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Role of Selected Fermentative Microorganisms on Cyanide Reduction, Protein Enhancement and Palatability of Cassava Based Food

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ABSTRACT

Cassava is a potential energy-rich food crops to make different food products in developing countries but limited by a shortage of protein content and the presence of toxic cyanogenic glycosides. Cassava-teff flour fermented with three pure starter cultures of Saccharomyces cerevisiae, Lactobacillus plantarum and Lactobacillus coryneformis. Two different inoculum levels (0.5 and 1.5 ml) were used. 300 g of cassava-teff flour were fermented with each of single starter cultures at 24 and 48 h. The analysis of pH, crude protein and cyanide content indicates fermentation samples with Lactobacillus plantarum and Lactobacillus coryneformis for 48 h with 1.5 ml inoculums resulted in highest pH reduction. Similarly, highest reduction cyanide was recorded with 1.5 ml inoculums of Lactobacillus coryneformis and Lactobacillus plantarum, while the least cyanide reduction was recorded in fermentation samples of Saccharomyces cerevisiae at 24 h of 1 ml of inoculum level, but this value is higher when compared upon boiling(47.77 mg/kg). The highest levels of crude protein were observed fermentation samples with 1.5 ml inoculum of Saccharomyces cerevisiae for 48 h. Regarding palatability, the panelist preferred that fermentation sample with 1.5 ml inoculums of Lactobacillus plantarum and Lactobacillus coryneformis at 48 h having the best taste with the score of 4.90 ± 0.17 and 4.87 ± 0.23 respectively over the control, while sample fermented with Saccharomyces cerevisiae was preferred in terms of flavor, texture and overall acceptability with score of 4.87 ± 0.23, 4.73 ± 0.11 and 4.67 ± 0.12 respectively over the control. Therefore, fermentative microorganisms are a promising candidate for improving nutritional and safety value of cassava based food and suggested as a choice of the processing method, as this method significantly reduced cyanide content, increased protein content and improved sensory properties of injera.

Keywords: *Cassava based food, cyanide, fermentative microorganisms, palatability, protein.*

INTRODUCTION

The challenge facing global food security due to increasing human population and climate change calls for some solutions. According to Tariku (2013), to meet both current and future challenges in developing countries, it is important that these countries should develop capabilities to use neglected and underutilized crops as alternatives to the current staple crops. This may be attained by using food fermentation technology. Cassava (*Manihot esculent Crantz*) is one of the underutilized food crops in African country like Ethiopia, where many people are afflicted with food shortage. It is the most important root crop in Africa, Asia, and Latin America (Girma *et al*., 2014; Labri *et al*., 2013). In Western and central African countries Garri, Attieke, and Fufu are among the most widely consumed traditional cassava fermented food.

In Ethiopia, cassava crop has been cultivated in South, Southwest and Western part of Ethiopia as an alternative food security crops (Girma *et al*., 2015), and known by a variety of local names like “Mita Boye” (Walaitigna),“Muka Furno”(Oromifa) and ”Mogo”(Amaro), where it dominantly grown, utilized and used as a food crop and playing a significant role in alleviating the food crisis during harsh weather condition (Abebe *et al*., 2014).

Cassava based traditional food products could become even more important in feeding additional segments of the increasing Ethiopian population in the future. However, consumption and processing of cassava in the country are in a primitive stage as compared to many African countries. It is consumed mostly by boiling root or rarely in the form of bread or injera after mixing cassava flour with crops such as maize, wheat or teff flour to make bread or injera (Tefera *et al.*, 2014).

Injera is primarily made from teff (*Eragrostis tef*). Teff protein essentially lacks gluten, the type found in wheat, so it is alternative food for consumers who suffer from wheat gluten allergies. However, teff is currently the most expensive grain to purchase in Ethiopia, because injera made of teff is the favorite diet of the citizens and usually considered as prestige in the community and also teff flour is exported to USA (Gebrekidan, 2016). Additionally, seasonal change due to global warming and increasing human population on limited land need an alternative solution.

Typically, cassava, corn, and sorghum flour are used as alternatives to teff when making injera. Cassava is one of the most drought-tolerant crops capable of growing on marginal soils. It is rich in carbohydrate (Labri *et al*., 2013). However, the existence of cyanogenic glucosides namely linamarin and lotaustralin especially in bitter varieties and limited protein content make it inferior.

Different studies were performed to improve the nutritional and safety value of cassava based food products in Ethiopia. According to Gebrekidan (2016), the blending ratio had a significant influence on the proximate composition and sensory acceptability of the cassava-teff injera product. According to Girma *et al*. (2015) cassava can be processed by drying, milling and then mixed with wheat or sorghum or teff to make bread or injera through traditional fermentation, accordingly the cyanide reduction may be obtained by drying, while protein enhancement was obtained by mixing of cassava flour with that of either wheat or teff flour. Although cassava is processed by fermentation during bread or injera preparation, there is no information on cyanide reduction, protein enhancement and sensory improvement of cassava based food by fermentative microorganisms. Microorganisms (lactic acid bacteria and yeasts) can serve as a substitute for protein enhancement because they contain a high amount of protein, and have a rapid growth rate. The growth of these microorganisms which is more rapid than that of higher plants makes them very attractive as higher protein crop. While only one or two-grain crop is grown per year, yeast may be harvested weekly and bacteria may be harvested daily (Gunawan *et al*., 2015; kaarel, 2003). It has the greatest advantage of cyanide reduction and protein enhancement. Despite its importance as a food and feed in of a *Woreda* of Walaita Zone, SNNPRS, Ethiopia, not much is known about the role of the fermentative microorganisms in cyanide reduction, protein improvement and palatability in locally produced cassava based food product. Therefore, this study was tried to investigate the contribution of fermentative microorganisms (lactic acid bacteria and yeast) in the reduction of cyanide, protein enhancement and palatability in cassava based food.

MATERIALS AND METHOD

Samples were collected from Offa Woreda of the Walaita zone which is found in SNNPRS, Ethiopia.

Experimental Design

The experiment was conducted using three selected pure cultures of cassava fermenting microorganisms i.e *Lactobacillus plantarum, Lactobacillus coryneformis,* and *Saccharomyces cerevisiae* and, each at 1 ml and 1.5 ml inoculums level, and two fermentation time (24 and 48 h) and the treatments were factorially arranged in completely randomize design (CRD) with three replications. The non-fermented cassava-teff flour was used as a control for all fermentation experiments.

Collection and Processing of Plant Materials

The two years edible cassava roots (5kgs) on which investigation carried out was collected from local communities of Busha kebele in Offa woreda and directly brought to the laboratory on the same day using containers with ice to keep them before processing. Teff grain (4 kg) was purchased from Arba Minch Town and ground.

Preparation of cassava flour was done according to Girma *et al*. (2014) with some modification. Undamaged and uniformly matured raw fresh cassava tubers were taken and washed with tap water to remove adhered soil. The tubers were peeled, cut into pieces and dried to reach moisture content less than 12% using oven glassware cabinet drier(Endecotts LTD, London England) at

temperature 65 °C for 24 h. The dried pieces were ground into flour by electrical grinder (Moulinex, A2424A, France) and kept in a refrigerator at a temperature of 4°C for the further process.

Teff flour was prepared as described by Gebrekidan (2016). Four kilograms(4 kg) of teff grain was manually cleaned and milled. The flour was kept in an airtight sealed plastic bucket at room temperature for the duration of the analysis. The blended mixture was prepared by mixing 50% teff flour with 50% cassava flour for making injera, protein analysis and cyanide determination.

Microbial Culture Preparation

Lactobacillus plantarum, *Lactobacillus coryneformis* and *Saccharomyces cerevisiae* previously isolated from fermented milk were obtained from Ethiopian Biodiversity Institute (EBI). *Lactobacillus plantarum* and *Lactobacillus coryneformis* apart from being widely used in the preparation of fermented milk have been reported as the predominant strains among the isolates of traditional sour cassava fermentation (Wakil and Bengamin, 2014). Similarly, *Saccharomyces cerevisiae* is known industrially as important yeast used in the production of a variety of fermented foods. Besides, all the strains have no history of pathogenicity.

Lactobacillus plantarum and *Lactobacillus coryneformis reactivated* using MRS agar medium and propagated using MRS broth medium (sharp, 1966). After an incubation period of 18 h at 30 °C, each of cell culture was centrifuged at 3500 rpm for 10 minutes. Then the cell pellets were washed with distilled water and resuspended in distilled water repeatedly until the optical density (OD) of cells reached 0.5 by spectrophotometer at 600 nm. Finally, 1 and 1.5 ml of the diluted sample were used as inocula.

Saccharomyces cerevisiae reactivated using YPD agar medium and propagated in YPD broth medium according to Frier *et al*. (2015). After the incubation period of 18 h at 25 °C cell culture was centrifuged at 3500 rpm for 10 minutes and suspension was discarded. Then the cell pellets were resuspended in distilled water repeatedly until the optical density (OD) of cells reached 0.5 by spectrophotometer at 600nm. Finally, 1 and 1.5ml of the diluted sample were used as inocula.

Fermentation and Injera Preparation

About 300 g of sieved cassava mixed teff flour were weighted into 13 separate 3 L plastic jerry can. Three hundred seventy (370 ml) of sterile distilled water was added into each plastic jerry can and stopped. Then 1 and 1.5 ml inocula of each of *Lactobacillus plantarum* and *Lactobacillus coryneformis* were inoculated aseptically to the 2 set of 4 separate plastic jerry can each containing 300 g of sterile cassava mush. The inoculation was accompanied by stirring using a sterile glass rod and allowed to ferment for 24 and 48 h at 30 °C, while 1 and 1.5 ml inocula of *Saccharomyces cerevisiae spore suspension* was added aseptically to the 1 set of 4 separate plastic jerry can each containing 300 g of sterile cassava mash and allowed to ferment for 24 and 48 h at a temperature of 25 °C. Samples were withdrawn at an interval of 24 and 48 h for the determination of pH (Tefera *et al*., 2014).

After fermentation, about 10% of dough was added into boiling water and waited for 2-5 minutes with continuous string and allowed to cool forming the "Absit" and added back to the fermenting dough (Alemayehu *et al*., 2016). After thorough mixing, the batter was fermented at room temperature for 2 h. "Absit" ensure injera to have proper texture and consistency by enhancing the dough –rising and gas forming process.

After fermentation, a portion of it further dried in a shade for 3 days. The dried product then milled to a powder. Finally, the powder was kept in a refrigerator at 4 °*C* until used for further analysis. The remaining portion of dough was thinned down to a thick batter and poured in a circular manner onto a hot clay griddle which is lightly oiled, then covered (to retain steam), and baked for approximate 2-3 min. The baked injera was then removed and kept in an airtight container (Blandino *et al*., 2003; Gebrekidan, 2016) until the palatability test. The non-fermented cassava mash was used as a control for all fermentation experiments.

Boiling

Boiling of raw fresh cassava roots were done according to the methods described by (Tilahun *et al*. 2013, Abebe *et al*. 2014). The raw fresh cassava roots were peeled, and placed into stainless steel pan and boiled for about 45 minutes. The cooked cassava roots were crushed and then shade dried for a week to less than 12

% moisture content. The dried pieces were ground into flour by electrical grinder (Moulinex, A2424A, France), sieved by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA), and kept in a refrigerator at a temperature 4 °C for further analysis.

Physicochemical Analysis of Cassava

Determination of Moisture Content

The moisture contents of the cassava root flour sample were determined according to the Association of Analytical Chemistry (AOAC, 1995) in triplicate. About 5 kg of chopped fresh cassava tuber was weighed and transferred in to previously dried and weighed glass dishes (porcelain crucibles). The dishes with cassava samples were placed in a thermostatically controlled oven and heated at 65 °C for 24 h to achieve a constant weight. The dishes were dried again for 30 minutes, cool down and weighed. The drying process was allowed to continue until no more weight loss was recorded between two successive readings. The moisture content then determined by difference and expressed as a percentage (Prapasri and Tee, 2011).

Determination of PH

The pH of the cassava based food was determined using a digital pH meter (TH009 (I) A, Arab emirate) in triplicate (Buck *et al*., 2002). The pH was measure before fermentation, after 24 and 48 h by pouring 100 ml of slurry from samples into 250 ml beaker.

Determination of Crude Protein Content

For studying the changes in protein content associated with fermented cassava-teff flour with Single starter cultures, inoculums level and time of fermentation was estimated by Lowry's method (1951) with minor modification. All the samples mentioned above were weighed 100 mg each and was extracted 50 mM Tris HCl buffer (pH 5.7) containing 5 mM MgCl2, 2mM K2HPO4, 1mM EDTA, 5 mM DTT, 2 mM KH2PO4, 5 mM DTT, 2% PVP, 20% glycerol, 10mM NaF, 10mM β-mercaptoethanol and 2mM PMSF. After homogenization, the samples were centrifuged at 4°C centigrade for 20 min at 12,000 rpm. The supernatant was taken and soluble protein content was determined. Bovine serum albumin (BSA Sigma) was used as a standard protein (5-50 µg/ml); absorbance of the samples was recorded at 750 nm.

Determination of Cyanide

Preparation of Test Samples for Analysis of Free Cyanide (as HCN Equivalent)

Residual cyanide levels of the flours of the cassava were determined in triplicate using the alkaline picrate method (Eleazu, 2012) in the modification. Five grams (5 g) of each sample was dissolved in 50 ml distilled water and allowed to stay overnight. The sample was filtered and the filtrate was used for cyanide determination. One milliliter (1 ml) of each sample filtrate and standard cyanide solution was measured into seven test tubes respectively and 4 ml of alkaline picrate solution (obtained by dissolving 1 g of picrate and 5 g of Na_2Co_3 in 200 ml of distilled water) was added to each and the whole set up was incubated in a water bath at a temperature of 50 °C for 5 minutes. After color development (dark red), the absorbance of each content in the test tubes was taken in to a spectrophotometer at 490 nm against a blank containing only 1 ml distilled water and 4 ml alkaline picrate solution (1 g of picrate and 5 g of sodium carbonate (Na2CO3) were dissolved in a warm water in 200 ml flasks and made up to 200 ml with distilled water).

The standard solution was prepared of varying concentrations (0.1 to 3 ppm) of standard KCN solution from a stock solution of KCN. The stock solution was prepared by dissolving 2.5 g of KCN crystals in 50 ml distilled water and made up to 100 ml in a volumetric flask . This gives a concentration of 10mg CN^-/liter. The KCN solutions in glass bottles were subsequently acidified with 20 % HCl acid solution in ratio 1:1 to release the free cyanide as HCN. Standard cyanide solution was measured into seven test tubes and 4 ml of alkaline pirate was added to each tubes and the whole set up was incubated in water bath at a temperature of 50°C for 5 minutes and was subsequently removed from the bath and kept on the laboratory at room temperature for 24 h. Thereafter, the absorbance of the solution was measured using a spectrophotometer at 490 nm wavelength against a similarly prepared blank developed without KCN solution. A standard calibration curve of absorbance against cyanide concentration in ppm HCN equivalent was plotted. The plot was subsequently used for the determination of cyanide concentration in the test samples.

The cyanide content of the flours was extrapolated from a standard curve using equation of the standard graph: y = 0.188x + 0.079 *10(R^2 = 0.998), where Y= unknown concentration of the sample, 0.188=slop of the graph, x=absorbance of the sample, 0.079= intercept, 10=dilution factor

Sensory Evaluation

Injera produced from the fermented cassava with pure culture and with no culture were subjected to sensory evaluation following the method of Larmond *et al*. (1977). Panelists were selected from the Arba Minch University postgraduate and undergraduate students. Samples were presented to panelists (4 male and 6 female) in random order during the test day. The sensory attributes were rated on a 5 point hedonic score scale Samples receiving an overall quality score of >3 were considered acceptable.

Statistical Analysis

The analytical determination was made in triplicate. The triplicates per treatment were evaluated for the effect of starter cultures, inoculums level and fermentation time on protein content, cyanide content and consumer acceptance of cassava based food. The data were analyzed using an Analysis of Variance (ANOVA). Statistical analysis was carried out using SPSS software version 20.0 where possible, mean comparisons were made using the Tukey HSD test at $P \leq 0.05$.

Table1. *Cyanide and Protein contents in teff flour, cassava flour and cassava- teff flour.*

Types of flour	Protein (%)	Cyanide(mg HCN/kg)
Teff flour	7.66±0.34^{a}	0.00±0.00^{c}
Cassava flour	1.17± 0.02^{c}	184.00±1.00^{a}
Cassava-teff flour	4.23± 0.03^{b}	159.00±1.17^{b}

Value represents the means±Standard deviation (n=3). Within the column, different letters indicate significantly different values (P < 0.05).

Table2. *pH, protein and cyanide at different inoculum size and microbes (time 48 h for all).*

Contents	Treatment with different microbes at different inoculum size						
	Control	*Lactobacillus plantarum*		*Lactobacillus coryneformis*		*Saccharomyces cerevisiae*	
		1 ml	1.5 ml	1 ml	1.5 ml	1 ml	1.5 ml
pH	6.45±0.19^{a}	3.91±0.06^{d}	3.60±0.10^{e}	3.93±0.05^{d}	3.62±0.02^{e}	5.43±0.11^{b}	5.12±0.01^{c}
Protein (%)	4.22±0.01^{e}	6.64±0.01^{d}	6.98±0.01^{c}	6.65±0.02^{d}	7.02±0.01^{c}	9.92±0.01^{b}	13.31±0.02^{a}
Cyanide (mg/kg)	151.84±0.16^{a}	8.86±0.02^{d}	5.54±0.06^{e}	8.51±0.27^{d}	5.29±0.50^{e}	18.47±0.20^{b}	9.64±0.03^{c}

Value represents the mean of three replicates ± Standard deviation (n=3). Within the raws, a different letter indicates significantly different values (P≤0.05).

Table3. *PH, protein and cyanide contents at different times and microbes (inoculum 1 ml for all).*

Contents	Treatment with different microbes at different time							
	Control		*Lactobacillus plantarum*		*Lactobacillus coryneformis*		*Saccharomyces cerevisiae*	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
pH	6.62±0.02^{a}	6.45±0.19^{a}	5.11±0.02^{d}	3.91±0.06^{e}	5.14±0.01^{d}	3.93±0.05^{e}	6.40±0.06^{b}	5.43±0.11^{c}
Protein (%)	4.23±0.01^{e}	4.22±0.01^{e}	4.98±0.03^{d}	6.64±0.01^{b}	4.99±0.02^{d}	6.65±0.02^{b}	5.59±0.01^{c}	9.92±0.01^{a}
yanide mg/kg)	156.46±0.02^{a}	151.84±0.16^{a}	58.51±0.72^{c}	8.86±0.02^{e}	57.55±0.19^{c}	8.51±0.27^{e}	69.14±0.04^{b}	18.47±0.20^{d}

Value represents the mean of three replicates ± Standard deviation (n=3). Within the raws, a different letter indicates significantly different values (P≤0.05).

Results and Discussion

Effect of Starter Cultures on PH, Cyanide and Protein Content at Different Inoculums Level and Fermentation Time

PH

There was a significance difference (p<0.05) in pH of fermented cassava-teff flour due to single starter culture (Table 1,2), size of inoculums (Table 3) and fermentation time (Table 4) except between *Lactobacillus plantarum* and *Lactobacillus coryneformis* using the same inoculum level and fermentation time. Cassava-teff flour fermented with each of 1.5 ml of *Lactobacillus plantarum, Lactobacillus coryneformis* and *Saccharomyces cerevisiae* at 48 h showed pH change from 6.72 ±0.02 to 3.60 ±0.01, from 6.70± 0.10 to 3.62 ±0.02 and from

6.72 ± 0.02 to 5.12 ±0.10 respectively (Table 3), while using 1 ml inocula of *Lactobacillus plantarum, Lactobacillus coryneformis and Saccharomyces cerevisiae* pH reduced from 6.66±0.02 to 3.91±0.06, 6.66±0.10 to 3.93±0.05 and from 6.71±0.10 to 5.43±0.11 respectively after 48 h fermentation time. As fermentation time increases, there was also reduction of pH in all starter cultures expect control (non-fermented sample), differing the extent of reduction depending on types of microorganisms, inoculum level and fermentation time. Similarly, Gunawan *et al.* (2015) showed that the optimum pH condition of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* was 3.5-4.5 and 3.5-6.0 respectively indicating that cassava fermentation by the action of a single species of micro-organisms can result in a significant reduction in pH. The decreases of pH during the fermentation of cassava-teff flour results from the production of an organic acid by lactic acid bacteria on the carbohydrate content of cassava root (Kobawila, 2005; Tefera *et al*., 2014; Victor and Chidi, 2010).

Table4. *pH, protein and cyanide at different times and microbes (inoculum 1.5 ml for all).*

	Treatment with different microbes at different time							
	Control		*Lactobacillus plantarum*		*Lactobacillus coryneformis*		*Saccharomyces cerevisiae*	
Contents	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
pH	6.62±0.02^{a}	6.45±0.19^{a}	5.02±0.02^{d}	3.60±0.10^{e}	5.01±0.20^{d}	3.62±0.02^{e}	5.70±0.00^{b}	5.12±0.01^{c}
Protein (%)	4.23±0.01^{e}	4.22±0.01^{e}	5.96±0.37^{d}	6.98±0.01^{c}	5.90±0.03^{d}	7.02±0.01^{c}	7.24±0.02^{b}	13.31±0.02^{a}
Cyanide(mg/kg)	156.46±0.02^{a}	151.84±0.16^{a}	56.38±0.38^{c}	5.54±0.06^{e}	55.19±0.46^{c}	5.29±0.50^{e}	67.57±0.88^{b}	9.64±0.03^{d}

Value represents the mean of three replicates ± Standard deviation (n=3). Within the raws, a different letter indicates significantly different values (P≤0.05).

Cyanide

Addition of single starter culture, inoculum level and time of fermentation exhibited significant ($p < 0.05$) differences on cyanide content of fermented cassava-teff flour, but there was no significant difference between *Lactobacillus plantarum* and *Lactobacillus coryneformis*(Table 2,3 and 4). The cyanide content of all fermented samples was reduced to lower levels compared to control (non-fermented). However, the extent of reduction varied with the type of microorganisms, size of inoculum and fermentation time. The cyanide content of the cassava-teff flour fermented at 48 h with 1.5 ml inoculums of *Lactobacillus corneformis* was the lowest (5.29±0.50 mg/kg) and followed by *Lactobacillus plantarum* (5.54±0.06 mg/kg). The variation in the reduction of cyanide content within given microorganism is attributed to differences in time of fermentation and the size of inoculum used. In cassava-teff flour fermented with 1.5 ml inoculum of *Lactobacillus plantarum* at the 24 h, cyanide contents reduced from 159.00±1.17 mg/kg to 56.38 ± 0.38 mg/kg and subsequently reduced to 5.54 ± 0.06 mg/kg after 48 h.

This shows that further more fermentation of cassava-teff flour with *Lactobacillus plantarum and Lactobacillus coryneformis* for 48 h caused significant reduction of cyanide content. The reduced cyanide content of fermented cassava-teff flour by *Lactobacillus plantarum and Lactobacillus coryneformis* were below the safe level recommended by WHO (1991). These findings are consistent with the results of Kobawila *et al*. (2005) who reported cyanide reduction drastically from 1158 to 339.6 mg/kg after 48 h of fermentation which corresponds to 70.67 % reduction only the difference is initial cyanide content. Fermentation of cassava flour by selected microorganisms result in microbial growth was shown to be essential for the efficient elimination of cyanogens (Tefera *et al*., 2014). From our investigation, *Lactobacillus plantarum* and *Lactobacillus coryneformis* appear to play an important role in cyanide detoxification, as already reported by Labri *et al.* (2013) and Tefara *et al.* (2014). This indicates that it is possible to significantly reduce the residual HCN content of cassava through fermentation using *Lactobacillus coryneformis* and *Lactobacillus plantarum*. The reduction in cyanide content could be attributed to the ability of the inoculated microorganism (*Lactobacillus plantarum* and *Lactobacillus coryneformis*) to produce linamarase which can hydrolyze linamarin and result in degradation of cyanogenic glycosides in to HCN which is subsequently converted in to formamide which is used as both a nitrogen and carbon source (Kobawila *et al*., 2005; Okafor *et al*., 1998).

According to Table 1, 2, 3 and 4 there was high efficiency of the *Saccharomyces cerevisiae*, next to *Lactobacillus plantarum* and *Lactobacillus*

coryneformis, in abating the cyanide levels that are seen after 24 and 48 h compared to control which contains high levels of cyanide (151.84±0.16 to 156.46±0.02 mg/kg), whereas, in cassava-teff flour inoculated with 1.5 ml *Saccharomyces cerevisiae*, cyanide level dropped from 159.00+1.17 mg/kg to 67.57±0.88 mg/kg and 9.64±0.03 mg/kg after 24 and 48 h respectively. This shows that Saccharomyces *cerevisiae* is capable of utilizing cyanogenic glycosides and the breakdown products, thus explaining why they are natural flora involved in cassava fermentation (Oboh and Elusiyan, 2007). The degradation might be due to enzymes linamarase, hydroxynitrile lyase and cyanide hydratase that catalyze the sequential degradation of cyanogenic glycosides into HCN which is subsequently converted into formamide which is used as both a nitrogen and carbon source (Okafar *et al.*,1998*)*.

Table5. *Comparison of the effect of the microbial fermentation using 1.5 ml inoculums at 48 h and boiling on cyanide content of cassava–teff flour.*

Treatment	Cyanide content	
	Final concentration (mgHCN/kg dw)	HCN reduction (%)
Lactobacillus plantarum	5.54±0.06^{d}	96.51
Lactobacillus coryneformis	5.29±0.05^{d}	96.670
Saccharomyces cerevisiae	9.10±0.03^{b}	94.40
Average	6.38±0.01^{c}	95.90
Boiling	96.10±0.70^{a}	47.77

A value represent means±SD (n=3).Within the column, different letters indicate significantly different values(P≤0.05).

Comparison of the Microbial Fermentation and Boiling on Cyanide Contents

The results indicate that the effect of microbial fermentation on cyanide content was significantly different($p<0.05$) from boiling as boiling accounted for about 47.77% of cyanide reduction whereas, the fermentation by the single starter cultures for 48 h accounted for 94.40 to 96.67% (Table 5). These results are consistent with Nambisan (2011) and Cardosan *et al.* (2005) that boiling (cooking) method could only reduce cyanide content 20-50%, suitable for the processing of sweet variety which contains small cyanide. The result was also in agreement with the results of Abebe *et al*. (2014) who reported that the maximum and minimum percentage reduction of cyanide for fermented cultivars of Gamo and 5553-19, and boiled cultivar of Hayek was observed to be 98 % and 51% respectively. This may be in boiling (cooking) enzymatic breakdown of linamarin is small due to heat denaturation of linamarase (Cardosa *et al.*, 2005). Whereas using microbial fermentation was a very efficient process for elimination of cyanide suggests that this method needs to be used for processing of the variety containing a high amount of cyanide (Labri *et al.*, 2013).

Crude Protein

Single starter cultures, inoculums level and time of fermentation had a significant effect ($p<0.05$) on the crude protein content of fermented cassava-teff flour. The protein content of fermented cassava-teff flour is significantly higher than that of control (Table 2, 3 and 4). The crude protein content of cassava-teff flour fermented with each of 1.5 ml of *Lactobacillus plantarum* and *Lactobacillus coryneformis* increased from 4.23± 0.03 % to 6.98 ± 0.01 and 7.02± 0.01 % respectively after 48 h. The extent of increasing protein content depends on types of microorganism, inoculum level and fermentation time which is consistent with the earlier report by Tefera *et al.* (2014) that fermentation of cassava based food by *Lactobacillus Plantarum* could have increased protein content up to 4.31%. Okafor *et al.* (1998) also given his observation that cassava mash fermented by *Lactobacillus coryneformis* increased lysine content 1.2 to 2.45 % after 48 h of fermentation. The difference is only initial protein content in cassava root that it would appear that the organisms may definitely play some role in increasing the protein content of fermented cassava teff flour because the protein content in the control (non-fermented) cassava-teff flour was consistently lower than the cassava-teff flour inoculated with *Lactobacillus plantarum* and *Lactobacillus coryneformis*. The increase in protein content of fermented cassava-teff flour may be because of some microorganisms which degrade cassava pulp by readily could have secreted some extracellular enzymes (proteins) in the cassava pulp (Oboh and Elusiya, 2007). Growth and proliferation of bacteria during fermentation time in the form of

single-cell proteins increases that may be possibly accounted for the apparent increase in protein content (Bonnop *et al.*, 2009).

The protein content in cassava-teff flour fermented with 1.5 ml inoculums of *Saccharomyces cerevisiae* (13.31±0.02 %) was higher than that of *Lactobacillus plantarum* and *Lactobacillus coryneformis* (Table 4). In our observation, further fermentation of cassava-teff flour with *Saccharomyces cerevisiae* for 48 h caused a significant ($p < 0.05$) increase in the protein content. The crude protein content of fermented cassava-teff flour by *Saccharomyces cerevisiae* showed in Table 3 and 4 was lower than that reported by Boonnop *et al.* (2009) who demonstrated that fermentation of cassava chips with *Saccharomyces cerevisiae* could increase crude protein content from 2 % to 32.4 %. The difference could probably attribute to the size of inoculums used and fermentation time. Similarly, the increase protein content also agrees with earlier reports (Oboh and Elusiya, 2007) that fermentation of cassava with *Saccharomyces cerevisiae* would increase protein content, indicating that *Saccharomyces cerevisiae* had the highest capability to enrich the crude protein content of cassava products.

The increase in protein content in fermented cassava-teff flour could be attributed to the ability of *Saccharomyces cerevisiae* to secret some extracellular enzymes such as amylases, linamarase, and cellulase into cassava mash during their metabolic activities which could lead to yeast growth (Bonnop *et al.*,2009). This high protein cassava product could very well serve as a protein source in animal diets provided it is economically viable.

Table6. *Sensory acceptability scores of injera at different times and microbes (using 1 ml inoculum for all).*

Quality attributes	Treatment with different microbes at different time							
	Control		*Lactobacillus planetarium*		*Lactobacillus coryneformis*		*Saccharomyces cerevisiae*	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Flavor	2.33±0.29^{d}	2.33±0.29^{d}	3.13±0.13^{c}	3.80±0.20^{b}	3.07±0.12^{c}	3.77±0.38^{b}	3.90±0.17^{b}	4.53±0.06^{a}
Color	4.17±0.29^{a}	4.17±0.29^{a}	4.30±0.26^{a}	4.43±0.40^{a}	4.33±0.06^{a}	4.47±0.06^{a}	4.50±0.50^{a}	4.67±0.29^{a}
Taste	2.60±0.17^{d}	2.60±0.17^{d}	3.87±0.12^{b}	4.67±0.31^{a}	3.83±0.06^{b}	4.57±0.40^{a}	3.13±0.23^{c}	3.83±0.15^{b}
Texture	2.27±0.23^{d}	2.27±0.23^{d}	3.07±0.40^{c}	3.87±0.12^{b}	3.07±0.06^{c}	3.83±0.29^{b}	3.90±0.17^{b}	4.47±0.42^{a}
Overall acceptability	2.77±0.25^{d}	2.77±0.25^{d}	3.56±0.06^{c}	4.17±0.29^{b}	3.53±0.06^{c}	4.17±0.29^{b}	3.93±0.12^{b}	4.40±0.34^{a}

Value represents the mean of three replicates ± Standard deviation (n=3). Within the raws, a different letter indicates significantly different values (P≤0.05).

Table7. *Sensory acceptability scores of injera at different times and microbes (using 1.5 ml inoculum for all).*

Quality attributes	Treatment with different microbes at different time							
	Control		*Lactobacillus plantarum*		*Lactobacillus coryneformis*		*Saccharomyces cerevisiae*	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Flavor	2.33±0.29^{d}	2.33±0.29^{d}	3.43±0.21^{c}	4.17±0.29^{b}	3.4±0.17^{c}	4.13±0.23^{b}	4.17±0.29^{b}	4.87±0.23^{a}
Color	4.17±0.29^{a}	4.17±0.29^{a}	4.43±0.21^{a}	4.17±0.29^{a}	4.40±0.17^{a}	4.53±0.06^{a}	4.67±0.29^{a}	4.83±0.28^{a}
Taste	2.60±0.17^{d}	2.60±0.17^{d}	4.27±0.25^{b}	4.90±0.17^{a}	4.27±0.23^{b}	4.87±0.23^{a}	3.47±0.57^{c}	4.23±0.25^{b}
Texture	2.27±0.23^{d}	2.27±0.23^{d}	3.40±0.10^{c}	4.17±0.47^{b}	3.40±0.20^{c}	4.17±0.29^{b}	4.13±0.23^{b}	4.73±0.11^{a}
Overall acceptability	2.77±0.25^{d}	2.77±0.25^{d}	3.87±0.12^{c}	4.43±0.21^{b}	3.87±0.23^{c}	4.40±0.34^{b}	4.13±0.23^{b}	4.67±0.12^{a}

Value represents the mean of three replicates ± Standard deviation (n=3). Within the raws, a different letter indicates significantly different values (P≤0.05).

Sensory Evaluation of Cassava Based Food (Injera)

Flavor

The sensory acceptability showed significance ($p<0.05$) differences between single starter culture and control on the flavor of produced injera. Analysis of variance also had shown significance ($p<0.05$) differences among single starter cultures (Table 6) at different fermentation time (Table 7) and inoculums level (Table 8) except between *Lactobacillus plantarum* and *Lactobacillus coryneformis*. The means scores of the flavor test of samples are appeared to improve with a longer period of fermentation of cassava-teff flour for each starter culture, the highest values being attained around 48 h of fermentation time. The score given to flavor acceptability were

highest for injera produced from 1.5 ml inoculum of *Saccharomyces cerevisiae* after 48 h fermentation time, which was 4.87±0.23. Regarding the inoculum level, the highest score observed in injera produced from a 1.5 ml inoculum level for all starter cultures. The flavor acceptability score of the control (none fermented) 2.33 ± 0.29 is lower when compared with injera produced from the fermented cassava-teff flour. The microbial activities which increased as fermentation continued might have accounted for the perceived differences in the flavor of the product fermented for different lengths of fermentation time. The flavor of food depends on the balance of volatile compounds those produced during fermentation. A vast number of volatile compounds may be synthesized and modulated by *Saccharomyces cerevisiae* during fermentation. In line with this finding, Tefera *et al*. (2014) reported that *Saccharomyces cerevisiae* was able to produce compounds such as organic acids, alcohols, aldehydes, and carbonyls which have imparted appealing flavor to the fermenting cassava.

A previous study by Hasan *et al*. (2018) on fermented rice also showed an increase in volatile compounds due to fermentation by yeasts and lactic acid bacteria. Similar to the current study it has been reported that *Saccharomyces cerevisiae* contributes to flavor development while fermenting rice for injera production.

Color and Taste

Regarding Color of cassava based food (injera) the highest score was 4.83 ± 0.28 and 4.67 ± 0.29 by 1.5 and a 1ml inoculum of *Saccharomyces cerevisiae* respectively at 48 h fermentation time, while the least score 4.17±0.29 was for control. However, Analysis of variance showed that using a single starter culture (Table 6), time of fermentation (Table 7) and addition of inoculum level (Table 8) had shown no significant difference (P >0.05) on the color acceptance of injera. Color acceptability scores of the single starter culture and the control (non-fermented) were all in the range of 4.17±0.29 and 4.83 ± 0.28 indicating that using single starter culture at different inoculums level and fermentation time does not change the color of injera.

The sensory score of taste appears greater than three in all cassava based food (injera) produced by fermentation except for control (non-fermented). Analysis of variance showed that using single starter culture, addition of inoculums level and fermentation time had shown significant ($p<0.05$) difference regarding taste attributes of cassava based food, but no significant difference ($p>0.05$) was detected between *Lactobacillus plantarum* and *Lactobacillus coryneformis* on taste attributes of cassava based food(Table 6,7 and 8). This indicates that all organisms seem to be responsible for the taste of the cassava based food as suggested by Odibo and Umeh (2014) and confirmed by the present study.

However, panelists rated the sample fermented with 1.5 ml inoculums of *Lactobacillus plantarum and Lactobacillus coryneformis* at 48 h as having the best taste with the score of 4.90 ± 0.17 (98%) and 4.87 ± 0.23 (97%) respectively, but the sample fermented with 1 ml inoculums of *Saccharomyces cerevisiae* at 24 h fermentation time as having lower taste with the score of 3.13±0.23 (62.6%). As the fermentation time increased, the scores for taste also increased in each starter culture. This result agrees with earlier reports by Tefera *et al.* (2014) that fermentation of cassava based food (chike) by *Lactobacillus mesenteroides* result in 72% score of taste, only a difference is inoculum level used. This might be possibly attributed to the fact that *Lactobacillus plantarum* and *Lactobacillus coryneformis* converts the sugars in fermenting substrate (primarily glucose and fructose) to lactic acid, acetic acid, ethanol, CO2 and other flavor compounds (Olaoluwa *et al.*, 2013).

Texture

As shown in Table 6, 7 and 8, there was a significant difference ($p<0.05$) in the texture acceptability scores between control and starter cultures as well as among starter cultures, inoculum levels and fermentation time of cassava based food. However, the least scores 3.07±0.4 and 3.07±0.06 were for the cassava based food fermented with 1 ml inoculums level of *Lactobacillus plantarum* and *Lactobacillus coryneformis* respectively at 24 h. The highest mean score 4.73±0.11 was observed for the cassava based food fermented with 1.5 ml *Saccharomyces cerevisiae* at 48 h fermentation time followed by 1.5 ml *Lactobacillus plantarum (*4.17±0.47*)* and *Lactobacillus coryneformis* (4.17±0.29*)*. This showed that the starter culture, inoculums level and fermentation time

influences the quality of dough thereby that of the texture of the injera. This indicates that *Saccharomyces cerevisiae* possesses cellular activities, where also found to be contributed to the modification of cassava texture during fermentation (Oboh and Elusiyan (2007).

Table8. *Sensory acceptability scores of injera using different inoculum size and microbes (time 48 h for all).*

Quality attributes	Treatment with different microbes at different inoculum size						
	Control	*Lactobacillus plantarum*		*Lactobacillus coryneformis*		*Saccharomyces cerevisiae*	
		1 ml	1.5 ml	1 ml	1.5 ml	1 ml	1.5 ml
Flavor	2.33±0.29^{e}	3.80±0.20^{d}	4.17±0.29^{c}	3.77±0.38^{d}	4.13±0.23^{c}	4.53±0.06^{b}	4.87±0.23^{a}
Color	4.17±0.29^{a}	4.43±0.40^{a}	4.63±0.15^{a}	4.47±0.06^{a}	4.53±0.06^{a}	4.67±0.29^{a}	4.83±0.28^{a}
Taste	2.60±0.17^{e}	4.67±0.31^{b}	4.90±0.17^{a}	4.57±0.40^{b}	4.87±0.23^{a}	3.83±0.15^{d}	4.23±0.25^{c}
Texture	2.27±0.23^{e}	3.87±0.12^{d}	4.17±0.47^{c}	3.83±0.29^{d}	4.17±0.29^{c}	4.47±0.42^{b}	4.73±0.11^{a}
Overall acceptability	2.77±0.25^{d}	4.17±0.29^{c}	4.43±0.21^{b}	4.17±0.29^{c}	4.40±0.34^{b}	4.40±0.34^{b}	4.67±0.12^{a}

Value represents the mean of three replicates ± Standard deviation (n=3). Within the raws, a different letter indicates significantly different values (P≤0.05).

Overall Acceptability

Overall acceptability scores exhibited significance ($p<0.05$) differences between starter cultures and control (Table 6). The inoculums level (Table 7) and fermentation time (Table 8) also exhibited significance ($p<0.05$) differences. However, there was no significance ($p<0.05$) difference between the *Lactobacillus plantarum* and *Lactobacillus coryneformis (*Table 7 and 8). The highest overall panelists acceptability 4.67±012 was recorded for the sample fermented with 1.5 ml inoculum of *Saccharomyces cerevisiae* at 48 h fermentation time, followed by 1.5 ml inoculums of *Lactobacillus coryneformis and Lactobacillus plantarum* which was 4.40±0.34 and 4.40±0.34 at 48 h fermentation time, while the lowest overall panelist acceptability 3.53±0.06 was recorded by 1 ml inoculums of *Lactobacillus coryneformis* at 24 h fermentation time.

This indicates that pure cultures of isolates had varying contributions to the overall acceptability of injera. This finding is in agreement with Tefera *et al*. (2014) who explained in his report that overall sensory score 3.48 for the sample fermented with *Saccharomyces cerevisiae* for 48 h with the addition of 0.75 ml inoculums showed preference by panelists compare to samples fermented with *Lactobacillus palantarum* and *Lactobacillus mesenteroides*. The difference is only types of food prepared and amounts of inoculum level used. This might be due to the improvement of the sensory quality of the product by *Saccharomyces cerevisiae*. A previous study by Hasan *et al*. (2018) on fermented rice flour has shown an increase in volatile compounds due to fermentation by yeasts and lactic acid bacteria. This volatile compound may significantly impact the overall quality of the product. Chelule *et al*. (2010) also explained in his report that fermentation makes the food palatable by enhancing its flavor which makes fermented food more popular than the unfermented one in terms of consumer acceptance.

CONCLUSIONS

From this study, fermentation using starter cultures was found to be effective and resulted in low toxic cyanide (HCN) compare to boiling. From inoculated pure single starter cultures, highest cyanide reduction was recorded with 1.5 ml of *Lactobacillus coryneformis* (5.29 ± 0.50 *mg/Kg),* followed by *Lactobacillus plantarum* (5.54 ± 0.06 mg/Kg) at 48 h fermentation time, while the least cyanide reduction was recorded by *Saccharomyces cerevisiae* (9.64±0.03 mg/Kg) at the same fermentation time with the same inoculum level, but in all cases the final concentration of cyanide was below WHO (1991) standard and safe for human consumption. From samples fermented by single starter cultures, *Saccharomyces cerevisiae* was identified as more efficient in improving the crude protein content of cassava-teff flour from 4.23 ± 0.23 % control (non-fermented) to 13.31 ± 0.02 % after 48 h at 1.5 ml inoculums level compare to lactic acid bacteria. Therefore, increasing the protein content of cassava for injera production using microbial cells is important and prospect for the transformation of cassava based food into the protein-rich food-stuff. Regarding sensory evaluation of cassava based food, sample fermented with 1.5 ml inoculums of *Lactobacillus*

plantarum and *Lactobacillus coryniformis at 48 h* was more preferred by the panelist in terms of taste, while sample fermented by 1.5 ml inoculums of *Saccharomyces cerevisiae* at 48 h was preferred in improving flavor, texture and overall acceptability.

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