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Article

Effect of Fermentation Time and Blending Ratio on Microbial Dynamics, Nutritional Quality and Sensory Acceptability of *Shameta*: A Traditional Cereal-Based Fermented Porridge for Lactating Mothers in Ethiopia

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Abstract: Ethiopia has one of the highest levels of malnourished lactating mothers in sub-Saharan Africa. Traditionally, different communities prepare foods solely for lactating mothers. For example, "*Shameta*" is one of the cereal-based fermented cultural foods exclusively produced for lactating mothers with the perception that it would support the health, increase strength, and promote the recovery process of mothers after childbirth. This study investigated the effects of fermentation time and blending ratio on the nutritional quality of "*Shameta*." Three levels of blending ratio of ingredients (Maize-Barley-Fava bean) and three levels of fermentation times were laid down in a completely randomized design (CRD). The study showed that lactic acid bacteria was the dominant group, followed by yeasts. Notably, the ingredient formulation ratio of Maize-Barley-Fava bean (81:5:5) had the highest LAB dominance with the highest crude fat (13.23 g/100g) content in all fermentation times (8, 10, and 12 days). However, the highest crude protein (16.56 g/100g) and mineral contents were observed in a ratio mix of 66:10:15 fermented for 12 days. The results of this study indicate that the nutritional quality of culturally prepared *Shameta* can be improved by optimizing fermentation time and ingredient compositions for fast recovery, increased strength, and improved health of lactating mothers.

Keywords: fermentation time; formulated *Shameta*; lactating mothers; blending ratio

1. Introduction

Traditional fermentation of foods often leads to improved nutritional quality [1]. The process enables the breakdown of complex compounds and the synthesis of vitamins and other growth factors for a better diet [2]. During lactic acid fermentation, the solubility of proteins and amino acids is enhanced [3], while the concentrations of anti-nutrient factors are decreased [4]. In addition, the fermentation increases mineral and trace elements' bioavailability by reducing the non-digestible material of plants such as glucuronic, polygalacturonic acids, cellulose, and hemicelluloses [5]. However, the nutritional quality of the final product can be affected by ingredient composition and fermentation time [6].

Globally, about 14 million adolescent girls become lactating mothers each year, and more than 90% of them are living in the developing world [7]. As a result of limited resources, lactating mothers in developing countries are often exposed to undernutrition due to increased physiological requirements and the multiple roles played by mothers [8]. Insufficient dietary diversity, unbalanced

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distribution of food within households, improper food storage and preparation, dietary taboos, and infectious diseases all contribute towards undernutrition among lactating mothers [9].

Some studies carried out in different parts of Ethiopia showed that the prevalence of underweight (BMI < 18.5 kg/m^2) among lactating women ranges from 20-40.6% [10,11]. In Ethiopia, an estimated number of 404,000 pregnant women and lactating mothers are at risk of malnutrition [7]. Another study also showed that one of every four lactating mothers in Ethiopia is undernourished [9].

To address this problem, in different parts of the country and communities in Ethiopia, several indigenous foods are exclusively produced for lactating mothers, usually following the spontaneous fermentation process. *"Shameta"* is one of the cereal-based fermented foods commonly produced by the Oromo community in the western part of Ethiopia. The production practices, utilization patterns, and nutrient contents are described elsewhere [12,13].

Cognizant of the significance of fermentation, lactating mothers in the western part of Ethiopia prepare and consume *Shameta* from the day they give birth.Unlike other staple foods, the community perceives "*Shameta*" as able to provide rapid recovery and strength for the mother and increase breast milk for the newborn. *Shameta* is mainly prepared from maize, barley, sorghum and wheat. Vegetable oil, spices, and herbs are also added as flavor enhancers and preservatives [13]. Unlike other fermented products in the country, *Shameta* preparation involves a double fermentation process with intermediate cooking between two fermentation stages [13]. The process involves one week in the first fermentation phase, followed by intermediate cooking to make porridge, and is eventually subjected to 20-30 days in the second fermentation phase [13]. Therefore, it is necessary to investigate the effects of the blending ratio of ingredients and fermentation time to enhance the benefits of *Shameta*, an affordable and alternative nutrient-rich diet source for mothers. Therefore, the purpose of this study is to determine the effects of ingredient mix ratio and fermentation time on microbial succession during fermentation time, investigate the dynamics in the physicochemical properties, and nutrient contents of ready-to-consume *Shameta*.

2. Materials and Methods

2.1. Experimental materials

In this study, maize (*Zea mays*), barley (*Hordeum vulgare*), and fava bean (*Vicia fava*) were used as significant ingredients bought from the local market of Nekemte town. Other ingredients, fenugreek (*Trigonella foenumgraecum*), black cumin (*Nigella sativa*), black cardamom (*Elettaria cardamomum*), and white cumin (*Cuminum cyminum*) were also bought from the same market. Rapeseed (*Brassica napus*) was also used as an oil source during *Shameta* preparation.

2.2. Experimental design and plan

The experiment was laid out in a complete factorial design consisting of two factors: the blending ratio of primary ingredients [12,13] and fermentation time [14]. Three blending ratios of maize: barley: fava bean (81:5:5, 71:10:10, and 66: 10: 15) and three levels of fermentation times (8, 10, and 12 days] were used based on our previous works [14].

2.3. Samples preparation

Preparation of maize, barley, and fava bean flours: Maize grain was sorted, cleaned, and washed by immersion in cold tap water, stirred very well, and the impurities were removed by decanting. The bran of barley was removed with the help of a wooden mortar and pestle. Kernels of maize and barley were then sun-dried and milled into flour using a flour miller to a sieve size of 0.5 mm. Finally, the flour was packed and stored in moisture and airproof plastic bags at room temperature.

The fava bean was sorted and cleaned, and a hammer mill removed the seed coat. Then, after dehulling, it was milled into flour using a flour miller to a sieve size of 0.5 mm. Finally, the fava bean flour was packed in air and moisture-proof plastic bags and stored at room temperature.

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Preparation of rapeseed: During the preparation of rapeseed, 1.18 kg of the seed was ground using a wooden mortar and pestle until oil was visible in the mortar. The ground rapeseed was transferred to a clay pot (6 L holding capacity) where hot water (3 L/1kg) was added and covered for three days at ambient temperature to allow equilibration and incubation for maximum potential to extract the oil. After three days of incubation, 1.2 L of hot water was added to the crude to allow the separation of the oil. The oil was eventually extracted from the crude by decanting the upper layer, followed by multiple pressing and decanting the residue using cloth and a sieve. Although commercially purified oils are available on the market. Pregnant mothers in the community prefer rapeseed oil extracted using traditional methods rather than commercially available oil.

Preparation of spices: Spices used in *Shameta* preparation such as fenugreek (*Trigonella foenum-graecum*) (51 g), black cumin (*Nigella sativa*) (25.25 g), black cardamom (*Elettaria cardamomum*) (26.56 g), white cumin (*Cuminum cyminum*) (23.67 g), and ginger (*Zingiber officinale*) (20.62 g) were roasted on the metal griddle, cooled to ambient temperature and ground using spice grinding machine. Garlic bulb and basil leaves were cleaned and washed fresh without size reduction. During the second fermentation stage, bulbs of garlic and basil leaves were placed in different layers of the porridge strata (Figure 1).

2.4. Composite flour for Shameta preparation

The flour composition was determined considering the availability of major ingredients and with the potential to improve the nutrient content of the final product (Kitessa et al. 2022a and b). The nutrient content considered for improvement was the proximate compositions and mineral contents.

2.5. Fermentation of composite ingredients

The above formulations (13.38 kg) were mixed with 18 L of tap water in a 30 L holding capacity plastic bucket to make the dough. The dough was then left to ferment for two days for the first fermentation stage, followed by cooking for 1:30 hrs to make the porridge. Extracted rapeseed oil and spice powders (147.1 g) were added before the porridge was fully cooked. For the second-stage fermentation, overnight cooled porridge to ambient temperature was transferred to 9 vessels (5 L capacity each). At the same time, garlic bulbs (30 bulbs) and basil leaves (12 basil branches) were placed (laid) at different strata of the porridge. Finally, the porridge was allowed to ferment for 12 days with intermittent sampling on three occasions during fermentation (on the 8th, 10th, and 12th days).

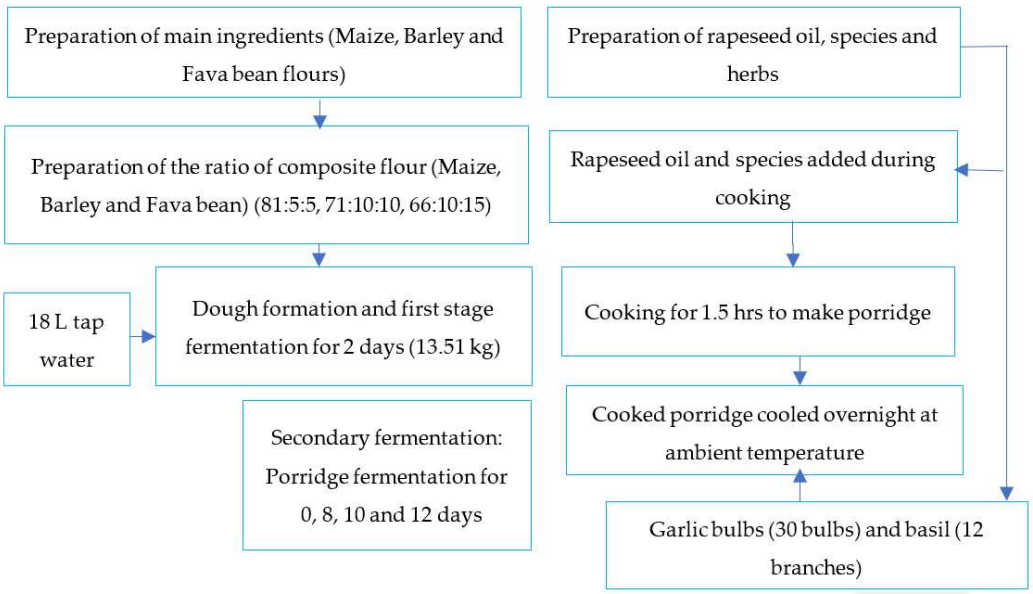

Figure 1. Flow diagram of preparation steps of *Shameta*.

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Zero-day fermentation implies the fresh porridge mixed with garlic bulbs and basil leaves in the vessel after overnight cooling before it was subjected to the second fermentation phase as a control sample to assess the impact of fermentation duration on the quality of *Shameta*. The duration of fermentation was selected based on the results of a previous report [14].

2.6. Determination of microbial dynamic in the second phase of fermentation

Counting dominant microbial groups during fermentation followed standard microbiological methods [15]. For microbial analysis, a 10g sample was aseptically transferred into 90ml sterile peptone water at zero, eight, ten, and twelve days and homogenized in a flask at 100 rpm for 10 min on a shaker (Compact shaker, D-72379 Hechingen, Germany). After homogenization, one ml of the sample was aseptically transferred into 9 ml of pre-sterilized peptone water and mixed thoroughly using a vortex mixer. The homogenate was serially diluted up to 10^{-7}, and 100 µl of the dilution was transferred to an agar medium and incubated at an appropriate temperature and duration to conduct the enumeration as indicated below.

Aerobic Mesophilic Bacteria (AMB) counts were inoculated onto Plate Count Agar (PCA); duplicate samples were incubated at 32°C for 48 hrs.

***Enterobacteriaceae* counts:** A 100 µl aliquot from appropriate dilution was spread plated in duplicate on Violet Red Bile Glucose agar and incubated at 32°C for 24 hrs, and purple/pink colored colonies surrounded by purple halos were counted as members of *Enterobacteriaceae*.

Total coliform counts: A volume of 100 µl aliquot of serially diluted sample was inoculated into Trypticase soy agar (TSA) (Oxoid) after 30 minutes of overlay with Violate Read Bile agar (VRBA) medium (BFCO). The plates were incubated at 37°C for 24 hrs.

Aerobic spore-forming bacteria counts (ASFB): For ASFB counts, 10 ml of appropriate dilution was heat treated in a water bath adjusted to 80°C for 10 min and cooled rapidly under tap water. Then, a volume of 100 µl aliquot from appropriate dilution was spread-plated on nutrient agar (NA) and incubated at 32°C for 72 hrs.

Lactic acid bacteria counts: From appropriate dilutions, 100 µl aliquots were spread plated in duplicate on pre-dried surfaces of MRS (de-Mann, Rogosa, and Sharp) agar (Oxoid) plates. The plates were incubated under anaerobic conditions using an anaerobic jar (BBL, anaerobic Jar System) at 30-32°C for 48 hours [16].

Staphylococci counts: A volume of 100 µl of the aliquot from appropriate dilution was spread plated on Mannitol Salt Agar and incubated at 32°c for 48hrs, and then, yellow colonies surrounded by red color were counted as staphylococci.

Yeast and mold count: Yeast and mold were counted by inoculating 100 µl sample on PDA (BFCO) medium supplemented with 200 mgL-1 chloramphenicol and incubated at 25°C for five days.

2.7. Sample preparation for analytical measurements

Except for moisture content determination, samples were dried at 50°C for 24 to 30hrs to reduce moisture content and converted into flour for further analysis. The flours were then packed into moisture-proof plastic bags and stored in air-tight containers at 4°C until used for analysis. All chemicals and reagents used were analytical grades.

2.8. Determination of pH and titratable acidity (TA.)

The pH was measured using a digital portable pH meter (pH-013, China) after homogenizing 5g of the *Shameta* sample in 20 ml distilled water, followed by pipetting 10 ml of the homogenized sample into a beaker [17]. The TA of "*Shameta*" was determined by homogenizing 2.5 g of the sample in 10 ml of distilled water and filtering it through the Whatman No. 1 filter paper [18]. In the filtrate, 3 to 5 drops of one gram per 100 ml phenolphthalein indicator were added, and samples were titrated with freshly prepared 0.1 mol/L NaOH solution until a faint pink color persisted for 30 seconds. The TA value was determined according to the following equation, considering lactic acid as a dominant organic acid in the sample.

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$$\% \text{ Lactic acid (wt/v)} = \frac{N x V_{NaOH} x Eq.\ wt x 100}{Vs(ml) x 1000}$$

where N = normality of titrant (mEq/ml), V_{NaOH} = Volume of titrant (ml), Eq. wt = Equivalent weight of predominant acid (mg/mEq, which is 90.08 for lactic acid), Vs = Volume of sample (ml) and 1,000 = factor relating mg to grams.

2.9. Determination of proximate composition and gross energy

Proximate composition, i.e., crude protein, fat, fiber, ash, and moisture content, was determined according to AOAC [19]. The utilizable carbohydrate content was determined by the method described by FAO [20], and gross energy was determined by Atwater's conversion factors [21].

2.10. Determination of minerals content

The contents of calcium (Ca), iron (Fe), and zinc (Zn) were determined by an atomic absorption spectrophotometer (SH 90273010, China) ([22] official method No985.35). Lanthanum was used to compensate for ionization interferences in the analysis of Ca.

2.11. Determination of anti-nutritional factors

2.11.1. Determination of condensed tannin contents

Condensed tannin contents in *Shameta* samples were determined according to Maxson & Rooney [23]. Briefly, ten ml of 1% HCl solution was added to methanol in a test tube with a screw cap, to which one gram of sample was added and mixed. In order to extract the condensed tannin in the cell wall matrix, the solution was shaken for 24 hrs at ambient temperature on a mechanical shaker [Hy-2(C), Shanghai, China], followed by centrifugation (sigma 2-16KC, UK) at 1,000 rpm for 5 min. Then, one ml of centrifuged supernatant was transferred to another test tube and mixed with 5 ml of vanillin-HCl reagent. For the standard preparation, the D (+)- catechin was weighed (40 mg)and dissolved in 1,000 ml of 1% HCl solution in methanol to make a series of 0, 10, 25, 40, 60, and 80 µg /ml. Finally, the absorbances of samples were measured at 500nm using a UV-VIS Spectrophotometer (JASCO V-630, Shimadzu Corporation, Tokyo, Japan). The condensed tannin content was determined from the standard curve of catechin and expressed as mg/100g.

2.11.2. Determination of phytate contents

Phytate content was determined as described by Vaintraub and Lapteva [24]. Accordingly, about 0.5 g of sample was weighed and mixed with 10 ml of 2.4% HCl, followed by further mixing using a mechanical shaker (Hy-2(C), Shanghai, China) for one hour at ambient temperature. After the sample was centrifuged (Sigma 2-16KC, UK) at 3,000 rpm for 30 min, the clear supernatant was taken for phytate determination. Three ml of the supernatant was mixed with one ml of Wade reagent and mixed thoroughly on a vortex for 5sec. The standard solution was prepared from sodium phytate in 0.2N HCl to make a series of 0.0, 5.5.0, 12.0, 25.0, 40.0, and 55.0 µg/ml. One ml of the Wade reagent was added to each sample in test tubes and mixed thoroughly using Vortex for 5 sec. The mixture was centrifuged for 10 min and measured at 500 nm using a UV-VIS spectrophotometer (JASCO V-630, Shimadzu Corporation, Tokyo, Japan). The phytate content was determined from the standard curve and reported in mg/100g.

2.12. Determination of total antioxidant activities

The antioxidant activities of the extract were determined by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assays, as described by Villano and others [25]. One mg of each extract was mixed with 4 ml solution (0.004 g DPPH in 100 ml methanol) and incubated in the dark at ambient temperature for one hour. The absorbance of the mixture was then measured at 517 nm using a UV spectrophotometer

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(Model SP9, PyeUnican UK). The concentrations for the standard calibration curve were 0.0, 6.50, 12.50, 25.00, 50.00, and 100.00 µg/ml. The concentration of total antioxidant activities was expressed in milligrams of ascorbic acid equivalent (AAE) per gram of sample from the calibration curve (R^2= 0.9973).

2.13. Sensory evaluation

Sensory evaluations of the formulated *Shameta* were carried out in the East Wallaga zone, Ethiopia. Based on data from this study, two *Shameta* samples were selected and prepared together with two more control samples. The control samples were prepared from different ingredients based on local experiences. For the evaluation, 55 untrained panelists of lactating mothers who have experience in the preparation and consumption of *Shameta* were randomly selected for sensory evaluation. The selected mothers were informed of the evaluation and grading methods of the samples. The samples were coded to separate one judge from the others. Sensory attributes such as aroma, color, taste, texture, and overall acceptability were analyzed. Sensory evaluation was conducted in the morning between 10.00 am and 11.00 am. A five-point hedonic scale was used as 5 = Like extremely, 4 = Like slightly, 3 = Neither like nor dislike, 2 = Dislike slightly, and 1 = Dislike extremely.

2.14. Data analysis

Microbial counts and physicochemical properties samples were analyzed using analysis of variance (ANOVA) and least significant difference (LSD). The samples were statistically analyzed using SAS (SAS Institute, Cary, NC, USA) version 9.3, and the significance threshold was $p \leq 0.05$. Fisher's least significant difference (LSD) was used to identify significant differences among means ($p \leq 0.05$). The coefficients of variation (CV) were calculated from the standard deviations and means of the parameters and expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Microbial dynamics of Shameta fermentation

There was a significant difference ($p \leq 0.05$) in mean microbial counts (log cfu/g) among fermentation times for all formulated samples (Table 1). In the present results, the mean counts (log cfu/g) of lactic acid bacteria (LAB) in MBF_1 increased from 3.75 to 8.41, 8.88, and 9.41 on days 8, 10, and 12 of fermentation, respectively. However, the growth rate between the 8^{th}, 10^{th}, and 12^{th} day was <2 log cfu/g. In the case of MBF_2 and MBF_3, the mean colony counts (log cfu/g) at the start of fermentation were 4.45 and 4.44 which later increased to 7.95 and 7.71 on the eighth day, respectively. However, colony counts were reduced on the 10^{th} and 12^{th} days of fermentation with a reduction rate of <2 log cfu/g. The colony counts of LAB in MBF_1 samples fermented for 8, 10, and 12 days were higher than the fermentation of other formulations (MBF_2 and MBF_3) for the same days of fermentation. The highest counts in MBF_1 samples might be due to the availability of more carbohydrate-rich substrates, including maize, compared to fava bean [26]. The availability of fermentable cereals and favorable conditions created during fermentation time may haveaffected the nature of microbial dynamics [27,28]. Nemo and Bacha [29[reported lower mean counts of LAB (6.87 log cfu/g) in *Borde*, whose fermentation time was shorter than that of MBF_1, formulation fermented for 12 days, but the mean count was greater than that of MBF_2 and MBF_3. Cereal fermentation is usually characterized by a complex microbial ecosystem dominated mainly by LAB species and yeasts [30].

Members of AMB can grow in an aerobic environment under moderate temperatures as an indicator of the quality of ready-to-eat foods and help to estimate the shelf life of stored products [31]. In the present finding, fermentation time showed significant difference ($p<0.05$) in AMB count , with the lowest colony count from the MBF_3 sample (3.85 logcfu/g) fermented for 12 days (Table 1).With an increase in fermentation time, the AMB count for MF_3 decreased from 5.54 to 3.85 log cfu/g. The drop in count might be associated with an increase in the LAB population and acidification

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of the medium as the fermentation proceeds. Similar to the present results, in many cereal-based fermented food products such as *Keribo*, *Akamu*, and sourdough used for bread preparation, AMB persists until the final stage of fermentation but with relatively lower counts [32–34]. In addition, a more or less comparable mean AMB count (3.36 log cfu/g) was observed for *Shameta* samples collected from households of lactating mothers that fermented for at least for two weeks [14].

On the other hand, Enterobacteriaceae and TC showed more or less similar trends and counts during the fermentation of all formulations. At zero hour of fermentation, the mean counts of both Enterobacteriaceae and TC showed a significant difference ($P< 0.05$) in all formulations; however, after the onset of fermentation, they showed no significant difference ($P>0.05$) with mean colony counts less than 2 log cfu/g. It might be associated with an increase in the dominance of LAB with an increase in fermentation time inhibiting their growth through the production of antimicrobial compounds regardless of variation in ingredients composition [28,35].

In addition to their role in foodborne illness, some members of the Enterobacteriaceae family are associated with food spoilage, which results in significant economic losses in the food industry [36]. Some of these microorganisms might contaminate flours and continue in the processing chain of inadequate cooking products and post-processing contamination [36,37]. In the current results, the primary source of Enterobacteriaceae in the control sample of all formulated products might be contaminants from the fermentation vessels , garlic bulbs, and basil leaves introduced as flavoring ingredients [37,38], furthermore, the garlic bulbantimicrobial effects could contributed to control microbial growth[39]. Coliforms are commonly used as indicators of the sanitary quality of foods and water, as some are pathogenic enteric bacteria [38,39]. Water used for washing fermentation vessels, bulbs, and basil leaves during the second fermentation round could be another source of coliforms counted from the formulations [39].

Table 1. Effect of fermentation fermentation time (days) on Microbial counts (log cfu/g) of *Shameta*.

FC	FT (day)	Mean count (log cfu/g) of different microbial groups							
		Lactic acid bacteria	Aerobic mesophilic bacteria	Enterobacteriaceae	Total coliforms	*Staphylococcus* spp.	ASFB	Yeast	Mold
MBF_1	0	3.75+0.05^{d}	4.45+0.02^{c}	4.17+0.01^{a}	3.85+0.02^{a}	3.23+0.01^{a}	3.14+0.01^{c}	2.51+0.01^{d}	3.36+0.02^{a}
	8	8.41+0.01^{c}	6.43+0.03^{a}	<2	<2	3.21+0.01^{a}	3.42+0.01^{a}	5.94+0.02^{b}	2.75+0.01^{b}
	10	8.88+0.01^{b}	5.11+0.01^{b}	<2	<2	3.14+0.02^{b}	3.30+0.01^{b}	6.65+0.02^{a}	2.53+0.01^{c}
	12	9.41+0.01^{a}	4.16+0.02^{d}	<2	<2	2.48+0.01^{c}	2.83+0.02^{d}	4.83+0.01^{c}	2.52+0.02^{c}
CV		0.35	0.42	0.48	1.04	0.44	0.42	0.32	0.32
LSD		0.06	0.05	0.01	0.02	0.03	0.03	0.04	0.04
MBF_2	0	4.45+0.03^{d}	5.20+0.2^{a}	3.75+0.02^{a}	3.30+0.01^{a}	3.81+0.01^{a}	3.29+0.01^{a}	2.52+0.01^{d}	2.52+0.02^{a}
	8	7.95+0.03^{a}	5.44+0.02^{a}	<2	<2	3.38+0.01^{b}	3.28+0.01^{a}	6.45+0.03^{a}	<2
	10	7.56+0.03^{b}	4.61+0.01^{b}	<2	<2	2.52+0.02^{c}	2.58+0.01^{b}	5.26+0.03^{b}	<2
	12	6.28+0.02^{c}	4.04+0.04^{c}	<2	<2	2.47+0.02^{d}	2.51+0.01^{c}	4.43+0.01^{c}	<2
CV		0.42	2.13	1.07	0.61	0.52	0.30	0.48	0.64
LSD		0.07	0.26	0.02	0.01	0.04	0.02	0.05	0.01
MBF_3	0	4.44+0.02^{d}	5.54+0.01^{a}	3.82+0.02^{a}	3.03+0.01^{a}	3.22+0.01^{a}	3.64+0.02^{a}	2.22+0.01^{d}	2.47+0.01^{a}
	8	7.71+0.01^{a}	4.15+0.02^{b}	<2	<2	2.83+0.02^{b}	2.55+0.01^{b}	5.94+0.02^{a}	<2
	10	7.46+0.03^{b}	3.95+0.03^{c}	<2	<2	2.22+0.01^{c}	2.51+0.01^{c}	4.97+0.02^{b}	<2
	12	6.45+0.04^{c}	3.85+0.01^{d}	<2	<2	2.13+0.01^{d}	2.07+0.01^{d}	4.25+0.02^{c}	<2
CV		0.42	0.44	1.05	0.66	0.51	0.49	0.23	1.01
LSD		0.07	0.05	0.02	0.01	0.03	0.03	0.04	0.01

ASFB = Aerobic Spore Forming Bacteria; FC = Flour composition; FT = Fermentation time; MBF_1 = Maize-Barley-Fava bean with ratio 81:5:5; MBF_2 = Maize-Barley-Fava bean with ratio 71:10:10 and MBF_3 = Maize-Barley-Fava bean with ratio 66:10:15; CV = Coefficient of variation. Different letters in superscript along a column indicate a significant difference ($p\leq0.05$).

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There was a significant difference ($p \leq 0.05$) in means colony counts of *Staphylococcus* species between flour compositions and fermentation time (Table 1). Compared to the control samples, the counts of *Staphylococcus* species decreased with fermentation time at all stages. However, the reduction rate was higher in samples fermented for 12 days than for the 8th and 10th days. The colony counts of *Staphylococcus* species in MBF_1, MBF_2, and MBF_3 reduced from 3.23, 3.81, and 3.22 log cfu/g in control samples (0 hour fermentation) to 2.48, 2.47, and 2.13 log cfu/g at 12 days of fermentation, respectively. This implies that as the fermentation time increased, the counts of *Staphylococcus* decreased by about one log unit. Some *Staphylococcus* species are pathogenic, while others are responsible for the spoilage of foods, with coagulase-negative staphylococci playing an essential role in food fermentation [40–42]. Generally, in the present finding, except for the MBF_2 sample that fermented for eight days, the microbial counts at all stages of fermentation for all formulations were below the borderline (3.28 log cfu/g) of *Staphylococcus aureus* and other coagulase-positive staphylococci in foods [43]. This implies that except for the MBF_2 sample fermented for eight days of fermentation time, others could be consumed with minor treatment with spices and salt, which are typical side dishes and appetizers during *Shameta* consumption.

The results of this study found a significant difference ($P < 0.05$) in the mean counts (log cfu/g) of aerobic spore-forming bacteria (ASFB) among samples at all fermentation time for all formulated samples (Table 1), except for fermentation time at zero hour and eight days for the MBF2 sample and fermentation time on the 8th and 10th day for the MBF3 sample. The absence of a significant difference between zero and eight days of fermentation time for the MBF_2 sample might be due to the growth of the microbes with a growth rate of more than 2 log cfu/g for specific days and then after reduced with a reduction rate of less than 2 log cfu/g. In this result, the highest colony count was observed in the MBF_1 sample (3.42 log cfu/g) fermented for eight days, and the lowest in the MBF_3 sample (2.07 log cfu/g) fermented for 12 days. This variation is due to the length of fermentation time and the inhibitory effect of secondary metabolites produced by LAB and yeasts against spoilage and pathogenic microorganisms [28,44]. Contrary to the present results, Anumudu and others [45] reported that ASFB could not persist until the final stage of fermentation of *Ogi*, cereal-based fermented porridge in Nigeria. However, in some cereal-based fermented food products, ASFB is reported to persist until the end of fermentation [46,47], although the duration of fermentation among the products differs, hence accounting for the observed variation.

The highest mean colony counts (log cfu/g) of yeast were recorded in the MBF_1 sample (6.65) fermented for ten days and the lowest in the MBF_3 control sample (2.22 log cfu/g). There was a statistically significant difference ($p \leq 0.05$) in counts of yeast between consecutive fermentation times (Table 1). In addition, even if there was no significant difference ($P > 0.05$) between control samples, the yeast count of the MBF_1 sample fermented for 12 days was the highest, while the counts of all formulated samples decreased as the proportion of maize decreased in the formulation. This effect might be due to the fast growth of lactic acid bacteria in carbohydrate-rich products, as lactic acid bacteria facilitate the growth of yeasts [48]. Many authors also explored the co-existence and symbiotic association between lactic acid bacteria and yeasts in spontaneously fermented foods [49,50], which was further confirmed by the presence of yeasts until the final stage of fermentation in cereal-based fermented foods such as *Kirario* in Kenya [51], *Akamu* and *Ogi* in Nigeria [34,52], and *Borde* in Ethiopia [53]. The role of yeasts in *Shameta* might be the production of flavor-enhancing and aroma-producing secondary metabolites and inhibiting the growth of mycotoxin-producing molds [34,54–56]. Nwokoro and Chukwu [34] reported that *Saccharomyces* spp. in pure culture did not produce ethanol from carbohydrates in maize fermentation. However, *Saccharomyces rouxii* and *Saccharomyces cerevisiae* are responsible for the organoleptic properties of the same product during fermentation.

In addition to these microorganisms, mold was also detected at the initial and final stages of fermentation in MBF_1 formulations. However, mold observed at the initial stage in MBF_2 and MBF_3 samples weren't observed on days 8, 10, and 12 of fermentation time, which agrees with previous work by Kitessa and others [14]. Unlike to samples prepared in the laboratory, mold was observed in all the samples collected from the households of lactating mothers, which aligns with the result of MBF_1 [14]. The reduction of oxygen and production of antimicrobial compounds by lactic acid

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bacteria and yeasts could be main reasons for the subsequent disappearance of molds during fermentation a [28,47]. Likewise, molds detected at the initial stage of fermentation of maize dough during *Ogi* and *Kenkey* production were subsequently eliminated at the end of fermentation [49,50]. However, in different works, molds were detected at the initial and final stages of the fermentation of '*Akamu*,' fermented for 72 hours [34], probably because of the short duration of fermentation. The occurrence of mold could not be always considered responsible for the spoilage , but there are some non-spoilage molds that could contribute to the flavor and overall digestibility of the fermented products [57].

3.2. Effect of flour composition and fermentation time on the chemical composition of Shameta

3.2.1. Effect of flour composition and fermentation time on pH and titratable acidity (TA)

The interaction effect of both factors on pH and TA are indicated in Table 2. The pH of control samples (unfermented porridge) was in the range of 5.22 to 5.26. However, with increased fermentation time, the pH decreased to values below four pH units for fava bean flour-rich samples (MBF_2 and MBF_3). *Shameta* samples collected from households of lactating mothers made from similar ingredients composition to MBF_1 resulted in a pH value of 3.9 [12]. Furthermore, the pH values of MBF_1 and MBF3 fermented for 12 days were in line with some Ethiopian traditional fermented foods such as *Azo* and *Cheka*, with values of 3.81 and 3.74, respectively, while the pH of MBF_1 fermented for the same days was slightly lower than *Borde* with the value of 4.2 [58–60]. The result of this study shows that the pH of the controlled study was more or less the same as the pH value of traditionally processed *Shameta* made at the household level.

The decrease in pH value with an increase in fermentation time is expected for fermented products due to the growth and dominance of LAB and yeast to produce lactic acid and other organic acids during the fermentation [61–63]. A significant change in pH values for all formulations was observed in the first week of fermentation. The pH value decreased from an average of 5.24 units to 4.29 units after eight days of fermentation. From a food safety point of view, the required pH reduction below 4.5 could be achieved within the first week of fermentation. However, the products fermented up to the 12th day to get the required nutritional values and sensory properties.

Change in titratable acidy (TA) negatively correlated with change in pH of the fermenting foods. As indicated in Table 2, the TA increased with a decrease in pH value with fermentation time. However, the TA values observed in this study were lower than the TA value (0.82%) of the *Shameta* sample collected from households of lactating mothers made using the same ingredient composition (MBF_1) as reported in a previous study [12]. The higher TA value associated with over-fermentation of the product could affect the sensorial property of the same product, resulting in low acceptability of the product by the product consumers. Both the pH and TA values recorded in this study and the previous work done on microbial dynamics during the fermentation of *Shameta* [14] indicate that a maximum of 12 days of fermentation of *Shameta* could be sufficient enough to attain the desired product maturity and safety.

Table 2. Effect flour compositions and fermentation time on pH and TA of *Shameta*.

Flour composition	Fermentation time (days)	pH	Titratable acidity (TA)
MBF_1	0	$5.26+0.03^{a}$	$0.37+0.01^{h}$
	8	$4.42+0.02^{b}$	$0.58+0.01^{f}$
	10	$4.21+0.21^{c}$	$0.63+0.03^{e}$
	12	$4.00+0.02^{d}$	$0.66+0.02^{d}$
MBF_2	0	$5.24+0.02^{a}$	$0.39+0.01^{gh}$
	8	$4.42+0.02^{b}$	$0.61+0.01^{e}$
	10	$4.02+0.02^{d}$	$0.68+0.01^{cd}$
	12	$3.98+0.01^{d}$	$0.69+0.01^{bc}$
MBF_3	0	$5.22+0.01^{a}$	$0.41+0.01^{g}$
	8	$4.04+0.04^{d}$	$0.63+0.01^{e}$

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	10	4.01+0.01^{d}	0.71+0.01^{ab}
	12	3.87+0.01^{e}	0.73+0.01^{a}
CV		1.27	2.22
LSD		0.09	0.02

MBF_1 = Maize-Barley-Fava bean with ratios of 81:5:5; MBF_2 = Maize-Barley-Fava bean with ratios of 71:10:10 and MBF_3 = Maize-Barley-Fava bean with ratios of 66:10:15; CV = Coefficient of variation, LSD = List significant different, Different letters in superscript along a column indicate a significant difference ($p \leq 0.05$).

3.2.2. Effect of flour composition and fermentation time on proximate composition

The highest moisture content value was observed in MBF_1 fermented for 12 days (66.32 g/100g), while the lowest was in the MBF_2 control sample (60.58 g/100g). Accordingly, all control samples (cooked porridge not fermented for the second round) have lower moisture content than porridge fermented for the second round '*Shameta*' (Table 3). This might be associated with the microbial breakdown of polysaccharides to simple sugar during fermentation, while there could be free water in the control samples as the large polymer polysaccharides do not bind water molecules [64,65]. Foods' moisture content levels significantly impact the aesthetic values: taste, texture, appearance, shape, and even safety of products. Accordingly, an excessive amount of water could facilitate the spoilage of food products as its excessive losses could also change the sensorial properties of foods, making the product unacceptable [66]. With regards to moisture content, almost all of the formulated products assessed in the study were near the category of intermediate moisture foods [67] in which some of the water bound that makes the products safe for some more weeks in combination with the inhibitory effects of low pH of the products (Table 2) and essential oils in spices and herbs added during preparation (Figure 1). The moisture contents of all *Shameta* formulations (MBF_{1-3}) were lower than the co-fermented *Ogi* made of maize soybean (67.35 g/100g) and millet soybean (67.86 g/100g). However, the values align with co-fermented *Ogi* made of sorghum soybean with a moisture content of 64.52 g/100g [68].

In the present study, the highest crude protein was observed in MBF_3 fermented for 12 days (16.56 g/100g), followed by the same sample fermented for ten days (16.32 g/100g). However, the lowest was observed in the MBF_1 control sample (12.22 g/100g), followed by the same sample fermented for eight days (12.82 g/100g). The difference might be associated with a higher proportion of fava beans than the MBF_1 sample. In addition, the prolonged fermentation time could contribute to the release of bound proteins and other macromolecules. It is possible to blend cereals' relatively poor protein quality with protein-rich food crops to achieve a nutritionally balanced diet [69]. Studies showed that the fermentation of cereals with lactic acid bacteria and yeast cultures increased the protein content of the fermented foods [70,71]. With high counts of LAB and yeast in the cereal-based fermented products, the microbial cell mass protein (commonly called single-cell protein) could also contribute to the total crude protein of the fermented product. Omemu and others [68] also indicated the incremental Effect of fermentation on the crude protein content of *Ogi*. However, crude protein contents in the present study are different than the values in co-fermented *Ogi* made of Maize-Soybean (6.53 g/100g), Millet-Soybean (6.64 g/100g), and Sorghum-Soybean (8.44 g/100g) at the ratio of 66.67:33.33 and fermented for 48 hrs. Whereas, the crude protein content (18.28 g/100g) of complimentary food made of formulation of maize, haricot bean, and cooked banana flour at the ratio of 30:60:10 and fermented for 36 hours is greater than the values in the present finding [72].

The proteins in human milk come from the diet and maternal body stores. The potential to supply the extra protein required by lactating mothers (20 g/day) among the formulations assessed in the present study is the highest in MBF_3 fermented for 12 days (82.8%), followed by the same sample fermented for ten days (81.6%). The extra protein requirement yield in the present finding is greater than the yield in *Borde* (48%), made of 100% maize fermented for four days, and average values of co-fermented *Ogi* (36.02%) made of Maize-Soybean, Millet-Soybean, and Sorghum-Soybean fermented for two days. However, it was lower than complimentary food (91.4%) made of maize, haricot bean, and cooked banana flour (30:60:10) fermented for 36 hrs [53,68,72]. The finding revealed

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that to improve the extra protein requirement yield of *Shameta*, supplementing primary ingredients (maize and barley) with legumes such as fava bean is a necessity.

Table 3. Effect of flour composition and duration of fermentation on proximate composition (g/100g) of *Shameta*.

Flour composition	Fermentation time (days)	Moisture content	CP content	CF content	Fiber content	Ash content	Carbohydrate content	Gross energy (kcal/100g)
MBF_1	0	61.34+0.34^{g}	12.22+0.02^{g}	12.46+0.05^{d}	3.61+0.01^{c}	2.45+0.01^{l}	72.20+0.82^{a}	449.79+0.11^{a}
	8	65.89+0.11^{b}	12.82+0.02^{f}	12.89+0.02^{b}	2.43+0.01^{j}	2.63+0.01^{j}	69.23+0.05^{b}	444.24+0.03^{ab}
	10	66.00+0.00^{b}	13.00+0.5^{f}	13.11+0.11^{a}	2.24+0.02^{k}	2.64+0.02^{j}	69.01+0.42^{bc}	446.05+0.47^{ab}
	12	66.32+0.02^{a}	13.52+0.02^{e}	13.23+0.01^{a}	2.21+0.01^{l}	2.61+0.01^{k}	68.43+0.06^{bcd}	446.87+0.02^{ab}
MBF_2	0	60.58+0.09^{i}	13.56+0.1^{e}	11.68+0.03^{e}	4.66+0.02^{b}	2.87+0.01^{e}	67.24+0.15^{bcd}	428.29+0.05^{cd}
	8	64.77+0.08^{def}	13.77+0.1^{de}	12.41+0.10^{d}	3.34+0.01^{b}	2.85+0.01^{f}	67.63+0.19^{bcd}	437.31+0.47^{bc}
	10	64.79+0.20^{de}	13.98+0.01^{d}	12.81+0.04^{b}	3.31+0.01^{f}	2.84+0.01^{g}	67.06+0.04^{bcd}	439.43+0.16^{b}
	12	65.11+0.11^{c}	14.68+0.1^{c}	12.63+0.06^{c}	2.98+0.01^{g}	2.82+0.12^{h}	66.89+0.14^{bcd}	439.94+0.30^{ab}
MBF_3	0	60.87+0.13^{h}	14.89+0.1^{c}	10.01+0.21^{g}	4.84+0.02^{a}	3.75+0.01^{a}	66.51+0.09^{cd}	415.73+1.13^{e}
	8	64.56+0.19^{f}	15.88+0.1^{b}	10.75+0.06^{f}	3.36+0.02^{d}	3.70+0.02^{d}	66.31+0.18^{d}	425.49+0.19^{de}
	10	64.66+0.33^{ef}	16.32+0.32^{a}	10.80+0.11^{f}	2.88+0.01^{h}	3.72+0.01^{c}	66.28+0.22^{d}	427.57+0.48^{cd}
	12	64.98+0.02^{cd}	16.56+0.1^{a}	10.66+0.01^{f}	2.86+0.01^{i}	3.73+0.02^{b}	66.19+0.11^{d}	426.91+0.14^{d}
CV		0.20	1.36	0.69	0.13	0.15	2.18	1.35
LSD		0.22	0.33	0.14	0.007	0.008	2.51	9.97

CP = Crude protein, CF= Crude fat, MBF_1= Maize-Barley-Fava bean with ratios of 81:5:5, MBF_2= Maize-Barley-Fava bean with ratios of 71:10:10, and MBF_3 = Maize-Barley-Fava bean with ratios of 66:10:15, CV=Coefficient of variation, LSD=List significant different, Different letters in superscript along a column indicate a significant difference ($p \leq 0.05$).

As indicated in Table 3, flour compositions and fermentation time showed significant differences in crude fat contents. The prolonged fermentation time from the 8th to the 10th and 12th day has provided better crude fat contents. The better crude fat content in MBF_1 could be due to the relatively high proportion of maize flour rich in fat content and rapeseed oil added during the preparation of *Shameta* (Figure 1). Studies have shown that maize is richer in fat content than fava beans [26,73]. Maize and rapeseed oil are rich in mono- and polyunsaturated fatty acids with good oxidative stability [74–76]. Fatty acids are considered to be a fundamental building material for the structural components of cells, tissues, organs, and synthesis of specific biologically active substances, and facilitate the absorption and transport of fat-soluble vitamins [77].

In general, the crude fat content in the present finding is far greater than what was reported from other cereal-based fermented foods in Ethiopia, including *Borde* (6.9 g/100g) made of maize [58] and *Cheka* (1.3 g/100g) [60] made of maize and taro leaves (approximately in the ratio of 70:30) and fermented for four days. Therefore, *Shameta* is an excellent candidate to promote supplementary fermented cereal-based food rich in fat to support the strength and health of lactating mothers. In addition, the better fat content, likely rich in mono- and polyunsaturated fatty acids, could contribute to better breastfeeding of newborns [78].

One of the ingredients of *Shameta*, barley, contains significant amounts of soluble fiber (beta-glucans), which microorganisms can ferment to produce short-chain fatty acids, an important energy source for the brain, muscles, and tissues [79,80]. In addition, the fatty acids contribute to lowering the pH to prevent the growth of pathogenic microorganisms and reduce peptide breakdown and toxin formation [81,82]. Fava bean is also a rich source of oligosaccharides, the third most abundant nutrient in breast milk behind lactose and fat, and serves as prebiotic soluble fibers for the infant's gut, ensuring proper immune responses [83,84].

The fiber contents of different formulations assessed in the current study were significantly different ($p \leq 0.05$) from each other (Table 3). The possible reduction in crude fiber content during the fermentation process could be attributed to the partial solubilization of cellulose and hemicellulosic

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materials in fermentation by the activities of microbial enzymes [85]. Although the reduction of fiber content during fermentation is sound, as high fiber content increases the viscosity of food, which reduces food intake, it plays an essential role in increasing the utilization of nitrogen and absorption of some other micronutrients [86,87]. The average fiber content of the current formulations is lower than complementary food made of maize, haricot bean, and cooking banana flour (4.21 g/100g) but significantly different than values in *Cheka* (1.1 g/100g), *Injera* (pancake-like bread made of teff) (2.8 g/100g) made of 100% teff (Eragrostis tef) fermented for 24 hrs and co-fermented *Ogi* (0.30 g/100g) [60,68,72,88].

The ash content of the present finding ranged between 3.75 and 2.45 g/100g for MBF_3 and MBF_1 control samples, respectively, with an average value of 3.05 g/100g. Initial ash content for the fresh porridges showed significant differences due to differences in the proportion of ingredients used to make the porridges. Porridge using a relatively higher fava bean composition (10 or 15%) showed better ash content than porridge with 5% fava bean. Variations in ash content might be associated with a higher mineral concentration in fava bean than in maize and barley flours [55,89]. However, the difference has little association with fermentation time compared to the effect of variation in ingredient composition.

The average ash content of all the different formulations is significantly different than values in a complementary food made from maize, haricot bean and cooking banana flour (2.23 g/100g), co-fermented *Ogi* (3.01 g/100g), *Cheka* (0.75 g/100g) and wheat-based *Borde* (0.78 g/100g), while it was lower than the maize-based *Borde* (3.7 g/100g) [58,60,68,72,90]. The variation could be attributed to differences in composition, preparation steps, and fermentation time. *Shameta* could contribute total ash, translating to a better mineral supply than other commonly consumed cereal-based fermented foods like *Cheka* and *Borde*.

Most of the values of carbohydrates in the present findings were not significantly different ($P>0.05$) from each other (Table 3). The highest value was observed in MBF_1 fermented for eight days (69.23 g/100g) and the lowest in MBF_3 fermented for 12 days (66.19 g/100g). The difference might be associated with the higher accumulation of carbohydrates in maize than in fava bean [26]. The recorded carbohydrate content is significantly different than value observed in *Azo* (16.6 g/100g) made of sorghum and endod (Phytolaca dodecandra) leaves (50:50) as significant ingredients fermented for 30 days, *Cheka* (9.6 g/100g), and co-fermented *Ogi* (22.26 g/100g), but lower than complementary food made of maize, haricot bean and cooking banana flour (71.16 g/100g) [59,60,68,72].

Carbohydrates are primary energy sources that comprise 55% of the total caloric intake [91]. Therefore, lactating mothers should consume at least 100 g/kg/day of carbohydrates from locally available food crops [91]. The Recommended Dietary Allowance (RDA) of carbohydrates for lactating women is 160 g/kg/day [92]. Accordingly, MBF_3 fermented for 12 days contributed 41.37% of carbohydrates. The current selected *Shameta* formulation (MBF_3) contributes the highest carbohydrate for lactating mothers when compared to other cereal-based fermented condiments, beverages, and porridges such as *Azo* (10.38%), *Cheka* (6%), and *Ogi* (13.91%); while slightly lower than complementary food made of maize, haricot bean and cooking banana flour (44.48%) [59,60,68,72].

Most of the values of gross energy in the present finding were not significantly different ($P>0.05$) from each other, with the highest value in MBF_1 fermented for 12 days (446.87 Kcal/100g) and the lowest in MBF_3 fermented for eight days (425.49 Kcal/100g) (Table 3). The gross energy in the present finding is significantly different from values in a complementary food made of maize, haricot bean, and cooked banana flour (397.11 Kcal/100g) and co-fermented *Ogi* (218.77 Kcal/100g) [68,72]. Although the increase in energy requirements during lactation is maximum compared to protein requirements, if the energy intake is low, protein will be used for energy production rather than its primary role [93]. According to the extra energy demand for exclusive breastfeeding from birth to six months postpartum (500 kcal/day), the MBF_3 fermented for 12 days provides 85.38% for lactating mothers. All gross energy values recorded in the current study are significantly different than values in *Injera* (76.8), *Azo* (18.3), and *Cheka* (18.8%)of the extra energy required for lactating mothers, respectively [59,60,94].

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3.2.3. Effect of flour composition and fermentation time on minerals contents

Calcium (Ca) is one of the essential mineral elements for better recovery and strength of lactating mothers. Calcium deficiency in maternal Nutrition could lead to hypertensive conditions, pregnancy disorders, lower blood pressure, and osteoporosis [95]. Results in Table 4 showed that flour formulations had a significant effect on Ca content than the effects of fermentation time. Porridge prepared from flour composition rich in fava beans results in better Ca content (15%) than others. Relatively high Ca content was observed in MBF_3 porridge fermented for 12 days (61.3 mg/100g), followed by the same formulation fermented for 10 days (60.7 mg/100g). However, the lowest Ca content was observed in the MBF_1 formulation (25.8 mg/100g) because of its small proportion of fava bean flour (5%). Even if fermentation time appears to have some effect on the Ca content, overall, the effect was not significantly high as with the composition of ingredients.

The results of this present study is in agreement with complementary foods made of maize, haricot bean, and cooking banana flours (30:60:10) with the value of 61.43 mg/100g; however lower than the average value in co-fermented *Ogi* (2073.54 mg/100g) made of Maize-Soybean, Millet-Soybean, and Sorghum-Soybean with a cereals-soybean ratio of 66.67:33.33 and fermented for 48 hrs [68,72]. According to the present analysis, the highest recorded Ca content could meet only close to 6.1% of DRA of Ca for lactating mothers. However, '*Injera*' made from 100% teff (Eragrostis tef) and fermented for 24 hours could meet 16.77% of DRA for lactating mothers [88]. *Cheka*, the other form of cereal-based fermented food made of maize and taro leaves (approximately 70:30) and fermented for four days, could meet only 1.47% DRA [60]. Even if there is an improvement of Ca content by more than double as compared to the control, there is a need to have additional Ca from other sources, including consumption of *Shameta* with *Injera*, to improve Ca contents for rapid recovery and strength of lactating mothers.

The iron (Fe) and Zinc (Zn) contents of the *Shameta* formulations were not significantly ($P>0.05$) affected by fermentation time (Table 4). In this present study, Ca, Fe and Zn contents improved with an increment in the proportion of fava bean flour in the mix from 5 to 15%. The highest Fe content (8.83 mg/100 g) and better Zn contents were observed in MBF_3 porridge fermented for 12 days (Table 4).

Table 4. Effect of flour composition and duration of fermentation on mineral contents (mg/100g) of *Shameta*.

Flour compositions	Fermentation time (day)	Calcium (Ca)	Iron (Fe)	Zinc (Zn)
MBF_1	0	$25.75+0.22^{h}$	$5.66+0.01^{f}$	$6.76+0.18^{e}$
	8	$27.90+0.19^{g}$	$6.81+0.01^{d}$	$7.47+0.46^{cd}$
	10	$27.98+0.23^{g}$	$6.83+0.01^{d}$	$7.50+0.47^{cd}$
	12	$28.12+0.22^{g}$	$6.84+0.01^{d}$	$7.51+0.46^{cd}$
MBF_2	0	$35.53+0.22^{f}$	$6.32+0.01^{e}$	$7.14+0.31^{de}$
	8	$36.86+0.04^{e}$	$6.87+0.01^{d}$	$7.67+0.16^{c}$
	10	$37.49+0.64^{d}$	$6.89+0.01^{d}$	$7.71+0.18^{c}$
	12	$37.93+0.71^{d}$	$6.89+0.01^{d}$	$7.73+0.20^{c}$
MBF_3	0	$59.15+0.56^{c}$	$7.06+0.05^{c}$	$8.24+0.21^{b}$
	8	$60.16+0.47^{b}$	$8.72+0.13^{b}$	$8.83+0.16^{a}$
	10	$60.66+0.19^{ab}$	$8.73+1.15^{b}$	$8.87+0.14^{a}$
	12	$61.27+0.42^{a}$	$8.83+1.16^{a}$	$8.89+0.14^{a}$
CV		0.89	0.84	3.03
LSD		0.63	0.10	0.40

MBF_1 = Maize-Barley-Fava bean with ratios of 81:5:5; MBF_2 = Maize-Barley-Fava bean with ratios of 71:10:10 and MBF_3 = Maize-Barley-Fava bean with ratios of 66:10:15; CV = Coefficient of variation; LSD = List significant different. Different letters in superscript along a column indicate a significant difference ($p\leq0.05$).

The Fe contents in most of the samples analyzed were different than values in complementary foods made of maize, haricot bean, and cooking banana flour (5.69 mg/100g) and *Ogwo* (0.34 mg $100g^{-1}$) made of malted sorghum, un-malted sorghum and potato (54.55:27.27:18.18) fermented for 48 hrs;

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however, the contents were lower than Fe contents of *Cheka* (18.3 mg $100g^{-1}$), and *Injera* (15.4 mg $100g^{-1}$) [60,88,96,97]. Iron deficiency revealed during lactation may not be only because of a lack of access to iron-rich foods. However, it could also be associated with complications related to iron status before pregnancy, hemorrhage after delivery, low-vitamin C diet, excessive consumption of tannin-rich foods, frequent pregnancies, and early pregnancy. According to this study, the Fe content in MBF_3 porridge fermented for 12 days could meet close to 98% of the recommended dietary allowance of Fe for lactating mothers.

Although the demand for Zn increases during lactation is required for many biological activities, it has been reported that the amount of zinc in breast milk is independent of Zn in diet [98]. Fermented porridge produced from MBF_3 samples fermented for different days could provide close to 74% of RDA of Zn for lactating mothers. These values are better than those reported from cereal-based fermented foods in Ethiopia, such as *Cheka* and *Injera*, which could provide only 7.67 and 20% of RDA, respectively [60,88]. Thus, the mineral composition of fermented products could be improved by considering the ingredients' compositions more than monitoring the fermentation time. Therefore, further improvement in mineral content can be achieved through home or industry-based formulations for better health and recovery of the mothers and their infants.

3.2.4. Effect of flour composition and fermentation time on anti-nutritional factors and antioxidant capacity

The adverse health effect of phytate in the diet is reducing the absorption of minerals such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}, especially Zn and Fe deficiencies were reported as a consequence of high phytate intakes [99]. A report indicated that a high level of dietary tannin (120 mg/kg) reduces protein absorption and damages the intestinal walls [100]. This study aimed to minimize the negative impact of phytate and tannin in traditionally produced fermented porridge. Results of the study showed that both formulation of flour to make the porridge and fermentation time have shown significant differences ($p \leq 0.05$) in terms of phytate and tannin contents. The concentrations decreased with a decrease in maize flour from 90% to 75% due to an increase in fava bean proportion.

Similarly, increased fermentation time significantly reduced the phytate and tannin contents. For instance, after 12 days of fermentation, the phytate content decreased by 84, 70, and 71% compared to fresh *Shameta* for MBF_1, MBF_2, and MBF_3, respectively (Table 5). Similarly, the tannin concentration decreased by 88, 78.5, and 78.5% after 12 days of fermentation for the same formulations. As expected for fermented food products, fermentation time's impact was significant compared to flour formulations.

Table 5. Effect of flour composition and duration of fermentation on anti-nutritional factors (mg/100g) and antioxidant activities.

Flour composition	Fermentation time (days)	Phytate	Tannin	Antioxidant activities (IC50) (mg AAE/g)
MBF_1	0	$1.76+0.03^{a}$	$1.58+0.01^{a}$	$10.16+0.23^{b}$
	8	$0.56+0.02^{e}$	$0.26+0.01^{h}$	$0.64+0.02^{c}$
	10	$0.34+0.02^{j}$	$0.20+0.00^{i}$	$0.44+0.01^{cde}$
	12	$0.28+0.01^{k}$	$0.19+0.00^{i}$	$0.31+0.01^{de}$
MBF_2	0	$1.34+0.02^{b}$	$1.26+0.02^{b}$	$10.89+0.19^{a}$
	8	$0.77+0.02^{d}$	$0.47+0.01^{d}$	$0.52+0.03^{cd}$
	10	$0.42+0.02^{i}$	$0.39+0.00^{e}$	$0.46+0.03^{cde}$
	12	$0.53+0.01^{fg}$	$0.34+0.02^{g}$	$0.19+0.05^{e}$
MBF_3	0	$1.22+0.02^{c}$	$0.98+0.01^{c}$	$11.15+0.45^{a}$
	8	$0.54+0.02^{f}$	$0.39+0.00^{e}$	$0.71+0.17^{c}$
	10	$0.52+0.01^{gh}$	$0.36+0.01^{f}$	$0.56+0.11^{cd}$
	12	$0.51+0.01^{h}$	$0.34+0.01^{g}$	$0.31+0.08^{de}$
CV		0.85	1.75	5.44
LSD		0.01	0.02	0.28

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MBF_1= Maize-Barley-Fava bean with ratios of 81:5:5; MBF_2= Maize-Barley-Fava bean with ratios of 71:10:10; MBF_3 = Maize-Barley-Fava bean with ratios of 66:10:15; CV=Coefficient of variation; LSD=List significant different. Different letters in superscript along a column indicate a significant difference ($p \leq 0.05$).

The phytate content recorded in the current study is significantly lower than its content in complementary food made of maize, haricot bean, and cooking banana flour (30:60:10) (36.99 mg/100g) fermented for 36 hrs and *Kutukutu* (12.4 mg/100g) made of 100% corn fermented for 24 hrs; while greater than co-fermented *Ogi* (0.2 mg/100g) made of Maize-Soybean, Millet-Soybean, and Sorghum-Soybean with a cereals-soybean ratio of 66.67:33.33 and fermented for 48 hrs [68,72,101]. Consumption of dietary phytate up to 500 mg/day leads to a 0.04 mg/day reduction in zinc absorption [102], while Ndie and Okaka [103] reported that levels of phytate between 23.5-130.65 mg/kg are high enough to be associated with health risk. However, the phytate contents in this study are below 1% after one week of fermentation of the product.

The tannin content recorded in this study is also lower than the content in complementary food made of maize, haricot bean, and cooking banana flour (31.32 mg/100g) but significantly different than value in co-fermented *Ogi* (0.13 mg/100g) [68,72]. According to Ndie and Okaka,[103], levels of tannins up to 108.3 mg/kg are high enough to be associated with health risks beyond reducing the bioavailability of nutrients. However, the tannin level for fermented porridge is significantly lower than 1% for all durations of fermentation. The observed lower value could be associated with the combined effects of first-stage fermentation, intermediate cooking, and second-stage fermentation of the product. The absence or lower value of tannin will increase the prevalence of high bioavailability of minerals and proteins, which is necessary for lactating mothers in support of their rapid recovery, strength, and health.

Consuming food products rich in phytochemicals during pregnancy and lactation is a critical component of dietary guidelines to boost phytochemicals and protect mothers and infants from oxidative damage and related diseases (104). The result showed that the MBF_3 sample fermented for 12 days had a better DPPH scavenging ability with a lower IC50 value than other formulations fermented at different fermentation times. However, in all formulations, the potential for scavenging activities increased as fermentation time increased. Adebo and Gabriela Medina-Meza [104] also reported that an increase in fermentation time increased the total phenolic contents and antioxidant activities of whole cereal grains. The ability of fermentation to improve antioxidant activity is primarily due to an increase in the number of phenolic compounds and flavonoids as a result of the structural breakdown of plant cell walls by microbial hydrolysis reaction [104,105]. Meanwhile, the variation of antioxidant activities in control samples might be due to the variation during the roasting of spices and intermediate cooking to make porridge [106–108]. Generally, in addition to provide protein and some minerals, the consumption of *Shameta* improves the health of lactating mothers and newborns by preventing oxidative stress.

3.3. Sensory properties

Sensory characteristics are one of the most essential and influential determinants of food preferences, especially in developing new food products [109]. In the present finding, the sensory attributes and mean sensory scores of *Shameta* products are indicated in Table 6. The aroma of *Shameta* samples ranged from 4.13 to 4.42. The values showed no significant ($P>0.05$) differences in aroma among the control samples and the formulated products. This might be due to added spices, herbs, and rapeseed oil during preparation [13]. Puleo and others [110] reported that the aroma of foods plays a pivotal role in the perception and liking of food products, especially in new product development. On the other hand, unlike a pleasing aroma, an offensive smell of foods is one of the primordial senses to avoid potential food hazards and contributes to the evolutionary function of disgust as a disease avoidance mechanism [111,112].

Color is the most critical food product-intrinsic sensory cues that help the consumers to expect the likely taste and flavor of foods [113]. That is why, since ancient times, adding coloring agents to foods and drink has been considered one of the practices to enhance the acceptability of foods by consumers [113,114]. In the present finding, the highest color value was scored (4.82) by the control

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samples due to their acceptance by the panelists associated with their experience of using *Shameta* for a long time. However, there was no significant (P>0.05) difference in color between the two formulated samples. The preference for whitish color *Shameta* and the addition of maize for coloring purposes during barley-based *Shameta* preparation is reported elsewhere [13]. The present finding is in close agreement with *Ogi* fortified with moringa leave powder, where the lowest color preference was recorded in formulated samples than in control samples [115]. However, during the sensory analysis of *Ogi* formulated with synthetic provitamin, the formulated sample recorded the highest score than the control sample [116].

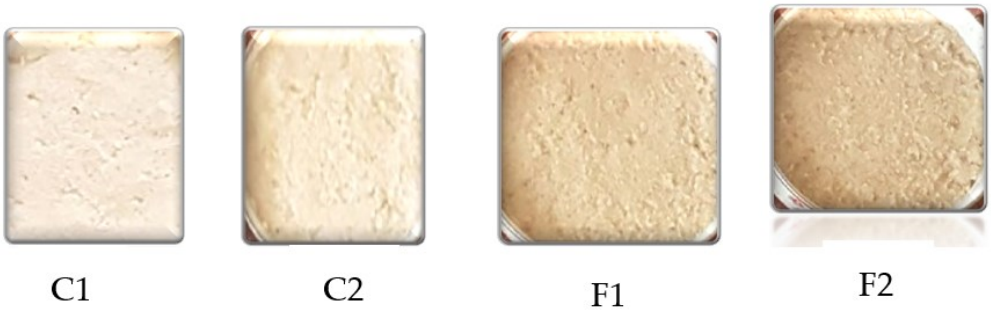
Table 6. Sensory scores of control and formulated *Shameta* samples (N = 55).

Formulation	Aroma	Color	Taste	Texture	Overall acceptability
C_1	4.13+0.86^a	4.82+0.39^a	4.55+0.50^a	4.55+0.50^a	4.45+0.50^a
C_2	4.18+0.88^a	3.91+0.40^b	4.07+0.26^b	4.36+0.52^a	4.02+0.24^b
F_1	4.30+0.71^a	3.41+0.89^c	3.96+0.69^b	4.05+0.49^b	4.05+0.35^b
F_2	4.42+0.68^a	3.40+0.89^c	3.91+0.67^b	4.09+0.51^b	4.02+0.30^b

C_1= 100% maize and fermented for 25 days; C_2= 90% Maize, 5% barley, 5% fava bean and fermented for 20 days; F_1 and F2= 75% Maize, 10% barley, and 15% fava bean, and fermented for 10 and 12 days, respectively. Different letters in superscript along a column indicate a significant difference ($p \leq 0.05$), and the same letters indicate no significant difference ($p > 0.05$).

Many intrinsic and extrinsic factors influence taste responses to food products [117]. In the present finding, the highest taste recorded was observed in C_1, followed by C_2. However, no significant ($p > 0.05$) differences existed between the values recorded in the formulated samples. In line with the present finding, the highest taste preference was recorded in the control sample rather than in formulated *Ogi* samples [115]. However, present finding is different from the study of Akinsola and others [116] as the highest taste preference was recorded in formulated samples compared to the control sample.

The texture of foods is derived from the structure of the food and consists of a complex set of sensory attributes important to food liking and choice [118]. In addition to processing methods such as fermentation, types of ingredients affect the texture of foods [13,14]. In the present finding, the highest texture value was scored (4.55) by the C_1 sample, followed by C_2 with no significant ($P > 0.05$) difference between each other. However, the highest texture preference recorded in *Ogi* was made by adding synthetic provitamin rather than the control sample [116]. Regarding overall acceptability, the value of C_2 was not statistically significantly different from the values of F_1 and F_2 formulated *Shameta* products. The present finding agrees with the work of Abioye and Aka [115], as the general acceptability scored for the control sample is more than for formulated products. However, this finding disagrees with the report of Akinsola and others [116].



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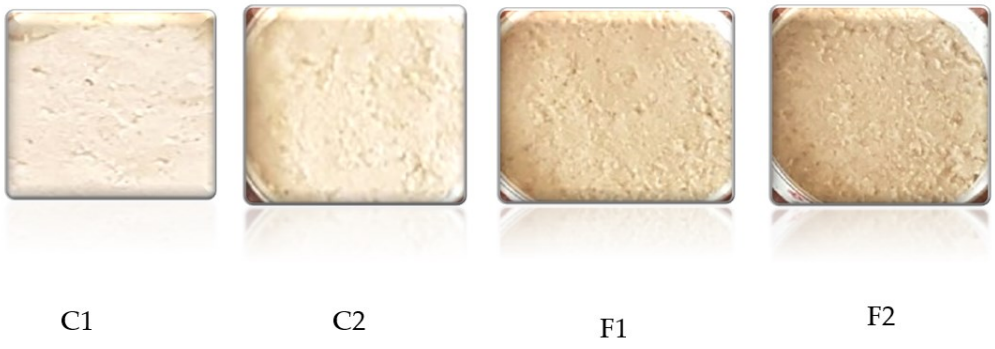

Figure 2. Control and formulated *Shameta* samples for sensory evaluation. C_1= *Shameta* made of 91% maize and fermented for 25 days; C_2= *Shameta* made of 81% maize, 5% barley, and 5% fava bean, and fermented for 20 days; F_1 and F_2 showed *Shameta* made of 66% maize, 10% barley and 15% fava bean, and fermented for 10 and 12 days, respectively.

4. Conclusions

The results of this study demonstrated that crude protein and mineral contents were affected by the proportion of formulation and fermentation time of *Shameta*. An increment in the fava bean flour ratio from 5 to 15% significantly improved the crude protein content. The improvement in calcium content was more than double compared to the control. Iron and zinc contents also significantly improved when the traditional maize- or barley-based formulation was enriched with 15% fava bean flour. Fermentation time also played a significant role in modifying the pH of the product to a safe range (3.89-4.01) to control the growth of potentially pathogenic microorganisms. The observed rapid growth of LAB contributed to the modification of the pH of the product to control the growth of members of the *Enterobacteriaceae*, TC, *Staphylococcus* spp., and ASFB. Extended fermentation time also significantly reduced the phytate and tannin contents below one percent after the first week of fermentation. In general, *Shameta* made with the help of LAB from a flour composition rich in fava bean (66:10:15) fermented for 10-12 days could contribute to better Nutrition, recovery, and strength for lactating mothers than traditionally produced *Shameta*. However, improving beyond this level of nutritional quality can be improved by developing appropriate starter cultures and optimizing other fermentation conditions such as temperature and pH.

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