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Microbial Properties of Milk and Traditional Fermented Milk Products in Ethiopia: A Review

Mihret Frew[1], Kiros Abebe[2]

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ABSTRACT

Milk and milk products play a vital role in human nutrition all over the world. In addition to being a nutritious food for humans, milk and milk products provide a favorable environment for the growth of spoilage and pathogenic microorganisms. Thus, this paper aims to review the microbial properties of milk and fermented milk products produced in different parts of Ethiopia. Microbial contamination may generally occur from within the udder, exterior to the udder, from the surface of milk handling and storage equipment and the traditional practice. The status of the cleanliness of the milkers could be the primary sources of the initial milk contamination. The reviewed dairy products are Milk, Ergo (naturally fermented milk), Kibe (traditional butter), Arera (defatted sour milk) and Ayib (Ethiopian cottage cheese). The existing microbial quality of dairy products produced in the rural part of Ethiopia was poor and did not meet the acceptable quality requirements. The problem was compounded from limited knowledge of the hygienic production, processing and handling of dairy products coupled with inadequate dairy processing infrastructures. Strict hygienic control measures along the value chain to improve the hygienic conditions of milk starting from production to consumption are necessary to provide safe products to the consumer.

Key words: Ethiopian fermented milk products, Milk quality, Microbial properties.

Dairy products play a fundamental role in human nutrition throughout the world. Therefore, milk and milk products must be high hygienic quality. In areas which are less developed and particularly in the hot tropics, good quality of the safe product is the most important but not easily accomplished (Zelalem and Faye, 2006; Asaminew and Eyassu, 2011). In addition to being a nutritious food for humans, milk provides an excellent culture medium for the growth and multiplication of many kinds of microorganisms (Walestra *et al.,* 2006; Parekh and Subhash, 2008).

Food spoilage is an enormous economic problem in the worldwide. Approximately one fourth of the world's food supply lost in the course of microbial activity. The highly perishable nature of milk and mishandling, the amount produced is subjected to high post-harvest losses in Ethiopia, which account for 20-35% (Getachew, 2003). The microorganisms mostly encountered in the dairy industry are bacteria, yeasts, moulds and viruses. Some of the bacteria (Lactic acid bacteria) are useful in milk processing, causing milk to sour naturally (Lingathurai and Vellathurai, 2010).

Milk, either raw or processed, is a familiar vehicle for a number of human pathogens and causes a health risk to consumers. If it is contaminated by any pathogens and subjected to temperature abuse, where these organisms can multiply to high counts and may produce toxins. Milk can also contain pathogenic bacteria, such as *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Listeria* spp., *Brucella* spp., *etc.* which are well known (Godefay and Molla, 2000; Zelalem, 2003). These pathogenic bacteria in milk emerged as major public health concerns, especially for those people who drink raw milk. The consumption of raw milk and milk products is also common in Ethiopia, which is not safe from a consumer health point of view as it may lead to the transmission of various diseases (Zelalem, 2003). On the other hand, bacteria can cause spoilage of the milk and poor quality products (Oliver *et al.,* 2005). Keeping fresh milk at high temperature together with unhygienic practices in the milking process may also result in microbiologically more inferior quality (Zelelem, 2010). The sources of milk pathogens (actually human pathogens found in milk) are primarily from poor sanitation, animal stress and animal sickness. Though fresh milk from cattle may have temporary germicidal or bacteriostatic properties, the growth of microorganisms is expected unless it is processed or well stored (Swai and Schoonman, 2011).

The safety of dairy products with respect to food-borne diseases is a great worry all over the world. This is especially true in developing countries where production of milk and

[1]Ministry of Agriculture, Addis Ababa, Ethiopia.
[2]Mekelle University, College of Veterinary Sciences, Mekelle, Ethiopia.

Corresponding Author: Kiros Abebe, Mekelle University, College of Veterinary Sciences, Mekelle, Ethiopia.
Email: kabebe52@gmail.com

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various dairy products take place under rather unsanitary conditions and poor production practices (Zelalem and Faye, 2006; Alganesh *et al.,* 2007; Asaminew and Eyassu, 2011). Consumers demand is safe, wholesome, nutritious and high quality milk and milk product that is produced and processed in a sanitary manner and is free from pathogens. However, inadequate dairy infrastructure together with limited knowledge of the hygienic handling of milk and milk products, the quality of smallholder milk and milk products in Ethiopia could be characterized to poor quality (Mennane *et al.,* 2007; Zelelem, 2010; Zelelem, 2012). Hence, understanding the existing situation concerning the microbial properties of milk and traditionally fermented milk products produced, marketed and consumed in Ethiopia is important to be able to make any improvement interventions. Therefore, the objective of this paper is to review the microbial properties of milk and traditionally fermented milk products in Ethiopia.

Microbial properties of milk and traditional fermented milk products in Ethiopia

The microbial content of milk and milk products is a major feature in determining its quality. It indicates the hygienic condition exercised during milking, cleanliness of the milking utensils, storage condition, means of transport and the cleanliness of the udder of the individual animal (Karmen and Slavica, 2008; Fatine *et al.,* 2012). The presence and multiplication of saprophytic bacteria in raw milk might change the milk composition and influence the milk products quality (Godefay and Molla, 2000). Milk produced from a healthy udder comprises few bacteria but it picks up many bacteria from the time it leaves the teat of the cow up to it is used for extra processing (O'Connor, 1994; Solomon *et al.,* 2013).

As stated in different studies, diverse quality tests can be performed quantifying bacterial population and other microorganisms present in milk and milk products, the major one being aerobic mesophilic bacterial count, colifom count, somatic cell count and standard plate count. They are useful to measure its sanitary quality and most grading of milk is on the basis of some method for estimating the numbers of bacteria (Collins *et al.,* 1995; Alehegn, 2004). Since dairy farms in the resource-limited countries like Ethiopia strive in the widespread presence of disease and compromised sanitary conditions, they milk of poor quality and higher public health risks (Alehegn, 2004). There are different types of traditionally fermented dairy products in Ethiopia, in which the exact form of lactic acid bacteria responsible for fermentation is unidentified due to uncontrolled and spontaneous fermentation. Most of these products are produced by smallholder producers where access to the required dairy infrastructure is limited. Where production and processing of milk and milk products take place under unhygienic conditions. However, these may contribute to the short shelf life of the products and also result in food poisoning (Ashenafi, 1990; Mogessie, 1990, O'Connor, 1994; Ashaye *et al.,* 2006). The various studies that have been carried out in different parts of the country on the microbial properties of milk and traditionally fermented milk products are summarized below.

Milk

In Ethiopia, milk is produced and supplied to consumer without being pasteurized and following the required quality standard. About 98% of the annual milk is produced by smallholder dairy farmers who live in rural areas. Dairy processing in the country is basically insufficient to smallholder level and poor hygienic qualities of products (Zelalem and Faye, 2006). The milk hygienic situations are diverse according to the production system, adapted practices, level of awareness and availability of resources (Zelalem, 2010). Milk is virtually a sterile fluid when secreted into the alveoli of the udder. Nevertheless, beyond this phase of production, microbial contamination might normally happen within the udder, outside of the udder and from the surface of milk handling and storage equipment. But the surrounding air, feed, soil, feces, grass as well as the traditional practices like the status of the cleanliness of the milkers could be the major sources of the initial milk contamination (Solomon *et al.,* 2013 and Haile *et al.,* 2012).

Milk provides a favorable environment for the growth of several microorganisms because of its high water content, nearly neutral pH and variety of available essential nutrients that render it as one of the excellent media for microbial growth and multiplication (Teka, 1997 and Soomro *et al.,* 2002). The bacterial contamination of milk not only decreases the nutritional quality, but also consumption of such milk impend the health of the society (Fadaei, 2014). The traditional milk equipment are often porous and consequently a reservoir for many organisms and difficult to clean (O'Connor, 1994 and Zelalem, 2003). All these reasons might increase the microbial load of milk produced in traditional practices of Ethiopia. Producers have to pay attention to the type as well as cleanliness of milk equipment. Milking equipment should be easy to clean; Aluminum and stainless steel equipment are mostly preferred (O'Connor, 1994 and Zelalem, 2003). Different studies have been conducted in different parts of the country and stated that the microbial counts of milk and milk products produced and marketed are generally much higher than the acceptable limits (Zelalem, 2010). The results showed that the total bacterial count (TBC) was in the range of 9.82 log cfu/ml - 4.57 log/cfu/ml as reported by (Abebe *et al.,* 2012; Asrat *et al.,* 2012 and Haile *et al.*, 2012). This result is similar to the findings of Godifay and Molla (2000) 8.23 $\log^{10}$ cfu/ml, Asaminew and Eyasu (2011), 7.58 $\log^{10}$ cfu/ml and Bruktawit (2016) 8.6 ± 1.11 $\log^{10}$ cfu/ml raw milk samples from different parts of the country.

However, raw milk samples from different parts of Ethiopia TBC counts is greater than 5 log cfu/ml which is higher than the given international standard set for the minimum acceptable level of bacterial count in milk (IFCN, 2006). This shows that the sanitary conditions in which milk has produced and handled are poor quality subjecting the product to microbial contamination and multiplication. Total bacterial count is a good indicator for monitoring the sanitary conditions of raw milk. High total bacterial loads may be due to contamination from lactating cow, milking equipment, storage containers, unsatisfactory hygiene/sanitation practiced at the farm level. Unsuitable storage condition, unclean udder/teats, poor quality of water used for cleanness and unhygienic condition of milkers increase TBC of raw milk (Chambers, 2002; Bukuku, 2013).

According to Godefay and Molla (2000) and Haile *et al.* (2012) aerobic mesophilic counts in milk sampled from different parts of the country is range between 7.28 and 10.28 log cfu/ml. This could be due to improper handling, storage and transport time after the milk leaves the dairy farms. Milk produced under sanitary circumstances from healthy cows should not contain more than 4.7 log cfu/ml (O'Connor, 1994). The coliform counts reported by Fekadu (1994); Alganesh (2002); Zelalem and Faye (2006); Asaminew and Eyassu (2011); Asrat *et al*. (2012); Abebe *et al*. (2012) in raw cow milk sampled from different areas of Ethiopia ranged between 4.03 log cfu/ml to 6.57 log cfu/ml. Coliform organisms contaminate raw milk from unclean milkers hands, improperly cleaned and unsanitized or faulty sterilization of raw milk utensils especially churns, milking machines, improper preparation of the cow's flecks or dirt, manure, hair dropping in to milk during milking, udder washed with unclean water, dirty towels and udder not dried before milking (Desalegn, 2014). The occurrence of coliform organisms in milk shows unhygienic conditions of production, processing or storage. Hence their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumers health (Godefay and Molla, 2000; Abebe *et al.,* 2012; Asrat *et al.,* 2012). Microorganisms found in raw milk are *Staphylococcus* spp.; *Streptococcus* spp, *Escherichia* spp. and *Bacillus* spp.. However, the occurrence of pathogenic strains in milk and milk products could be hazardous for consumers (Godefay and Molla, 2000; Zelalem *et al.,* 2007a).

Ergo (Spontaneously fermented whole milk)

Ergo is the most common dairy product in Ethiopia and it is traditionally made by natural/spontaneous/fermentation of milk at ambient temperature for 2-3 days, without the addition of starter cultures. However, the temperature and duration of incubation varies from place to place depending on the existing environmental conditions (Assefa *et al.,* 2008). Ergo resembles set yoghurt and has semi-solid, smooth and uniform appearance and usually has a white milk color with pleasant aroma and taste when prepared carefully. Ergo is the basic raw material for the production of most of the Ethiopian dairy products (Almaz *et al.,* 2001; Assefa *et al.,* 2008).

According to O'Connor (1994); Almaz *et al.* (2001) depending on the storage temperature, ergo can be stored for 15-20 days. The relatively low pH of ergo ranging from 4.3 to 4.5 is the main factor that enables the storage stability of the product (Gonfa *et al.*, 2001). The product is common and is consumed in all parts of Ethiopia by all age groups (Yonad, 2009). The production of ergo is usually done by a natural fermentation process, as the consequence various species of microorganisms involved and pay a lot to the basic end characteristics of ergo. The quality and safety of a fermented product entirely depend on type of lactic acid bacteria (LAB) that are involved in the fermentation process (Anteneh *et al.,* 2011). In the time course of ergo fermentation microorganisms such as *Streptococcus, Lactobacillus, Lactococcus and Leuconostoc* are carried out the souring process (Gonfa *et al.,* 1999; Almaz *et al.,* 2001). The authors' also detected fairly high numbers of micrococci spore formers and coliforms during the first 14 to 16 hours of fermentation. The lactococci species are the most dominant one throughout the fermentation that reached colony counts as high as 10 cfu/ml at the end of the fermentation process. At the same time, the aerobic mesophilic bacteria have also shown similar colony counts and the yeast population has increased up to 10 cfu/ml at 24 hours.

In Ethiopia, most households do no attempt to control the fermentation process of milk and milk products produced under traditional systems generally have poor qualities and do not meet the standard quality requirements set by many regulatory agencies (Ashenafi, 2002). Fekadu (1994) and Zelalem (2010) observed that the mean yeast and mould counts exceeded 4.6 and 8 log cfu/ml of Ergo sampled from different parts of the country. These values are much higher than the acceptable value <10 cfu/ml for yoghurt (Mostert and Jooste, 2002). Gadaga *et al. (*1999) reported that the presence of different species of yeast in milk and milk products may result in the spoilage of the product or on the other hand could contribute to the enhancement of the flavor of fermented milk products. *Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Citrobacter freundii and Enterobacter sakazakii* were isolated from Ergo samples collected from smallholder producers in the central Ethiopia (Zelalem *et al.,* 2007a).

Different researchers reported that the average total bacterial count (TBC) of Ergo was generally high ranging from 7.71 and 9 log cfu/ml samples collected from different parts of the country (Fekadu, 1994; Mogessie, 1995 and Zelalem, 2010). The coliform count averaging at 6.57 log cfu/ml was reported by Zelalem and Faye (2006). Also lower values of coliform count reported were less than 4.4 log cfu/ml. Coliform bacteria present in milk and milk products is

suggestive of fecal contamination and unsanitary practices during production, processing and storage (Fekadu, 1994; Zelalem, 2010; Muluken *et al.,* 2016).

The traditional milk processing techniques involve smoking of processing utensils. The importance of smoking milk containers is improving the shelf life of ergo. The practice of smoking milk containers slow down fermentation, improve flavor characteristics and prevent the growth of pathogenic/spoilage microorganisms. The total count of non-lactic acid bacteria in milk in the non-smoked containers reached a relatively high level (>10^8 cfu/ml) within 12 hours, whereas milk drawn in the smoked container required more than 24 hours to attain the same level. Likewise, the growth of coliforms and LAB was slow in the smoked containers. The common plant species used for smoking the milk containers is an African olive tree (*Olea africana*) (Ashenafi and Fekadu, 1994; Ashenafi, 1996).

Kibe (Traditional ethiopian butter)

Processing cream into butter is not common in Ethiopia and instead ergo is the base for traditional butter making (O'Connor and Tripathi, 1992). The traditional Ethiopian butter (Kibe) is constantly prepared from Ergo and not from cream (O'Connor *et al.,*1994). Kibe has an attractive appearance with a white to light yellowish color. Similar to the factory processed butter, it is semi-solid at room temperature. It has a pleasant taste and odor when it is fresh, but with increased storage at ambient temperatures results in putridity and rancidity or changes occur in odor and taste, unless refrigerated or further processed into Neter Kibe (Traditional ghee) by boiling with spices (O'Connor *et al.,* 1993). Without further processing, it is used for hairdressing and as a skin cosmetic mainly by women. Kibe has a relatively good keeping quality and is the most stable of all traditionally processed fermented milk products except Neter Kibe (Traditional ghee). Neter Kibe has a shelf life of more than one year without any change (Gonfa *et al.,* 1991). In addition to direct consumption as a side dish, they are used as a cooking oil for food preparation after processing into Neter Kibe and also for roasting coffee beans in special traditional ceremonies (Gonfa *et al.,* 2001; Yonad, 2009).

According to Yilma *et al.* (2007) about 21 liters of milk were required to produce 1 kg of butter (83% total solids) and the average fat recovery was 90%. Kibe has 17.2% moisture, 1.3% protein, 81.2% fat, 0.1% carbohydrate, 0.2% ash, 0.024% calcium and 0.0015% iron (EHNRI, 1997). The moisture content of the traditional Kibe produced in Ethiopia ranges from 20 to 43% compared to the international saleable standard for butter of 16%. Spoilage occurs when stored at room temperature for a long time, probably by putrefactive microorganisms (Zelalem, 2010). Different studies showed that microorganisms having lipolytic activity are highly responsible for disorders such as rancidity or loss of flavor (Almaz *et al*., 2001; Wondu, 2007; Zelalem, 2010). The average total bacterial counts in butter ranged from 6.18 - 7.25 log cfu/g sampled from different parts of the country (Zelalem, 2010). In addition to this the TBC of fresh butter sampled from rural and public butter markets in Addis Ababa ranged from 8.27 to 4.7 log cfu/g of butter (ILCA, 1992). These values are higher than the acceptable limit of 4.69 log cfu/g (Mostert and Jooste, 2002). This indicates Kibe produced from different part of the country was poor quality. The average Enterobacteriaceae and coliform counts were greater than 4 log cfu/g of butter sampled from different parts of the country (Zelalem, 2010). The genera identified from butter include *Klebsiella, Escherichia*, *Enterobacter* and *Klyuvera* in their order of abundance. *Escherichia coli* was the dominant isolate identified by Zelalem *et al.* (2007a).

In general, the Enterobacteriaceae and coliform count reported in the country were higher than the acceptable value of <10 cfu/g (Mostert and Jooste, 2002). Researchers reported that average yeast and mould count of butter ranging between 4.3 and 8 log cfu/g sampled from different parts of the country (Wondu, 2007; Asfaw, 2008; Zelalem, 2010). The majority of the moulds species growing in butter are *Thamnidium, Cladosporium and Aspergillus* (Zelalem, 2010).

Arera (Defatted Sour Milk)

Arera is a liquid product that remains after the removal of Kibe after churning of fermented whole milk (Ergo). It has a thin and smooth consistency and basically contains the casein and whey portion of the milk. Its color, taste and aroma are similar to those of ergo. It is either consumed as it is or cooked to produce Ayib. According to EHNRI (1997), Arera comprises 91.5% moisture, 3.1% protein, 1.4% fat, 3.4% lactose and 0.6% ash.

The consumption of Arera depends on the standard of living of the family and is mainly used to supplement the diets of children and the elderly in rural areas. When surplus amounts are obtained, it is given to calves, lactating cows and dogs. Arera has a shorter shelf life compared to all other fermented milk products (24-48 h) even when smoke is applied to the equipment used for its storage due to the high moisture content of the product (Almaz *et al.,* 2001; Gonfa *et al.,* 2001). The average counts of total bacteria, Enterobacteriaceae and coliforms were ranging from 9, 4.7 and 4.2 cfu/ml, respectively, of Arera sampled from different parts (Rahel, 2008; Zelalem, 2010). These values are higher than the acceptable value of <10 cfu/gm (Mostert and Jooste, 2002). Different species of bacteria were identified in Arera samples collected during both dry and wet seasons from different parts of the country, which include: *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *E. sakazakii*,

E. coli and some species of *Salmonella* (Zelalem *et al.,* 2007a).

Ayib (Traditional Ethiopian cottage cheese)

Ayib is a soft cheese prepared in various parts of Ethiopia. Ayib is made from the buttermilk resulting from the churning of sour whole milk (ergo) by heating the defatted sour milk (Arera) to coagulate the curd (O'Connor, 1994; Binyam, 2008). The product is white, acidic in nature and consumed locally as a side dish or blended with various spices according to the common practices of the various ethnic groups in the country. Ayib contributes to the overall nutrition of the people and forms part of the staple diet. It contains 76% water, 14% protein, 7% fat and 2% ash (O'Connor and Tripathi, 1992; O'Connor, 1994). Other studies showed that ayib comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash and 3.1% soluble milk constituents and the yield at least 1 kg of Ayib from 8 liters of milk (12.5%) (O'mahony, 1988; FAO, 1990). Fekadu and Abrahamsen (1997) also analyzed various Ayib samples produced by small-holders in different parts of the country and found out 80-81% moisture, 13.4-16% protein, 1.9 -2.0% fat and 0.75-0.87% minerals.

Ayib is a product that has a short-shelf life because of its high moisture content. Different researchers reported that the product is acidic in nature (pH of 3.3-4.6). However, storage stability is still 2-3 days at ambient temperature and 7 days at refrigeration temperature (°C). The keeping quality of Ayib can be improved by heating the curd to at least 75°C with accompanying removal of as much whey as possible, adding salt and storing in airtight container (Mogessie, 1992; O'Connor, 1993 and Gonfa *et al*., 2001). Cooking of the curd is also practiced which is expected to decrease the microbial load prior to consumption of the product. However, reports showed that the bacteria species identified in Ethiopian cottage cheese from samples collected from different parts of the country had been found to contain pathogenic and spoilage microorganisms such as aerobic mesophilic bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella* spp., *Escherichia coli, Enterobacter* spp., yeasts, *Listeria* spp. and *Klyuvera* species (Ashenafi, 2006; Zelalem *et al.,* 2007b; Mekonen *et al.,* 2011 and Eyassu, 2013).

The sources of contamination could be from handlers, water sources, utensils used for processing and possibly from packaging materials. The safety of cheese with respect to food borne diseases is a great concern around the world (Ashenafi, 2006). This is especially true in Ethiopia, where the consumption of Ethiopian cottage cheese (Ayib) which is typically manufactured in small dairy farms under poor hygienic conditions (Alehilign, 2004). The microbial quality of Ethiopian cottage cheese samples had high counts of mesophilic aerobic bacteria, yeasts and enterococci counts over 8 log cfu/g (Ashenafi, 2006; Zelalem *et al.,* 2007b; Binyam, 2008; Seifu *et al*., 2013). Mogessie (1990) also reported that the microbiological quality of Ayib samples collected from an open market in Awassa had counts of mesophilic aerobic bacteria, yeasts and enterococci of 10^8, 10^7 and 10^7 cfu/g, respectively. Over 60% of the samples had psychotropic count of 10 log cfu/g and about 55% of the samples were positive for coliforms and fecal coliforms. *Bacillus cereus* and *Staphylococcus aureus* were isolated at varying frequencies but at low levels (10^2-10^3 cfu/g). The maximum limit of aerobic mesophilic bacterial count which is usually employed to show the hygienic quality of food, for raw milk intended for processing is 5 log cfu/ml and intended for direct human consumption is 4.69 log cfu/ml (Collins *et al.,* 1995).

The coliform counts of Ayib samples in different parts of the country ranged between 2 and 5.70 log cfu/g (Ashenafi, 2006; Zelalem *et al.,* 2005; Binyam, 2008; Seifu *et al.,* 2013) with variances being a function of differences in the source of samples and handling conditions. In all cases, the values are higher than the recommended level of <10 cfu/g (Mostert and Jooste, 2002) indicating the poor hygienic conditions practiced during production, processing and handling of milk and milk products.

CONCLUSION

Milk and milk products play a vital role in human nutrition in urban, semi-urban and urban population of Ethiopia. The traditional fermented milk and milk products reviewed in this paper includes: Milk, Ergo (Naturally fermented milk), Kibe (Traditional butter), Arera (Defatted sour milk) and Ayib (Ethiopian cottage cheese). The microbial properties of milk and traditional fermented milk products made from small holder dairy producers were poor in quality. This is due to mainly the lack of hygienic practices followed by producers during milk production, milking, cleaning and handling of the milk containers, lack of cooling facilities and infrastructures, unimproved milk processing materials and limited knowledge on the hygienic practice of milk and milk products result in the contamination of the milk and fermented milk products. In general, high level of total bacterial count (TBC) and total coliform count (TCC) in the products indicate the poor hygienic conditions practiced during manufacturing. Several research results showed that the quality of milk and milk products in smallholder dairy farms were substandard as compared to the recommended level of milk quality standards. The commonly used traditional milk processing methods include smoking of processing utensils that slow down fermentation, improves flavor characteristics and prevent the growth of pathogenic and spoilage microorganisms.

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