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SENSORY PROPERTY EVALUATION ON CASSAVA BASED FOODS THROUGH SINGLE AND MIXED STARTER CULTURE OF MICEOBIAL FERMANTATION

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Abstract
This study was carried out to investigate the sensory property of chike (forge made from cassava powder) and improve the traditional processing method and nutritional quality of the traditional cassava snack (chike). Cassava root (ManihotesculentaCrantz) of white variety was processed into powder. The cassava powder of 30g was inoculated with 0.75ml of starter in single and mixed and to produce cooked cassava forage (chike). These products were analyzed to determine the color, odor, taste and overall acceptability of the product. The results obtained for the color odor taste and overall acceptability showed that odor and overall acceptability of chike fermented with S. cerevisiae were more preferred than all other samples including the unfermented ones, while samples fermented with Leu. mesenteroides produced more preferred taste, color, odor and overall acceptability in chike. Among mixed cultures, the sample fermented with S. cerevisiae, L. plantarum and Leu. mesenteroides for 48hrs with addition of 0.75ml of inoculum level was the most preferred in terms of color and with reasonably high ratings but still with no significant difference ($p>0.05$) in terms of odor, taste and overall acceptability when compared with other fermented samples.

Keywords: Cassava Chikelactobacillus plantarum, leu.mesentroides, S. cerevisiae

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INTRODUCTION

Producing enough food, in a sustainable manner, to meet the needs of an increasing global population is one of the greatest challenges we face. Recent estimates are that food production will need to double by 2050 (Baulcombe*et al*, 2009). Most of the world's population relies on a plant-based diet. Cassava (*Manihotesculenta*Crantz) is the third most important source of calories in the tropics (FAO,2010) and the sixth most important food crop after sugar cane, maize, rice, wheat and potato, in terms of global annual production (FAOSTAT, 2010). Due to its versatile nature, it is frequently referred to as the "drought, war and famine crop" to much of the developing world (Pearce, 2007). While it is already a major staple crop, it has the potential to be an important part of the solution to improving food security in a time of climate change (Gallo and Sayre, 2009). It can grow on poor soils, is easily propagated, requires little cultivation and can tolerate periodic and extended periods of drought (El-Sharkawy,2009). The main food product is the tuberous roots, which can be retrieved from the soil up to three years after maturity (Lebot, 2009), allowing crops to be abandoned during periods of agricultural and social instability. This provides an important form of "insurance" against social disruption, prolonged droughts, or other periods of stress and unrest. Thus, cassava is an important means by which food production could be increased without the use of large amounts of agricultural inputs (e.g, fertilizers, water and pesticides). Cassava is also emerging as an important large-scale agricultural crop for use as a bio-fuel (Gille*et al.*,2010) and a source of industrial starch (Lebot, 2009), although these are not considered here. According to the Food and Agriculture Organization, cassava is the third most important source of calories in the tropics, after rice and corn (F A O, 2002). It is usually processed traditionally to obtain different relatively shelf stable intermediate and final products for various food applications. These products include "gari," a roasted fermented cassava meal, "agbelima," which is a fermented cassava mash, the dried cassava chips known as "kokonte," and which is further processed into cassava flour. Tapioca is a minor product or by-product from cassava processing. For industrial use, cassava is processed to obtain starch. This intermediate product can be used for producing sugar, acetone, and alcohol. Some people believe that Brazil can produce 20% of the alcohol it needs for motor fuel from cassava. *Chike*" is a traditional cassava-based cooked forage product which is popular in areas of Southern and Orromiya regions, Ethioipa. It is a little known traditional product of cassava on which little or no investigation has been carried out. The need to improve the sensory property of

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cassava processing has been identified (Oyewole' 2003). Some of the ways of modifying the traditional process of producing *chike* (traditional cooked cassava forage) is introduction of adding value to the traditional snack. The objectives of this research work were to investigate the sensory property and improve the traditional processing method of the traditional cassava forage (*chike*), and the consumer's acceptability of the improved product.

MATERIALS AND METHODS

The peeled cassava tubers (2 kgs) were cut into cylindrical pieces and steeped in 4 litter of sterile distilled water for 72 hours. The resulting soft cassava tubers were hand pulverized and sieved using a sieve of about 1.00 mm mash size. The sieved mash was allowed to sediment for 12 hours before the tap water was decanted. The sediment mash was then placed in jute bag and pressed to remove the water. The resulting wet product was further dried in a single layer at 65oC for 48 hours in a cabinet dryer. The dried cake was then milled to powder by mortar and pestle. Finally the powder was kept in refrigerator at 4oC until used for further analysis (Oyewole, 1991).

Selection of starter microorganisms from fermented cassava.

Three isolates which were dominant during the fermentation were selected. *L. plantarum* and *L. mesenteroides*and *S. cerevisiae*. The two bacterial isolates belong to lactic acid bacteria that are commonly isolated from foods. *L. plantarum*and *L. mesenteroides*, apart from being widely used in the preparation of fermented milks, have been reported as the predominant strains among isolates of traditional sour cassava fermentation (Figueroa *et al*., 1995). Similarly, *S. cerevisiae* is known industrially as important yeast used in the production of a variety of fermented foods. Besides, all the three isolates have no history of pathogencity (Colar, 1996). A similar procedure was employed in selection of starter cultures of fermented maize bread by previous researchers (Edem and Sanni, 2008).

***Saccharomyces cerevisiae* (S.C)**

Growth medium containing yeast extract (1%), peptone (2 %), and glucose (2%) was prepared using three Erlenmeyer flasks of 250 ml capacity. Spore suspensions of *S. cerevisiae* were also prepared using sterilized peptone water in to the respective agar slants. The resulting suspensions were adjusted with sterile peptone water using a spectrophotometer to give a concentration of 10^6 – 107 cfu/ml and subsequently used as inocula. About 20 gm of cassava flour was then added into each of the three flasks and the moisture content was adjusted to about 25%. After

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autoclaving, the three flasks were inoculated with 0.25 ml, 0.5 ml, 0.75 ml of *S. cerevisiae* spore suspension and incubated at 25^{o}C (optimum growth temperature). Samples were then withdrawn for analysis after 24, 36 and 48 hrs of fermentation.

***Lactobacillus plantarum* (L.P) and *Leuconostocmesenteroides* (Leu.M)**

The growth medium used for slants of *Lactobacillus plantarum* and *Leuconostocmesenteroides* was MRS medium. 10 ml of sterile peptone water was added to 18-24 hrs held culture slants of *Lactobacillus plantarum* and *Leuconostocmesenteroides*, followed by aseptic agar surface scrapping under vigorous shaking (Adeyel, 1986). From the resulting suspensions, 0.25 ml, 0.5 ml, and 0.75 ml of each of *L. plantarum* and *Leumesenteroides* were added aseptically to each of the two sets of three flasks containing 20 g of sterile cassava mush and allowed to ferment for 24, 36 and 48 hrs. The incubation temperature and the moisture contents were adjusted to 30^{o}C and 25%, respectively. After fermentation the water was pressed out and used for further analysis.

Sensory Evaluation of Cassava Inoculated With Single Starter Culture.

Sensory Evaluation

Sensory evaluation of the samples fermented with single selected culture, a combination of selected cultures and with no culture was done at the time with equal amount of sample divided in labeled plastic trays. Then the samples were evaluated by assessors from Gambella ATVET College students of Meshenger zone. The samples were evaluated by 30 students (20 female and 10 male students). The selection of these students was done using purposive sampling technique to include only those who were familiar to the cassava products. Evaluation was done on a five point hedonic scale with respect to color, odor, taste and overall acceptability following the methods of Larmond (1977). The student panelists were instructed to taste the samples and rinse their mouth before and after each sample taste. During tasting, the students were also advised to chew the samples but not to swallow. They were requested to express their feelings about the samples by scoring the organoleptic attributes using the Hedonic scale (1= dislike extremely, 2 = dislike, 3 = neither like nor dislike, 4 = like and 5 = like extremely). To avoid different mouth feel, 15 minutes break was given and students were served with biscuits and soft drinks throughout the tasting session.

The student's evaluated *chike* made from equal proportion of 40g fermented and unfermented cassava flour sample stapled with cereal flour maize was placed into boiling distilled water

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150ml, stirred and left to boil for 5 minutes until a thick porridge was formed. The porridge was immediately served in treys. In all cases, the students were allowed to independently evaluate and score the samples.

Statistical analysis

All the measured variables were subjected to the analysis of variance for complete randomized design using SAS software. Three way ANOVA was used to compare results among fermented cassava and unfermented control. The least significance difference (LSD) at 5% was used to separate significant differences by different treatment means.

RESULTS AND DISCUSSION

Isolation, Identification and Selection of Starter Microorganisms from Fermented Cassava

Three isolates that were dominant during the fermentation were also persistently appearing at the end of the fermentation of cassava. Microscopic and macroscopic examination indicated that the isolates were bacteria and yeast. Further morphological, physiological and biochemical tests led to the identification of the two isolates, i.e. GCLAB3 and GCLAB4, as *L. plantarum* and *L. mesenteroides*(Zhang *et al*., 2000), respectively, and the third isolate (GCY3) as *S. cerevisiae* (Kurtzman, 1988). The two bacterial isolates belong to lactic acid bacteria that are commonly isolated from foods. *L. plantarum and L. mesenteroides*, apart from being widely used in the preparation of fermented milks, have been reported as the predominant strains among isolates of traditional sour cassava fermentation (Figueroa et al., 1995). Similarly, *S. cerevisiae* is known industrially as important yeast used in the production of a variety of fermented foods. Besides, all the three isolates have no history of pathogenicity (Colar, 1996). Hence, as a result of these desirable characteristics, they were selected in this study as starter microorganisms for further study in the in-vitro fermentation of cassava. A similar procedure was employed in selection of starter cultures of fermented maize bread by previous researchers (Edem and Sanni, 2008).

Sensory Evaluation of Cassava flour Inoculated with single Starter Cultures

The effect of single starter culture, time of fermentation and addition of 0.75ml of inoculums level on sensory quality of *chike.*

Analysis of variance showed that the interaction effect of single starter culture, time of fermentation and addition of 0.75ml of inoculum level had a significant ($P<0.05$) difference on the odor and taste and highly significant ($P<0.001$) difference on overall acceptability of *chike* (Table 1). In contrast, both the main and interaction effect of starter culture, fermentation time

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and addition of 0.75ml of inoculum level made no significant ($p>0.05$) difference on the color of *chike*.

Table 1 shows the result of sensory evaluation carried out on *chike* (product made from cooked cassava) fermented with three single starter culture, in three different fermentation times with the addition of 0.75ml of inoculums size. The panelists preferred sample fermented with *S. cerevisiae* for 48hrs with 0.75ml of inoculum size. They rated the odor of *chike* produced under this treatment condition as the best giving it a score of 3.60 (72%). The microbial activities which increased as fermentation progressed might have accounted for the perceived differences in the odor of the product fermented for different lengths of fermentation time. In line with this finding Torner*et al*. (1992) reported that *S. cerevisiae* was able to produce compounds such as organic acids, alcohols aldehydes and carbonyls which have imparted appealing flavor to the fermenting cassava. Similarly, Oyewole (1990) reported that the yeast flora whose population increased with the increase in period of fermentation contributed significantly to the odor of fermented cassava. Samples fermented with *L. plantarum* for 48 hrs with addition of 0.75ml of inoculum size were rated least by the panelists with a score of 2.46 (49.2%). This might be due to production of unpleasant odor by lactic acid bacteria during fermentation. This is in agreement with Edema and Sanni (2008) who reported hetrofermentetative lactobacilli usually produce sharp and unpleasant odors during fermentation and this may be responsible for the low sensory acceptance (odor) perceived for cassava fermented with *L. plantarum.*

The panelists rated the sample fermented with *Leu. mesenteroides* for 48 hrs at an inoculum level of 0.75ml as having the best taste with the score of 3.76 (75.2%) . This might be possibly attributed to the fact that *Leu. mesenteroides* converts the sugars in fermenting substrate (primarily glucose and fructose) to lactic acid, acetic acid, ethanol, CO2, and other flavor compounds (Lu *et al*., 2010). Samples fermented with *L. plantarum* for 24hrs with 0.75ml inoculum level scored 2.73 (54.6%) and was identified as the least preferred compared to other fermented samples and the unfermented control that scored 2.99 (59.8%) in terms of *chike'*s taste.

In terms of overall acceptability, compared to samples fermented with other combinations of treatments and unfermented samples, the panelists rated 3.48 (69.6%) and showed preference for the samples fermented with *S. cerevisiae* for 48hrs with the addition of 0.75ml inoculum level. This might be due to improvement of the organoleptic property of the product by *S. cerevisiae*.

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This finding is in agreement with Sanni (1993) who indicated about the role of *S. cerevisiae* in fermented foods and beverages showing that besides having many beneficial effects it also improves the flavor, texture, overall acceptance and the shelf-life of the products. The least score, i.e. 2.63 (52.6%), was recorded in the sample fermented with *L. plantarum* for 36hrs with the addition of 0.75ml inoculum level when compared to the unfermented samples which were rated 2.96 (59.8%) and all other samples fermented by other starter cultures. This might be due to the fact that this particular lactic acid bacterium produced high concentration of organic acid which strongly acidified the fermented cassava product. The least preference of samples fermented with *L. plantarum* in this study is in agreement with the findings of Braumane*t al.* (1996) who reported that the volatile fatty acids along with lactate were responsible for low acceptability of fermented cassava. The effect of single starter culture, time of fermentation with addition of 0.75ml inoculum level on color of *chike* was non-significant. In general, the sensory evaluation of *chike*showed that all the pure cultures of isolates had varying contributions to odor, taste and overall acceptability with *S. cerevisiae* playing a major role in enhancing odor and overall acceptability and *Leu. mesenteroides* only in the taste of *chike.*

Table 1: The effect of single starter culture, time of fermentation and addition of 0.75ml inoculum size on sensory quality of *chike*

Mean squares						
Treatment			**Parameters**			
Microorganism	**Time(hrs)**		**Color**	**Odor**	**Taste**	**Overall acceptance**
S C	24		3.56^{ab}	2.9^{b-e}	2.96^{c-f}	2.66^{cd}
	36		3.12^{ab}	3.10^{a-c}	3.30^{a-c}	3.46^{a}
	48		3.53^{ab}	3.60^{a}	3.63^{a-d}	3.48^{a}
L P	24		3.70^{ab}	2.85^{de}	2.73^{ef}	2.73^{cd}
	36		3.73^{ab}	2.66^{de}	3.33^{a-f}	2.63^{d}
	48		3.10^{ab}	2.46^{e}	3.30^{a-e}	3.00^{a-d}
Leu. M	24		3.56^{ab}	2.66^{de}	2.96^{d}	3.40^{ab}
	36		3.76^{ab}	2.90^{b-e}	3.70^{a-b}	3.33^{a-c}
	48		3.31^{ab}	3.40^{a-c}	3.76^{a}	2.96^{a-d}
Unfermented			3.33	3.31	2.96	2.96
LSD(0.05)			0.86	0.72	0.75	0.78
CV (%)			28.15	27.96	29.05	26.07

LSD = least significant difference, CV = coefficient of variances, ,*SC = Saccharomyces cerevisiae*, *LP = Lactobacillus plantarum*, *LeuM = Leuconostocmesenteroides*
Ino. = inoculum,
Mean values with the same letters of superscripts along the columns are not significantly different ($p<0.05$)

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Sensory Evaluation of Cassava flour Inoculated with Mixed Starter Cultures

The effect of mixed starter culture, time of fermentation and 0.75ml of inoculum level on sensory qualities of *chike*

The statistical analysis of the sensory evaluations by panelists showed that the interaction of mixed starter culture time of fermentation and addition of 0.75ml inoculum level had significant ($p< 0.05$) difference on color and taste of *chike* and highly significant ($p<0.001$) difference on odor and overall acceptability of *chike* (Table 2).

The result of the effect of mixed starter culture, time of fermentation and addition of 0.75ml of inoculum level on sensory quality of *chike* is presented in Table 2. The sample fermented with mixed starter culture of *S. cerevisiae, Leu. mesenteroides*and *L. plantarum* for 24hrs with addition of 0.75ml inoculum size was rated as 4.06 (81.2%) and was preferred by panelists as the best when compared to samples fermented with other combinations and the unfermented control in terms of chike color. In contrast, the sample fermented by the combination of *S. cerevisiae* and*Leu. mesenteroides* for 48 hrs was rated as 3.13 (62.6%) and was considered as the least preferred when compared with samples fermented with other combinations and the unfermented control which was rated 3.33 (66.6%).

With regards to *chike* odor, the sample fermented with a mixture of three of the microbial species for 48 hrs with the addition of 0.75ml inoculum was rated 3.96 (79.2%) was and preferred as best odor by panelists, while on the other hand, the sample fermented with *L. plantarum and Leu. mesenteroides* for 24 hrs showed the lowest score, i.e. 2.46 (48%), compared to the rest of the fermented products and the unfermented control which was rated 3.31(62.6%). These findings also indicated that as time of fermentation increased, the preference for chike odor also increased. This is in line with the findings of Achinewhu et al. ,1998; Achinewhu and Eke (2002) who reported that duration of the fermentation time was improved the odor and general acceptability of garri and emphasized on the need to ferment cassava mash for a minimum of 48 hrs.

The panelists rated the sample fermented with a mixture of *S. cerevisae and L. plantarum* for 48hrs with inoculum size of 0.75ml as 3.83 (76.6%) and considered the product (*chike*) as having the best taste. In contrast, the sample fermented for 24 hrs with a mixture of *L. plantarum and Leu. mesenteroides* was rated 2.60 (52%) and accordingly identified as the least preferred in taste when compared to other fermented samples and the unfermented sample which was rated 2.99

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(59.2%) Regarding overall acceptability of *chike*, the sample fermented with the combination of the three microorganisms for 48hrs at 0.75ml inoculum level showed the highest score, i.e. 3.93 (78.6%), while the sample fermented by a mixture of *L. plantarum and Leu. mesenteroides* for 24hrs with the addition of 0.75ml of inoculum was rated 2.46 (49.2%) and was subsequently perceived as the least preferred by the panelists.

In general from samples fermented by mixed starter cultures, the sample fermented with three combinations (i.e. *S. cerevisiae*, *L. plantarum*and*Leu. mesenteroides*) for 48hrs with addition of 0.75ml of inoculum level was the most preferred in terms of color and with reasonably high ratings but still with no significant difference in terms of odor, taste and overall acceptability when compared with other samples fermented by mixed culture.

Table 2: The effect of mixed starter culture, time of fermentation and addition of 0.75ml of inoculum size on sensory quality of *chike*

Mean Squares					
Treatment		**Parameters**			
Microorganisms	**Time(hrs)**	**Color**	**Odor**	**Taste**	**Overall acceptance**
S C, L P and *Leu M*	24	4.06^{a}	3.40^{a-g}	3.46^{a-e}	3.90^{a}
	36	3.96^{ab}	3.80^{ab}	3.70^{ab}	3.73^{a-d}
	48	3.76^{a}	3.96^{a}	3.80^{a}	3.93^{a}
S C and *Leu M*	24	3.56^{a-c}	2.86^{d-i}	2.96^{c-f}	2.63^{f-h}
	36	3.53^{a-c}	3.03^{c-i}	3.31^{a-f}	3.50^{a-e}
	48	3.13^{c}	3.60^{a-e}	3.53^{a-d}	3.46^{a-f}
L P and *Leu M*	24	3.76^{ab}	2.46^{hi}	2.60^{ef}	2.46^{gh}
	36	3.70^{a-c}	2.46^{hi}	3.33^{a-f}	2.67^{d-h}
	48	3.16^{c}	2.80^{e-i}	3.30^{a-f}	3.00^{b-h}
L P and *S C*	24	3.76^{ab}	2.66^{g-i}	2.96^{c-f}	3.50^{a-e}
	36	3.50^{a-c}	2.99^{d-i}	3.76^{ab}	3.33^{a-g}
	48	3.33^{c}	3.40^{a-h}	3.83^{a}	2.96^{c-h}
Unfermented		3.33	3.31	2.96	2.96
LSD(0.05)		0.87	0.80	0.83	0.82
CV (%)		27.59	29.46	28.15	28.82

LSD = least significant difference, CV = coefficient of variances,
SC = Saccharomyces cerevisiae, *LP = Lactobacillus plantarum*, *Leu. M = Leuconostocmesenteroides*, Ino. = inoculum,
Mean values with the same letters of superscripts along the columns are not significantly different ($p<0.05$)

CONCLUSION

The sensory evaluation of *chike* in this study showed that cassava fermented with single starter culture of *S. cerevisiae* was more preferred by panelists in terms of odor and overall

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acceptability, while cassava fermented by *Leu. mesenteroides* was preferred in improving the taste of the product. *Chike* prepared from cassava fermented by a mixed starter culture of the three microorganisms for 48 hrs at an inoculum level of 0.75ml was the most preferable in terms of color, while at the same time showing high values for odor, taste and overall acceptability which were comparable with results of other treatments.

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