteriorative bacteria [17]. They are used as starter culture, a product with high viable microbial counts, and when added to certain foods, they accelerate fermentation, leading to a final product with the desired change in aroma, texture, and flavour profile [19, 41]. To enhance the fermentation

TABLE 2: Microbial dynamics and their roles in fermenting onset mass.

S. no.	Product	Microbial dynamics	Their status and roles during fermentation	References
1	Fermenting kocho/onset mass	Acetic acid bacteria	(i) Increase as fermentation days increase (ii) Aeration favours the growth of AAB (iii) Flavors produced by AAB are used as a preservative for fermented mass (i) Increase at the onset and middle of the fermentation day and decrease as the fermentation day proceeds to end	[7, 9, 20, 42]
		Lactic acid bacteria	(ii) Produce acidic metabolites that allow the reduction of pH (iii) Used as a starter culture for onset fermentation	[19, 20, 35]
		Enterobacteriaceae	(i) Counts of Enterobacteriaceae decreased as fermentation days increased (ii) The acidic environment created in fermentation mass allows the reduction of Enterobacteriaceae counts	[8, 35]
		Yeast and molds	(i) Yeast and mold count slightly decrease as fermentation proceeds to an end (ii) They play a crucial role in degrading starch and imparting antimicrobial metabolites that allow the growth of lactic acid bacteria	[8, 43]
		Clostridium spores	(i) Anaerobic Clostridium increased as fermentation days increased (ii) They enhance the breakdown of amino acids into ammonia, which results in a reduction in pH	[13, 19, 44, 45]

process, inoculation of starter culture is used, though the starter should exclude and compete with the natural microbe [55]. The use of starter culture reduces the duration of fermentation and allows its completion in a short period of time [19, 36].

Andeta et al. [19] and Weldemichael et al. [36] revealed promising strains of LAB suitable for starter culture for enset fermentation. Strains of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* are validated as potential starter inoculum for enset fermentation [19]. *Leuconostoc* and *Lactococcus* spp. are the most abundant LAB in the initial phases of the fermentation, while *Lactobacillus*, *Weissella*, and *Bifidobacterium* spp. were dominant at the later stages of fermentation [8]. *L. mesenteroides* occurred dominantly at the onset of kocho fermentation and is used as an initiator for the fermentation process, while *Prevotella paludivivens*, *Lactobacillus* sp., and *Bifidobacterium* are less abundant species at the beginning of fermentation [13].

As the number of LAB increases during kocho fermentation, the sugar content of kocho decreases due to an increase in the fermentation period [9, 58]. In kocho fermentation, species of LAB, mainly *L. mesenteroides*, are responsible for decreasing moisture content and pH while increasing titratable acidity [13]. This might be the heterofermentative characteristics of *L. mesenteroides* that allow the production of carbon dioxide, ethanol, and organic acids [59], as cited in [60]. This reflects the fact that they are acid-producing microorganisms [19]. LAB present in kocho fermentation is also useful for the development of volatile compounds, which enhance the sensory quality of kocho products [36]. The findings of Karssa and Papini [63] also revealed similar results: an increment in LAB counts allows for a reduction in pH value and an increase in titratable acidity. Strains of LAB starter culture are responsible for the development of kocho quality [20]. The mutual interaction of *L. plantarum* and *L. mesenteroides* during kocho fermentation causes the degradation of starch content, reduction of pH value, and increase in titratable acidity that inhibit pathogenic microbes and improve the desirable organoleptic characteristics of kocho [42].

All microbial counts, including LAB, decreased as fermentation progressed [8, 35]. Dibaba et al. [37] also revealed similar findings that reflect the increase in LAB count until 30 days of fermentation and its decline at 60 days of fermentation. This might be the low pH value's inhibitory effect on the microbes inside the fermenting system [13, 38].

2.6.4. Yeasts and Molds. Yeast and molds are a large and diverse group of food-borne microbes that include several hundred species that cause various degrees of deterioration and decomposition on foods [62]. Identification and characterization of yeast species in kocho fermentation are useful in improving nutritional quality, maintaining hygiene, and ensuring the safety of kocho products [7]. Yeast has potential to break down sugars into acid, carbon dioxide gas, and other flavour compounds during vegetable fermentation. Moreover, the acid produced during fermentation provides

vegetable tartness and inhibits pathogenic microbes' growth [63]. Identified species of yeast such as *Filobasidilla neoformans*, *Candida*, and *Trichosporon* are harmful microbes to the health of human beings [42]. Therefore, safety and hygienic requirements are important during enset processing periods [14]. The characterization of yeast inside kocho fermentation is crucial for the formulation of starter culture and for improving, standardizing, and modernizing the quality of traditional enset fermentation and preparation [14].

Enough amount of oxygen is required for the growth of yeast during the fermentation of kocho [7]. However, as counts of yeast increase during Kocho fermentation, starch degradation also enhances and imparts antimicrobial metabolites such as ethanol, organic acids, and vitamins, which play a crucial role in the growth of LAB [43]. Counts of yeast and mold slightly decrease as fermentation days proceed to end in kocho fermentation [52]. This could result from the anaerobic conditions of the fermenting enset mass. This is in agreement with the finding of Dibaba et al. [37] that revealed counts of yeast and mold were reduced due to anaerobic conditions of *gamma* fermentation or a lack of sufficient oxygen during fermentation periods.

2.6.5. Clostridium Spores. *Clostridium* is composed of a large spectrum of Gram-positive, mesophilic, and anaerobic species. The *Clostridium* sp. bacteria acts in various environments, providing agro, ecological benefits in plant growth promotion and participation in industrial processes, and replacing in both cases chemicals harmful to the environment [64]. Counts of anaerobic *Clostridium* spores increased as fermentation days increased for different enset accessions [13]. Inoculation of LAB starter inoculum inhibits *Clostridium* spores' growth during enset fermentation more than control or uninoculated cultures [19]. *Clostridium* species play a great role in incrementing pH values during enset fermentation. Proteolytic *Clostridia* were known to breakdown amino acids into ammonia and thereby increase the pH [8, 44, 45]. Supportably, it was shown that saccharolytic *Clostridia*, *Clostridium tyrobutyricum*, increased the pH of a fermenting enset [60]. *Clostridium* spores increased during kocho fermentation [7].

2.7. Bioactive Compounds Produced by Microorganisms during Kocho Fermentation. During kocho fermentation, organic acids such as lactic acid, acetic acid, and propionic acid are among the bioactive substances that are produced [7]. During the fermentation process, acetic acid bacteria (AAB) and lactic acid bacteria (LAB) create these organic acids. Bacteriocins are antimicrobial peptides that LAB also makes; they prevent the growth of harmful bacteria [36]. Other bioactive substances such as phenolic compounds, flavonoids, and tannins are also produced during kocho fermentation in addition to organic acids and bacteriocins [42]. The antioxidant qualities of these substances can aid in preventing diseases brought on by oxidative stress.

3. Conclusion

Enset is a plant resource with significant potential to provide a starch-rich food called *Kocho*. It also contains low protein and mineral content in terms of nutritional quality and is a staple or costaple food in terms of food security. The dynamics of the microbial population and changes in physicochemical parameters during enset fermentation ensure the quality of the product. There is a microbial population in *Kocho* fermentation that provides desired sensory character, improves nutritional quality, enhances product shelf-life, and eliminates pathogenic agents. Monitoring microbial and physicochemical dynamics inside fermenting kocho results in good-quality products of enset-based food.

Data Availability

All data generated and analyzed during this study are included in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

Authors' Contributions

Dereba Workineh Seboka conceptualized, validated, and visualized the study; provided the resources; curated the data; administrated the project; and wrote the original draft. Abay Tabor Bejiga provided the resources, curated the data, and validated and visualized the study. Debela Jufar Turunesh reviewed the article and investigated, validated, and visualized the study. Andualem Arimo Turito provided the resources and investigated, validated, and visualized the study. Abayeneh Girma provided the resources; curated the data; investigated, validated, supervised, and visualized the study; and reviewed and edited the article. All authors read and approved the final version of the manuscript.

References

- [1] J. Matheka, J. N. Tripathi, I. Merga, E. Gebre, and L. Tripathi, "A simple and rapid protocol for the genetic transformation of *Ensete ventricosum*," *Plant Methods*, vol. 15, no. 1, pp. 130–217, 2019.
- [2] G. Yemata, "Ensete ventricosum: a multipurpose crop against hunger in Ethiopia," *The Scientific World Journal*, vol. 2020, Article ID 6431849, 10 pages, 2020.
- [3] J. S. Borrell, M. Goodwin, G. Blomme et al., "Enset-based agricultural systems in Ethiopia: a systematic review of production trends, agronomy, processing and the wider food security applications of a neglected banana relative," *Plants, People, Planet*, vol. 2, no. 3, pp. 212–228, 2020.
- [4] T. M. Olango, B. Tesfaye, M. Catellani, and M. E. Pè, "Indigenous knowledge, use and on-farm management of enset (*Ensete ventricosum* (Welw.) Cheesman) diversity in Wolaita, Southern Ethiopia," *Journal of Ethnobiology and Ethnomedicine*, vol. 10, no. 1, pp. 41–18, 2014.
- [5] G. Birmeta, *Genetic variability and biotechnological studies for the conservation and improvement of ensete ventricosum*, PhD Dissertation, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2004.
- [6] G. Mulaw and A. Tesfaye, "Technology and microbiology of traditionally fermented food and beverage products of Ethiopia: a review," *African Journal of Microbiology Research Review*, vol. 11, no. 21, pp. 825–844, 2017.
- [7] K. Dekeba Tafa and W. Abera Asfaw, "Role of microbial dynamics in the fermentation process of Ethiopia traditional food: kocho," *Cogent Food & Agriculture*, vol. 6, no. 1, Article ID 1840007, 2020.
- [8] A. F. Andeta, D. Vandeweyer, E. F. Teffera et al., "Effect of fermentation system on the physicochemical and microbial community dynamics during enset (*Ensete ventricosum*) fermentation," *Journal of Applied Microbiology*, vol. 126, no. 3, pp. 842–853, 2019.
- [9] A. Boshia, A. L. Dalbato, T. Tana, W. Mohammed, B. Tesfaye, and L. M. Karlsson, "Nutritional and chemical properties of fermented food of wild and cultivated genotypes of enset (*Ensete ventricosum*)," *Food Research International*, vol. 89, pp. 806–811, 2016.
- [10] S. W. Fanta and S. Neela, "A review on nutritional profile of the food from enset: a staple diet for more than 25 per cent population in Ethiopia," *Nutrition & Food Science*, vol. 49, no. 5, pp. 824–843, 2019.
- [11] B. Mohammed, G. Martin, and M. K. Laila, "Nutritive values of the drought tolerant food and fodder crop enset," *African Journal of Agricultural Research*, vol. 8, no. 20, pp. 2326–2333, 2013.
- [12] M. T. Yirmaga, "Improving the indigenous processing of kocho, an Ethiopian traditional fermented food," *Journal of Nutrition & Food Sciences*, vol. 3, no. 1, 2013.
- [13] A. F. Andeta, D. Vandeweyer, F. Woldesenbet et al., "Fermentation of enset (*Ensete ventricosum*) in the Gamo highlands of Ethiopia: physicochemical and microbial community dynamics," *Food Microbiology*, vol. 73, pp. 342–350, 2018.
- [14] B. Gizaw, Z. Tsegay, and B. Tilahun, "Isolation and characterization of yeast species from ensete ventricosum product; Kocho and Bulla collected from Angacha district," *International Journal of Advanced Biological and Biomedical Research*, vol. 5, no. 1, pp. 246–252, 2016.
- [15] T. Hunduma and M. Ashenafi, "Traditional enset (*Ensete ventricosum*) processing techniques in some parts of West Shewa Zone, Ethiopia," *Journal of Agriculture and Development*, vol. 2, no. 1, pp. 37–57, 2011, <http://opendocs.ids.ac.uk/opendocs/handle/123456789/8730>.
- [16] T. Hunduma, "Ethiopian indigenous knowledge of traditional foods and beverages processing: needs for modern food processing technology and transformations," in *National Conference on Science, Technology and Innovation for Prosperity of Ethiopia (NCSTI-2012)*, pp. 16–18, Bahir Dar, Ethiopia, May 2012.
- [17] M. Elifu, "Isolation and characterization of starter culture bacteria for ensete ventricosum fermentation," MSc Thesis, Arba Minch University, Arba Minch, Ethiopia, 2015.
- [18] M. Haile, "Comparison of the nutritional and microbial composition of kocho from wild and cultivated enset from bonga, ethiopia," Msc Thesis, Addis Ababa University, Addis Ababa, Ethiopia, 2015.
- [19] A. F. Andeta, D. Vandeweyer, E. F. Teffera et al., "Traditional starter cultures for enset fermentation: unravelling their production and microbial composition," *Food Bioscience*, vol. 29, pp. 37–46, 2019.

- [20] H. Weldemichael, Weldeselassie, S. Admassu Emire, and M. Alemu, "Selection and characterisation of the predominant *Lactobacillus* species as a starter culture in the preparation of kocho, fermented food from enset," *Food Science and Biotechnology*, vol. 28, no. 4, pp. 1125–1134, 2019.
- [21] Ethiopian Biodiversity Institute (Ebi), *Government of the Federal Democratic Republic of Ethiopia: Ethiopia's Fifth National Report to the Convention on Biological Diversity Ethiopian Biodiversity*, Addis Ababa University, Addis Ababa, Ethiopia, 2014.
- [22] H. Berhanu, Z. Kiflie, S. Feleke, and A. Yimam, "Chemical and morphological analysis of enset (*Ensete ventricosum*) fibre, leaf, and pseudostem," *Lignocellulose*, vol. 5, no. 2, pp. 139–151, 2016.
- [23] Z. Tsegaye and B. Gizaw, "Community indigenous knowledge on traditional fermented enset product preparation and utilization practice in Gedeo zone," *Journal of Biodiversity and Environmental Sciences*, vol. 5, pp. 214–232, 2015.
- [24] V. Heuzé, H. Thiollet, G. Tran, P. Hassoun, and F. Lebas, "Enset (ensete ventricosum) corms and pseudostems. feedipedia, a programme by inrae, cirad, afz and fao," 2017, <https://www.feedipedia.org/node/21251>.
- [25] S. A. Brandt, A. Spring, C. Hiebsch et al., *The Tree against Hunger. Enset-Based Agricultural Systems In Ethiopia*, American Association for the Advancement of Science, Washington, DC, USA, 1997.
- [26] A. Tsegaye, *On Indigenous Production, Genetic Diversity and Crop Ecology of Enset (Ensete Ventricosum (Welw.) Cheesman)*, Wageningen University and Research, Wageningen, Netherlands, 2002.
- [27] M. Atlabachew and B. S. Chandravanshi, "Levels of major, minor and trace elements in commercially available enset (*Ensete ventricosum* (Welw.), Cheesman) food products (Kocho and Bulla) in Ethiopia," *Journal of Food Composition and Analysis*, vol. 21, no. 7, pp. 545–552, 2008.
- [28] A. Debebe, B. S. Chandravanshi, and T. Wondimu, "Metallic nutrients in enset (*Ensete ventricosum*) corm cultivated in Wolliso and Wolkite towns in Ethiopia," *Sinet: Ethiopian Journal of Science*, vol. 35, no. 2, pp. 71–80, 2012.
- [29] M. Sahle, K. Yeshitela, and O. Saito, "Mapping the supply and demand of Enset crop to improve food security in Southern Ethiopia," *Agronomy for Sustainable Development*, vol. 38, no. 1, 2018.
- [30] H. Bekele, "Effect of enset (ensete ventricosum (wele) cheesman) variety and fermentation on nutritional composition, anti-nutritional factors, physicochemical characteristics and functional property of bulla," MSc Thesis, Addis Ababa University, Addis Ababa, Ethiopia, 2015.
- [31] B. Garedew, A. Ayiza, B. Haile, and H. Kasaye, "Indigenous knowledge of enset (*Ensete ventricosum* (Welw.) Cheesman) cultivation and management practice by Shekicho people, southwest Ethiopia," *Journal of Plant Sciences*, vol. 5, no. 1, pp. 6–18, 2017.
- [32] D. T. Balcha, B. Kulig, O. Hensel, and E. Woldeesenbet, "Mechanical properties of enset fibers obtained from different breeds of enset plant," *International Journal of Aerospace and Mechanical Engineering*, vol. 15, no. 1, pp. 7–14, 2021.
- [33] A. Tsegaye, "Identification and analysis of nutritional components of Enset (*Ensete ventricosum*) landraces suitable for corm production in Southern Ethiopia," *Ethiopian Journal of Development Research*, vol. 37, no. 2, pp. 1–27, 2015.
- [34] A. Y. Tadesse, A. M. Ibrahim, S. F. Forsido, and H. T. Duguma, "Nutritional and sensory quality of complementary foods developed from bulla, pumpkin and germinated amaranth flours," *Nutrition & Food Science*, vol. 49, no. 3, pp. 418–431, 2019.
- [35] T. H. Karssa, K. A. Ali, and E. N. Gobena, "The microbiology of Kocho: an Ethiopian traditionally fermented food from Enset (*Ensete ventricosum*)," *International Journal of Life Sciences*, vol. 8, no. 1, pp. 7–13, 2014.
- [36] H. Weldemichael, S. Admassu, and M. Alemu, "Optimization of enset fermentation in the production of kocho using response surface methodology," *Acta Universitatis Cibiniensis. Series E: Food Technology*, vol. 22, no. 2, pp. 67–75, 2019.
- [37] A. H. Dibaba, A. C. Tuffa, E. Z. Gebremedhin, G. G. Nugus, and G. Gebresenbet, "Microbiota and physicochemical analysis on traditional kocho fermentation enhancer to reduce losses (*Gammar*) in the highlands of Ethiopia," *Microbiology and Biotechnology Letters*, vol. 46, no. 3, pp. 210–224, 2018.
- [38] B. A. Gashe, "Spoilage organisms of kocho," *MIRCEN Journal of Applied Microbiology and Biotechnology*, vol. 3, no. 1, pp. 67–73, 1987.
- [39] M. Ashenafi, "A review on the microbiology of indigenous fermented foods and beverages of Ethiopia," *Ethiopian Journal of Biological Sciences*, vol. 5, no. 2, pp. 189–245, 2006.
- [40] H. W. Weldemichael, *Development of Starter Culture for Kocho a Traditional Fermented Food of Ethiopia*, Addis Ababa University, Addis Ababa, Ethiopia, 2018.
- [41] W. H. Holzapfel, "Appropriate starter culture technologies for small-scale fermentation in developing countries," *International Journal of Food Microbiology*, vol. 75, no. 3, pp. 197–212, 2002.
- [42] G. Birmeta, A. Bakeeva, and V. Passoth, "Yeasts and bacteria associated with kocho, an Ethiopian fermented food produced from enset (*Ensete ventricosum*)," *Antonie van Leeuwenhoek*, vol. 112, no. 4, pp. 651–659, 2018.
- [43] K. H. Steinkraus, "Classification of fermented foods: world-wide review of household fermentation techniques," *Food Control*, vol. 8, no. 5-6, pp. 311–317, 1997.
- [44] D. R. Buxton and P. O'Kiely, "Preharvest plant factors affecting ensiling," *Silage science and technology*, vol. 42, pp. 199–250, 2003.
- [45] Y. Oladosu, M. Y. Rafii, N. Abdullah et al., "Fermentation quality and additives: a case of rice straw silage," *BioMed Research International*, vol. 2016, Article ID 7985167, 14 pages, 2016.
- [46] R. J. Gomes, M. D. F. Borges, M. D. F. Rosa, R. J. H. Castro-Gómez, and W. A. Spinosa, "Acetic acid bacteria in the food industry: systematics, characteristics and applications," *Food Technology and Biotechnology*, vol. 56, no. 2, pp. 139–151, 2018.
- [47] D. H. Kim, K. Dong Lee, and K. Choon Choi, "Role of LAB in silage fermentation: effect on nutritional quality and organic acid production-an overview," *AIMS Agriculture and Food*, vol. 6, no. 1, pp. 216–234, 2021.
- [48] M. S. Donnenberg, "Mandell, douglas, and bennett's principles and practice of infectious diseases," *Jama*, vol. 304, 8th edition, 2015.
- [49] K. G. Mladenović, M. Ž. Grujović, M. Kiš et al., "Enterobacteriaceae in food safety with an emphasis on raw milk and meat," *Applied Microbiology and Biotechnology*, vol. 105, no. 23, pp. 8615–8627, 2021.
- [50] G. J. Leyer, L. Wang, and E. A. Johnson, "Acid adaptation of *Escherichia coli* O157: H7 increases survival in acidic foods," *Applied and Environmental Microbiology*, vol. 61, no. 10, pp. 3752–3755, 1995.
- [51] R. Nuguse, "Identification, isolation and characterization of suitable lactic acid bacterial strain for minimizing

- fermentation time of kocho,” MSc Thesis, Addis Ababa University, Addis Ababa. Ethiopia, 2017.
- [52] A. F. Andeta, F. E. Teffera, F. W. Misganaw et al., “Development and validation of lactic acid starter cultures for enset (*Ensete ventricosum*) fermentation,” *Lebensmittel-Wissenschaft und-Technologie*, vol. 115, Article ID 108462, 2019.
- [53] B. J. Stoecker, Y. Abebe, M. J. Hinds, and G. E. Gates, “Nutritive value and sensory acceptability of corn- and kocho-based foods supplemented with legumes for infant feeding in Southern Ethiopia,” *African Journal of Food, Agriculture, Nutrition and Development*, vol. 6, no. 1, pp. 1–19, 2006.
- [54] T. Bintsis, “Lactic acid bacteria: their applications in foods,” *Journal of Bacteriology and Mycology*, vol. 6, no. 2, pp. 89–94, 2018.
- [55] N. J. Gardner, T. Savard, P. Obermeier, G. Caldwell, and C. P. Champagne, “Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures,” *International Journal of Food Microbiology*, vol. 64, no. 3, pp. 261–275, 2001.
- [56] R. Di Cagno, R. Coda, M. De Angelis, and M. Gobbetti, “Exploitation of vegetables and fruits through lactic acid fermentation,” *Food Microbiology*, vol. 33, no. 1, pp. 1–10, 2013.
- [57] C. Chen, S. Zhao, G. Hao, H. Yu, H. Tian, and G. Zhao, “Role of lactic acid bacteria on the yogurt flavour: a review,” *International Journal of Food Properties*, vol. 20, no. 1, pp. S316–S330, 2017.
- [58] A. Nigatu and B. A. Gashe, “Effect of heat treatment on the antimicrobial properties of teff dough, injera, kocho, and paradise and the fate of selected pathogens,” *World Journal of Microbiology and Biotechnology*, vol. 14, no. 1, pp. 63–69, 1997.
- [59] J. M. Jay, M. J. Loessner, and D. A. Golden, *Modern Food Microbiology*, Springer Science & Business Media, Berlin, Germany, 7th edition, 2008.
- [60] E. Gorrens, *Fermentation of Enset (Ensete Ventricosum) in the Gamo Highlands of Ethiopia: Introduction of Sauerkraut Jars*, MSc thesis KU Leuven, Geel, Belgium, 2018.
- [61] T. Karssa and A. Papini, “Effect of clonal variation on quality of Kocho, traditional fermented food from enset (*Ensete ventricosum*), Musaceae,” *International Journal of Food Science and Nutrition Engineering*, vol. 8, no. 3, pp. 79–85, 2018.
- [62] T. Valerie, E. S. Michael, P. B. Mislivec, H. A. Koch, and R. Bandler, “BAM chapter 18: yeasts, molds, and mycotoxins,” 2021, Available at: <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-mycotoxins>.
- [63] J. Eifert, R. Boyer, and R. Williams, “Vegetable fermentation what is fermentation?” *Journal of Food Science & Technology*, vol. 10, pp. 1–18, 2013.
- [64] G. G. O. Figueiredo, V. R. Lopes, T. Romano, and M. C. Camara, “Clostridium,” in *Beneficial Microbes in Agro-Ecology*, Academic Press, Cambridge, UK, 2020.