



Review on effect of fermentation on physicochemical properties, anti-nutritional factors and sensory properties of cereal-based fermented foods and beverages

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Abstract

Fermentation is the oldest biotechnology in which a metabolic process carried out without the involvement of oxygen. It is one of the food processing methods that improve the nutrient contents and sensorial properties with potentially reducing or eliminating pathogenic microorganisms and natural toxins. The aim of this review is to compare, contrast and summarize the scientific data on the effect of fermentation on physicochemical properties, anti-nutritional factors and sensory properties of cereal-based fermented foods and beverages. The results of this review showed that fermentation improves the nutritional value of some proximate composition such as crude protein and fat contents, while decreases the carbohydrate and crude fiber contents. It also improves the bioavailability, antioxidant activities and sensory properties of cereal-based foods and beverages. This review concluded that fermentation improves the nutritional quality of proximate composition, bioavailability of minerals and phytochemicals, and decrease the anti-nutritional factors of cereal-based fermented foods and beverages.

Keywords Chemical composition, Cereal-based products, Fermentation, Sensory properties

Introduction

Fermentation is the oldest biotechnology in which a metabolic process carried out without the involvement of oxygen (Ray and Joshi 2014). It is one of the food processing methods that improve the nutrient contents and sensorial properties with potentially reducing or eliminating pathogenic microorganisms and natural toxins

(Abegaz et al. 2002; Hasan et al. 2014). Fermentation has been practiced for millennia; however, till today, it is a chosen processing method due to its shelf life enhancement and improving nutritional values of food products with enhancing the health of human bodies by providing probiotics (Anal 2019; Baruah et al. 2022). Like the fermentation method, the ultra-processing method and the E-numbers improve the shelf life of food products, although their products are associated with negative health effects (Asioli et al. 2017; Monteiro et al. 2019).

Spontaneous fermentation commonly occurs under uncontrolled conditions, while modern fermentation is carried out under controlled conditions, which results in

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Table 1 Effect of fermentation on proximate composition of cereal-based fermented foods and beverages

Name of products	Raw materials	Fermented end products	References
Protein contents			
<i>Akuma</i>	12.8	18.5	Nwokoro and Chukwu 2012
<i>Doklu</i>	8.1	7.1	Assohoun et al. 2013
<i>Borde</i>	8.7	9.55	Ashenafi and Mehari 1995
<i>Shamita</i>	9.0	10.37	Ashenafi and Mehari 1995
<i>Ragi</i>	7.6	8	Padmaharish et al. 2018
<i>Medida</i>	7.10	8.64	Kabeir et al. 2004
<i>Injera</i>	11.93	13.03	Asrat 2022
<i>Bushera</i>	7.79	11.63	Muyanja et al. 2003
<i>Kocho</i>	2.86	4.43	Tamiru et al. 2021
Carbohydrat contents			
<i>Akuma</i>	68.1*	37.4*	Nwokoro and Chukwu 2012
<i>Akuma</i>	5.3**	17.6**	Nwokoro and Chukwu 2012
<i>Doklu</i>	60.1	60.1	Assohoun et al. 2013
<i>Medida</i>	89.42	87.08	Kabeir et al. 2004
<i>Kocho</i>	32.56	40.49	Tamiru et al. 2021
Fat contents			
<i>Doklu</i>	0.61	0.18	Assohoun et al. 2013
<i>Borde</i>	4.6	6.88	Ashenafi and Mehari 1995
<i>Shamita</i>	1.9	6.85	Ashenafi and Mehari 1995
<i>Ragi</i>	1.5	1.5	Padmaharish et al. 2018
<i>Medida</i>	1.26	0.64	Kabeir et al. 2004
<i>Injera</i>	3.20	3.03	Asrat 2022
<i>Kocho</i>	0.01	0.11	Tamiru et al. 2021
Ash contents			
<i>Borde</i>	1.6	3.66	Ashenafi and Mehari 1995
<i>Shamita</i>	2.1	5.92	Ashenafi and Mehari 1995
<i>Medida</i>	1.05	1.64	Kabeir et al. 2004
<i>Medida</i>	1.61	1.55	Asrat 2022
<i>Bushera</i>	2.49	2.21	Muyanja et al. 2003
<i>Kocho</i>	3.62	1.44	Tamiru et al. 2021
Fiber contents			
<i>Ragi</i>	3.7	3.8	Padmaharish et al. 2018
<i>Medida</i>	1.17	2.00	Kabeir et al. 2004
Co-fermented M/C	5.34	2.31	Oyarekua 2011
Co-fermented S/C	4.94	4.35	
<i>Injera</i>	2.81	2.74	Asrat 2022

*Indicate starch, ** indicate reducing sugar, M/C=millet and cowpea blend, S/C=sorghum and cowpea blend

consistency in the quality and safety of products (Abe-gaz et al. 2002; Skřivan et al. 2023). In spontaneous fermentation, the fermenting microorganisms come from the environment or back slopping (Ashenafi and Mehari 1995; Bacha 1997). However, in modern fermentation, microorganisms are selected and/or improved starter cultures (Adrio and Demain 2006; Skřivan et al. 2023). During the fermentation process, microorganisms usually consume carbohydrates as major substrates for metabolic processes. However, most fermented foods and beverages contain a complex mixture of carbohydrates, proteins, and fats undergoing modifications simultaneously by the action of microorganisms and their enzymes (Assohoun et al. 2013; Hasan et al. 2014).

Fermented products can play an important role in contributing to the livelihoods of rural and peri-urban dwellers through enhanced food security, and income generation via a valuable small scale enterprise option (Marshall and Mejia 2011). There is a diversity of fermentable substrates available year-round so the activity can provide a regular income. Today, fermentation is still widely practiced as a household or village-level technology in many countries (Holzapfel 2002). Therefore, the aim of this review is to compare, contrast and summarize the scientific data on the effect of fermentation on physicochemical properties, anti-nutritional factors and sensory properties of cereal-based fermented foods and beverages.

Effect of fermentation on proximate composition of cereal-based fermented foods and beverages

Fermentation improves the nutritional quality of foods and beverages (Table 1). According to the findings of some authors, fermentation increases the essential amino acids of fermented food and beverage products (Lee et al. 1999; Agrahar-Murugkar and Subbulakshmi 2006). Similarly, Svanberg and Lorri (1997) and Mugula et al. (2003) reported that the fermentation of cereals with lactic acid bacteria and yeast cultures has been shown to increase the protein content of fermented foods and beverages. Natural fermentation of maize and soybean increases total soluble solids and non-protein nitrogen and slightly increases the protein contents of end products (Kiers et al. 2000; Nwokoro and Chukwu 2012). During natural fermentation to produce *Kinema*, there was little change in the crude protein and fats of the product (Shrestha and Noomhom, 2002). Like-wise, many researchers reported the increment of protein content during cereal-based spontaneous fermentation such as *Akamu*, *Borde*, *Shamita* (beverage), *Ragi*, and *Medida* (Ashenafi and Mehari 1995; Kabeir et al. 2004; Nwokoro and Chukwu 2012; Padmaharish et al. 2018).

The protein content of complementary foods prepared from millet, sorghum, pumpkin, and amaranth seed

flours was reported to increase during 36 h of fermentation (Simwaka et al. 2017). Similarly, the protein content of 'Hawaijar' a fermented soybean product, increased after 72 h of fermentation (Chaudhary et al. 2018). Bacha (1997) reported that the concentration of soluble protein increased in *Borde* and *Shamita* at 12 h of fermentation when the products were ready for consumption. Also, Kabeir et al. (2004) compared the protein content of spontaneously fermented and *Bifidobacterium Longum* BB 536 fermented *medida* in which the protein contents of *Bifidobacterium Longum* BB 536 fermented *Medida* increased twice that of the spontaneously fermented counterpart. However, Marko et al. (2014) who studied the performance of lactic acid fermentation on eight samples of cereal and pseudo-cereal flours concluded that the total protein content did not significantly change during fermentation in the majority of the cereal substrates.

The proteolysis of protein during fermentation produces peptides and amino acids which increase the soluble protein contents; however, as the fermentation continues amino acids metabolized to ammonia and flavor compounds which decrease protein contents (Pranoto et al. 2013). Some authors reported that the extent to which proteins are increased or decreased depends on fermentation conditions and types of microorganisms (Ikeda and Nakagawa 2003; Onwulata and Konstance 2006; Omafuvbe 2008; Pranoto et al. 2013). Most of the hydrolytic enzymes produced by *Escherichia coli*, *Saccharomyces cerevisiae*, *Pseudomonas dacunhae* and *Cryptococcus luredii* are used to produce optically pure D and L- amino acids in higher concentrations (Ikeda and Nakagawa 2003). Omafuvbe (2008) reported that proteolytic activities in *Dawadawa* fermented at the optimum temperature of 35°C were the highest; but there were no proteolytic activities at 25°C.

The major carbohydrate in cereals is starch, one of the major sources of energy (calories) for people around the world (Chaves-López et al. 2014). Fermentation activates starch-hydrolyzing enzymes such as α -amylase and maltase which degrade starch into maltodextrins and simple sugars leading to a decrease in the level of starch contents in fermented foods and beverages (Bacha 1997; Blandino et al. 2003; Osman 2011; Belay and Awraris 2014). Gudeta and Admassu 2017 also reported that the reduction of carbohydrates during fermentation might be due to increased microbial activities that use energy in metabolizing carbohydrates.

Several reports show that fermentation decreases the original carbohydrate content in due courses of fermentation. For instance, fermentation decreases the carbohydrate content of composite complementary food products made of Sorghum-Amaranth, Millet-pumpkin, Sorghum-Pumpkin-Amaranth, and Millet-Pumpkin-Amaranth (Simwaka et al. 2017). The total carbohydrate

content was reduced during cereal and pseudo-cereal flour fermentation with *Lactobacillus plantarum* (Marko et al. 2014). Similarly, in 'Akuma' And 'Medida', fermented porridges commonly consumed in Nigeria and Sudan, respectively, the carbohydrate content decreased with fermentation time (Kabeir et al. 2004; Nwokoro and Chukwu 2012).

Fermentation increases the crude fat contents of cereal-based fermented products such as 'Borde', an Ethiopian low-alcoholic beverage commonly consumed in the central and southern parts, and 'Doklu' produced and consumed in Côte d'Ivoire (Bacha et al. 1998; Assohoun et al. 2013). This might be due to the mass balance between fat and carbohydrate during fermentation or the formation of short-chain fatty acids by some types of microorganisms (Onyango et al. 2005; Morrison and Preston 2016). Shresth and Noomhom, 2002 reported that 'Kinema' fermentation led to substantial increases in the free fatty acids of the product. In contrast, the crude fat content of composite *Ogi* made from Millet-Cowpea and Sorghum-Cowpea decreased during fermentation time (Oyarekua 2011). The increment of some fatty acids and decrement of crude fat content might be due to the lipolytic activities during fermentation time (Tanasupawat et al. 2015). Marko et al. (2014) studied the effect of lactic acid fermentation with *Lactobacillus plantarum* on different cereals and pseudo-cereal flours in which a significant reduction in total lipids was observed during 24 h of fermentation time. The fat content of 'Medida', a Sudanese fermented thin porridge, decreased when spontaneously fermented but increased when fermented with an appropriate starter culture called *Bifidobacterium Longum* BB 536 (Kabeir et al. 2004).

The effects of fermentation on the ash contents have mixed findings with some researchers reporting that fermentation increases the ash contents of fermented products (Ashenafi and Mehari 1995; Kabeir et al. 2004); while others reported that fermentation decreases ash content (Oyarekua 2011; Assohoun et al. 2013). Simwaka et al. (2017) reported that the ash content of composite complementary food made of Sorghum-Amaranth, Millet-pumpkin, Sorghum-Pumpkin-Amaranth, and Millet-Pumpkin-Amaranth flour decreased during fermentation. Similarly, Igbabul et al. (2014) reported that during the fermentation of cocoyam flour, the ash contents decreased gradually with fermentation. However, the ash contents of *Borde* and *Shamita* increased from 1.6 to 3.66 and 2.1–5.92%; respectively, during fermentation for 24 h (Ashenafi and Mehari 1995). Thus, there was no conclusive information on the effects of fermentation on the ash content of the final fermented product.

Dietary fiber is defined as the sum of lignin and polysaccharides such as arabinoxylans, β -glucans, cellulose, resistant starch, fructans, and lignin that are not digested

by the human digestive enzymes (Dhingra et al. 2011; Bach Knudsen et al. 2017). Like carbohydrate contents, fermentation decreases the content of dietary fiber due to the enzymatic breakdown of the fiber structure by lactic acid bacteria (Vázquez and Murado 2008). During the fermentation of cocoyam flour, co-fermented millet and cowpea mixture, and *Ogi*, the fiber contents decreased throughout fermentation (Oyarekua 2011; Igbabul et al. 2014). In the same way, Ojokoh et al. (2013) reported that the fiber content of composite flour made of breadfruit and cowpea decreased during fermentation.

Fermentation decreases the molecular weight of β -glucans and modifies the residues ratios of fermented food products which have an impact on the physiological activities of polysaccharides (Lu et al. 2019; Tsafarakidou et al. 2020). Xiao et al. (2020) reported that fermentation by *Lactobacillus plantarum* dy-1 altered the state of β -glucan from a compact form (rod-shaped) in the raw barley to a smooth sheet-like structure in the fermented barley which may contribute to enhancing water adsorption or the molecular binding ability of the end product.

Table 2 Effect of fermentation on mineral contents of cereal-based fermented foods and beverages

Name of products	Name of minerals	Raw materials	Fermented end products	References
<i>Doklu</i>	Ca	0.05	0.03	Assohoun et al. 2013
	P	0.17	0.14	
	Na	0.01	0.01	
<i>Ragi</i>	Ca	43.91	40.5	Padmaharish et al. 2018
Fermented maize flour	Ca	0.05	0.03	Assohoun et al., 2013
	P	0.17	0.14	
	Na	0.01	0.01	
Co-fermented of millet and cowpea	P	127	128	Oyarekua 2011
	Ca	40.5	31.6	
	Na	2.0	5.6	
	Fe	7.9	34.7	
	Zn	3.4	1.41	
Co-fermented of sorghum and cowpea	Mg	133.8	16.2	Oyarekua 2011
	P	1812	178	
	Na	2.0	4.0	
	Ca	49.16	20.6	
	Mg	217.6	56.6	
<i>Injera</i>	Fe	6.1	14.2	Asrat 2022
	Zn	3.6	1.4	
	Fe	6.81	7.32	
	Zn	3.03	3.20	
	Ca	78.44	82.83	
Complementary fermented food	Ca	54.42	43.75	Feyera et al. 2020
	Fe	5.14	4.21	
	Zn	4.41	5.24	

Effect of fermentation on mineral contents of cereal-based fermented foods and beverages

Minerals from plant sources have very low bioavailability as they are found complex with non-digestible materials such as cell wall polysaccharides and anti-nutritional factors (Reddy 2001; Schlemmer et al. 2009). Although the fermentation process enables to release of the bounded minerals, the effect of fermentation on mineral content is controversial (Table 2). It increases the bioavailability of iron, calcium, zinc, phosphorus, magnesium, and sodium in food products due to decreased phytates (Pranoto et al. 2013; Day and Morawicki 2016). Production of phytase enzyme during fermentation hydrolysis the phytate and produces various inositol and phosphates. Prolonged fermentation decreases the tannin due to microbial phenyl oxidase action; the transformation of tannins to phenols occurring during fermentation increases phenol content (Emmambux and Taylor 2003).

Svanberg and Lorri (1997) reported that fermentation by lactic acid bacteria was observed to improve the iron bioavailability of fermented products of sorghum. Microbial strains affect the contents of minerals during fermentation. During controlled fermentation of *Medida* using *B. longum* BB 536, the calcium content of the product was recorded as 378.67 mg/L which was much higher than the value in spontaneously fermented *Medida* (3.70 mg/L) (Kabeir et al. 2004). However, the iron, magnesium, and zinc content of *Medida* fermented by the same strain (*B. longum* BB 536) were lower than the spontaneously fermented product. Oyarekua (2011) reported that the iron content of composite *Ogi* made of Millet-Cowpea and Sorghum-Cowpea increased during fermentation from 7.9 to 34.7 mg/100 g, and 6.1 to 14.2 mg/100 g, respectively. This might be due to the increment of the bioavailability of minerals during fermentation as phytates that form complexes with minerals degraded by phytase (Sripriya et al. 1997; Pranoto et al. 2013).

Effect of fermentation on anti-nutritional factors of cereal-based fermented foods and beverages

Fermentation is an important process that significantly lowers the content of major anti-nutrients of cereal grains such as phytic acid and tannin (Sindhu and Khetarpaul 2001). Some microorganisms play a role by degrading the anti-nutritional factors of foods during fermentation time (Igbabul et al. 2014; Ngongola-Manani, 2014). Simwaka et al. (2017) reported the phytate content of Sorghum-Amaranth, Millet-Amaranth, Sorghum-Pumpkin, Millet-pumpkin, and Millet-Pumpkin- Amaranth flours were reduced by 60.91%. Phytic acid contents also decreased during the fermentation of different maize cultivars (Cui et al. 2012). They reported that phytic acid content was reduced by 5.4, 18.8, 23.6, and 24.3% after fermentation

for maize cultivars of J₂, B, S, and J₃, respectively. The loss of this phytic acid during fermentation is due to the activities of endogenous phytases from raw materials and those produced by fermentative microorganisms (Hotz and Gibson 2007). The optimal temperature for phytase activity has been known to range between 35 °C and 45 °C (Sindhu and Khetarpaul 2001).

Tannin levels were reduced during lactic acid fermentation; as a result, iron bioavailability of cereal-based fermented foods increased (Nout and Ngoddy 1997). The fermentation of formulated Sorghum-Amaranth, Millet-Amaranth, Sorghum-Pumpkin, Millet-pumpkin, Sorghum-Pumpkin-Amaranth, and Millet-Pumpkin-Amaranth flours slightly decreased tannin contents (Simwaka et al. 2017). A decrease in tannin is due to the production of the tannase enzyme by *Lactobacillus* spp. during fermentation (Molin 2008). However, Elyas et al. (2002) reported the absence of any change in the tannin content of fermented dough of millet after 36 h of fermentation at room temperature.

Effect of fermentation on pH and titratable acidity of cereal-based fermented foods and beverages

During fermentation, the pH of fermented food and beverage products decreases as the amount of organic acid increases (Table 3). This could be accounted to microbial activities, especially of lactic acid bacteria that convert carbohydrates into different organic acids such as lactic acid, acetic acid, and butyric acid (Assohoun et al. 2013; Marko et al. 2014; Ukwuru et al. 2018). Among lactic acid bacteria, lactobacilli are the most important organisms that produced acidity and flavor during dough fermentation (Corsetti and Settanni 2007).

Production of acid during the natural fermentation of maize led to a significant reduction in pH which contributes to the enhancement of the shelf-life of products as well as the elimination of enteric pathogens (Mensah 1997). Beugre et al. (2014) evaluated the effect of fermentation time on the physico-chemical properties of maize

flour. The result showed that the total acidity of maize flour increased from 37.97 to 71.59 with an increase in the time of fermentation, while its pH decreased considerably from 6.67 to 3.85. As it is well known, when pH decreases, the titratable acidity increases. This could be due to acid-producing microorganisms breaking down sugars to produce different acids and other secondary metabolites (Ukwuru et al. 2018).

Effect of fermentation on phytochemical contents of cereal-based fermented foods and beverages

Phytochemicals are plant secondary metabolic products important in human nutrition and health which are produced in phenylpropanoid biosynthesis and shikimate pathways during the growth of plants (Golzarand et al. 2014). In cereal grains, most polyphenol compounds are bound with cell wall polysaccharides. Bounded polyphenols are not bioavailable (Adom and Liu 2002) as health-promoting factors. Fermentation is considered one of the best processes to enhance the release of such bound phenolic and flavonoids to increase the antioxidant activities of fermented food products (Sandhu et al. 2016; Martins et al. 2011). Kariluoto et al. (2006) reported that fermentation has a positive influence on the phytochemicals of cereals, but the degree of influence depends on the species of microorganism involved in fermentation. On the other hand, Zheng and Shetty (2000) reported that improvement in phenolic compounds is due to the action of enzymes such as β -glucosidase, α -amylase, and lactase.

Fermentation increases the phenolic and flavonoid contents of food products with an increase in antioxidant properties, whereas there is a decrease in anti-nutritional factors (Prabhu et al. 2014). Djordjević et al. (2010) reported an increase in antioxidant activity from 45.0 to 50.4% in the rye, 36.6 to 42.9% in barley, and 31.0 to 35.9% in wheat after fermentation with *L. rhamnosus*. Cai et al. (2012) also reported that oats fermented by three different fungi i.e., *Aspergillus oryzae* var. *effuses*, *Aspergillus oryzae*, and *Aspergillus niger* for 3 days at 25 °C improved

Table 3 Effect of fermentation on pH and titratable acidity of cereal-based fermented foods and beverages

Name of fermented products or substrates	Initial total titratable acidity (%)	Final total titratable acidity (%)	Initial pH of products	Final pH of products	Organic acid produced	References
Doklu	0.02	0.4	5.4	2.7	Lactic and acetic acids	Assohoun et al. 2013
Borde			6.01	3.84		Abegaz et al. 2002
Ibyer	0.14	0.15	6.16	5.69		Kure and Wyasu 2013
Wheat flour fermentation	4.90	1437.5	7.00	4.90	Lactic acids	Marko et al. 2014
Millet flour fermentation	957.3	1998.9	6.62	4.95	Lactic acids	Marko et al. 2014
Maize flour fermentation	0.3	0.4	3.2	2.7	Lactic acid and acetic acid	Assohoun et al. 2013
Akamu	0.48	0.79	6.6	3.9	-	Nwokoro and Chukwu 2012
Korefe	0.84	3.2%	5.18	4.0	Lactic acids	Getnet and Berhanu 2016
Kenkey	0.45	1.35	4.8	4.2	-	Fadahuni et al. 2012

the phenolic acid profile of the original substrate. Djordjević et al. (2010) demonstrated that *Lactobacillus rhamnosus* releases total phenolics more efficiently than *Saccharomyces cerevisiae* during fermentation of cereals. Different reports indicate that β -glucosidase produced by *L. plantarum* cleave glucoside bonds between phytochemicals and sugars, releasing them in simple utilizable forms (Dueñas et al. 2005; Marko et al. 2014). Cui et al. (2012) reported that the polyphenol contents significantly increased after the fermentation of different maize cultivars. They reported that the total phenolic content of untreated four maize samples was 0.98, 0.95, 0.91, and 0.96 mg GAE/g which increased to 1.19 (22%), 1.17 (22.5%), 1.10 (21.6%) and 1.18 mg GAE/g (23.4% increase) for J₃, J₂, S, and B maize cultivars, respectively.

Effect of fermentation on sensory properties of cereal-based fermented foods and beverages

Cereal grains are considered to be one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals, and fiber for people all over the world; however, the sensorial properties of their products are inferior as compared to animal products (Blandino et al. 2003). Fermentation improves cereal-based food and beverage products' texture, taste, and aroma (Blandino et al. 2003). In addition, the report indicated that during cereal fermentation different types of volatile compounds are produced and contribute to the development of complex flavors in fermented foods. The presence of aromas represented by diacetyl, acetic acid, and butyric acid makes fermented cereal-based products more appetizing. The proteolytic activity of fermenting microorganisms often in combination with malt enzymes may produce precursors of flavor compounds, such as amino acids, which may be deaminated or decarboxylated to aldehydes (Mugula et al. 2003) as flavor enhancer compounds.

The development of sensory characteristics during fermentation is due to the biochemical processes where the starter cultures produce enzymes that modulate the flavor profile of the product (Senanayake et al. 2023). In the first stages of fermentation, the enzymes produced break down complex nutrients into simple compounds (Smit et al. 2005). Some of these simple compounds are peptides, amino acids, fatty acids, glucose, maltose, and galactose, which act as precursors for the development of sensory characteristics during fermentation (Bintsis 2018). As the fermentation progresses, the starter cultures continuously convert these simple compounds into different organic compounds such as organic acids, alcohols, esters, and ketones, which give the final product a unique flavor and aroma (Liu et al. 2020).

Conclusion

This review concluded that fermentation improves the crude protein and fat contents, and the bioavailability of minerals and phytochemicals. However, it decreases the carbohydrate and crude fiber contents, while the finding on the effect of fermentation on ash content is not conclusive. Fermentation also improves the sensory properties of cereal-based foods and beverages as the sensory properties of cereals are inferior as compared to animal products. Fermentation decreases the anti-nutritional factors such as tannin and phytic acid which are high in cereal crops. Therefore, consumption of fermented foods and beverages has greater benefits than their raw materials, especially for vulnerable groups.

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DAK: Conceptualization, designing, validation, writing, review analysis and editing.

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