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A Study on Possible Use of Calcium Propionate and Ascorbic Acid in the Production of Cassava Bread

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Abstract: **There is little or no information on the use of legal additives in the production of cassava bread, a baked product made with cassava flour and most often in combination with wheat flour. In this study, calcium propionate (anti moulding agent) and ascorbic acid (a food antioxidant) were assessed as food additives in cassava bread samples made with cassava flour from two cassava varieties (TME419 and TMS30572) with 60%, 80% and 90% wheat flour inclusion. The molds associated with the spoilage of bread were studied. Results showed that 0.6% calcium propionate inclusion in the bread samples could make the bread to have safe level of molds even at four days post processing storage at ambient temperature (26-29ºC). The studies on colony count in breads at different storage intervals showed that treatment containing 0.6% calcium propionate 0.3% ascorbic acid proved to be most effective against control of count. Most molds isolated belonged to the genera Rhizopus, *Aspergillus*, and *Pencillium*. After analyzing the samples, *Rhizopus* was found to be the most occurring fungi in bread.**

The fresh bread samples with the additives were also generally acceptable by the consumers (sensory panelists) in an organoleptic evaluation with the ratings of the samples having 80-90% wheat flour inclusion not being significantly different (P=0.05) with that of 100% wheaten bread.

Keywords: **Cassava flour, cassava bread, food additives, calcium propionate, ascorbic acid, mould.**

1. INTRODUCTION

In recent years, scientists in Nigeria have developed a baked product known as cassava bread that is largely made with cassava (*Manihot esculenta*) flour alone or as composite with common wheat (*Triticum aestivum*) flour (Shittu *et al*, 2007; Oti and Ukpabi, 2007). Generally, common wheat flour inclusion in cassava bread production enhances consumers' acceptability of the baked product. This is based on the fact that cassava flour has no gluten and the presence of gluten in wheat flour makes it most suitable for production of yeast leavened products such as wheaten bread (Tarar *et al*., 2010). Considerate efforts have been made to promote the use of composite flours (from locally grown crops in Nigeria to replace a portion of wheat flour in bread production) so as to reduce the demand for imported wheat and stimulate the use of locally grown non-wheat agricultural produce as floury food materials. High Quality Cassava Flour (HQCF) has gained popularity in the West African sub region over the past few years as a raw material in the production of baked products such as bread (Eddy *et al*., 2007; Komlaga *et al*, 2012).

Oti and Ukpabi (2007) reported the possibility of 20% substitution of cassava flour to wheat flour in large scale bread production in Nigeria. Similarly Komlaga *et al*. (2012) reported high level of consumer acceptability of bread baked with 10% and 20% cassava flour substitution in Ghana. However, they observed that bread baked with ≥30% substitution was not highly acceptable to consumers when compared to those baked with 100% wheat flour. Although bread is regarded as the second most highly consumed non-indigenous food product after rice in Nigeria, it has limited shelf life. Bread spoilage mainly results from mold contamination, (moldiness) especially *Penicillium* and *Aspergillus* (Smith *et al*., 2004; Pateras, 2007). Bread is prone to rapid microbial spoilage, particularly mold growth, due to post-baking contamination during cooling, slicing and wrapping, which greatly limits its shelf life. Thus methods of mold control are of great importance to the bakery industry (Smith *et al.*, 2004). In addition to substantial economic losses associated with fungal spoilage, there are concerns regarding potential hazards of mycotoxin contamination, which could affect human health (Legan, 1993).Moldiness caused by molds and ropiness due to bacterial contaminations are the major factors responsible for microbial spoilage of bread (Pyler and Gorton, 1988). However the dominant spoilage flora varies with the type of bread, the storage temperature and the season. At present, wheaten breads often spoil within 48 hours of production. The use of techniques such as attention to hygiene within the bakery and use of preservatives and other additives are currently being employed to improve consumer acceptability and the shelf life of baked bread made with wheat flour (Alais and Linden, 1999).

Presently, there is dearth of information on the use of relevant additives in the production of cassava bread. The aim of this study was therefore to evaluate the possible use of calcium propionate (anti roping and molding agent) and ascorbic acid (a food antioxidant and bread improver) in producing cassava bread samples with varying levels of wheat flour inclusion. The antimicrobial activities of propionates in wheaten bread are known to inhibit the growth of molds and bacteria without having any damaging effects on the leavening yeasts (Alais and Linden, 1999).

2. MATERIALS AND METHODS

Samples Preparation:

High Quality Cassava Flour (HQCF) samples were processed from TME419 and TMS30572 cassava genotypes using the method described by Ukpabi (2009) and Obasi *et al* (2016).The flow chart for the production High Quality Cassava Flour is shown in Figure 1.

Preparation of composite flours:

Wheat flour was mixed with HQCF at several ratios: 90:10, 80:20, and 60:40 at the Post Harvest Technology Program of the NRCRI. The 90:10, 80:20, and 60:40 flour mixtures were used to prepare 10%, 20%, and 40% NRCRI cassava bread, respectively. The control bread was prepared with 100% wheat flour.

Bread dough was prepared using different blends of wheat/cassava flour, i.e. 10%, 20%, 40%, cassava bread samples using the Master's baker recipe in Nigeria as described by Ukpabi (2010) as shown in Table 1. Two different baking preservatives (0.3% ascorbic acid and 0.6% Calcium Propionate) were incorporated to dough mixtures. 100% wheat bread without any preservative was used as control.

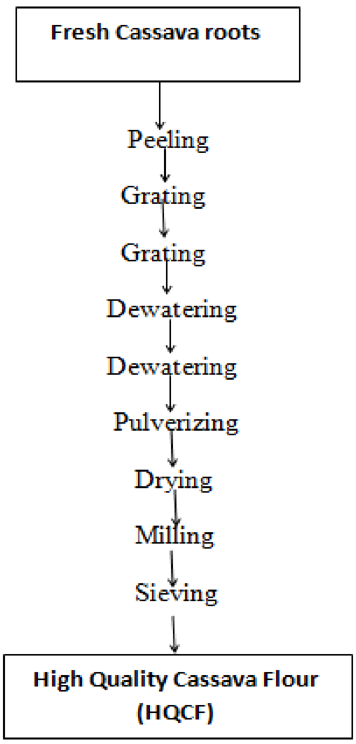

Fig. 1: Flow chart for the production of cassava flour for baked products.

Shelf life determination:

The bread produced was packed in the conventional transparent polyethylene bags and stored at room temperature (27±2°C). The bread was observed daily through the transparent polythene bags to determine when spoilage would occur. The total mould load was determined at the beginning of spoilage on the 1st, 4th, and 8th, day by carefully and aseptically taking 10g of the moldy outer layer of the bread using sterile scalpel and homogenizing in 90ml of 0.1%(w/v) sterile peptone water for 3 min in a stomacher (A.J. Seward and Co.London). Ten fold serial dilutions were subsequently prepared by transferring 1ml of the homogenate into 0.1% (w/v) peptone water as diluents. Further serial dilutions were carried out and 1ml of appropriate dilution was aseptically plated using the pour plate technique on Potato dextrose agar supplemented with chloramphenicol. At the end of the incubation period (3-5 days) the colonies were enumerated and expressed as colony forming units per gram (cfu/g) (Vanderzannt and Splittstoesser, 1992). The different colonies on each plate were isolated, purified, and stored on PDA slants for further characterization and identification. The fungi were identified under the light microscope (x40) and recorded using the scheme of Barnet and Hunter.

Sensory evaluation:

A 9- point Hedonic scale (1-9) was used for a sensory evaluation of the freshly prepared bread samples using 20 semi-trained sensory panelists and following the procedure as outlined by Iwe (2007) . The panelists were pre-trained to score for appearance, color, taste, aroma, texture and general acceptability independently and objectively with a pre-trial scoring test. Scoring was done on 9-point Hedonic scale where 9 represents like extremely, 5 neither like nor dislike and 1 = dislike extremely.

Statistical analysis:

Statistical Package SAS version 8e software (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Differences between samples in each item were tested using analysis of variance (ANOVA) and Duncan's Multiple Range as a post-hoc test when the analysis of variance indicated significant differences in the means.

3. RESULTS AND DISCUSSIONS

Tables 1-2 show the antimicrobial effect of calcium propionate on the experimental cassava bread samples made with 3 different substitution levels of cassava flour (10%,20% and 40%) and stored for eight (8) days at ambient room temperature (26-29ºC). The obtained results showed that the inclusion of calcium propionate to cassava bread recipe reduced mold growth to maximum storage of 4days at room temperature. The maximum number of viable mold colonies was recorded in the control 100% wheaten ($7x10^3$ cfu/g), while the counts for loaves made with composite flours (wheat/cassava) did have more than the control (Table 1). However, fungal growth was not observed during the first (0-1) day in all the bread samples. But fungal growth was observed in the samples from the fourth day till the eight day. The mould load increased progressing as the period of storage increased. The eight day therefore showed the highest fungal count for all examined samples.

Table 1: Effect of Calcium Propionate on the Mold Counts of the stored Bread Samples

Bread sample	Day1 (cfu/g)		Day4 (cfu/g)		Day 8 (cfu/g)	
	TMS 30572	TME 419	TMS 30572	TME 419	TMS 30572	TME 419
10% cassava flour inclusion	NG	NG	NG	$6.0x10^3$	$9.0x10^7$	$9.0x10^7$
20% cassava flour inclusion	NG	NG	$1x10^3$	$3.5x10^3$	$9.0x10^7$	$9.0x10^7$
40% cassava flour inclusion	NG	NG	NG	$3.5x10^3$	$13.5x10^7$	$17.25x10^7$
100% wheat flour	NG	NG	$7x10^3$	$7x10^3$	NG	NG

NG=no growth

Different preservatives effectively controlled fungal growth to a count between 0 to 10x 10^3 in bread samples prepared from the two cassava varieties respectively. The results at day 4 are in line with findings of Masood *et al.* (2001) who observed the maximum number of colonies range of 6-8 x 10^3 cfu with the use of additives like Calcium propionate (0.15%), Lactic acid (0.10%) and Acetic acid (0.10%).The microbial population at day4 in all the bread samples were within the recommended limits (10^5 Cfu/g) for microbial contamination in ready-to-eat food as stipulated by the

International Commission on Microbiological Specification for Foods (ICMSF, 2012). Preservatives help to reduce or prevent wastage of food through spoilage caused by microorganisms. Longer shelf life enables a greater variety of products to be kept in store and in the home saranraj and geetha(2010) The majority of moulds isolated from this study belong to *Rhizopus stolonifer*, *Apergillus niger* and *Penicillium* sp as shown in (Table 3).

One of the most common fungi involved in spoilage of bread is *Rhizopus stolonifer*, often referred to as the 'bread mould'. Most common source of microbial spoilage is due to mold growth. According to the previous studies (Banwart, 2004) bread molds like *Mucor* and *Rhizopus* are found to grow first during bread spoilage. This is followed by some other fungi like *Aspergillus*, *Penicillium* and *Fusarium* sp. Molds are the primary spoilage organisms reported in baked foods, with *Aspergillus*, *Penicillium*, and *Eurotium* being the most commonly isolated genera. These organisms synthesize toxic secondary metabolites called mycotoxins that have serious health implications The presences of *Aspergillus niger*, *Rhizopus stolonifer* and *pencillium* spp have been reported as an indication of contamination as they are common spoilage organisms of carbohydrate containing food Ezeama and Amajor,(2015).

Table 2 Frequency of occurrence of fungi

Fungal Isolates	Frequency (%)
Rhizopus sp	60
Aspergillus sp	40
Pencillium sp	20

Table 3 Cultural morphological characteristics and identification

Fungal Isolate	Cultural Characteristics	Morphological Characteristics
Rhizopus sp	White cottony mycelia, with black dots and covers the entire plate	Sporangiospores are produced inside a spherical sporangium. Columella is present on the top of the sporagiophore. Root-like rhizoids are found
Aspergillus sp	Yellow or yellowish green colonies with distinct margin	Conidiophores arise from a footcell. Club shaped vesicles at top of the conidiophores. Conidia are found in chains.
Penicillium sp	Fast-growing colonies in green colour with dense conidia	Conidiophores arise from a footcell. Club shaped vesicles at top of the conidiophores. Conidia are found in chains.

The Preference Test data show that the level pf substitution affected the degree of acceptance/preference of cassava bread. Bread prepared by using 10% and 20% HQCF substitution had a higher rating compared to samples prepared using 40% substitution. However, inclusion of ascorbic acid improved the texture and taste of bread baked using 40% substitution with cassava flour from TME 419 variety. Ascorbic acid is generally used in the food industry as an enhancer to improve the sensory attributes such as texture, colour and taste.

Table 4: Preference Test of TME419 Cassava Flour Bread Samples with Ascorbic Acid

Bread sample	Appearance	Taste	Aroma	Texture	Color	General Acceptance
10% cassava flour inclusion	7.30^{a}	6.20b^{a}	6.35^{a}	6.20^{b,a}	7.20^{a}	6.90^{a}
20% cassava flour inclusion	6.05^{a}	6.00^{b}	5.95^{a}	6.35^{b,a}	6.30^{a}	6.15^{a}
40% cassava flour inclusion	6.00^{a}	6.00^{b}	6.10^{a}	6.35^{b}	6.35^{a}	6.65^{b}
100% wheat flour	7.14^{a}	6.95^{b}	6.55^{a}	6.60^{a}	7.15^{a}	6.80^{a}

Values in a column with the same letter are not significantly different (P=0.05)

Table 5: Preference Test of TMS30572 Cassava Flour Bread Samples with Ascorbic Acid

Bread sample	Appearance	Taste	Aroma	Texture	Color	General Acceptance
10% cassava flour inclusion	7.30[a]	6.00[a]	6.40[a]	6.30[a]	7.3[a]	6.95[b,a]
20% cassava flour inclusion	7.20[a]	6.90[a]	6.75[a]	6.75[a]	6.75[a]	7.20[a]
40% cassava flour inclusion	6.70[a]	6.15[a]	6.00a	6.00[a]	6.00[a]	6.15[b]
100% wheat flour inclusion	7.14[a]	6.95[b]	6.55[a]	6.60[a]	7.15[a]	6.80[a]

Values in a column with the same letter are not significantly different (P=0.05)

Though the fresh bread samples were generally liked by the test panelists, there is a need to undertake a sensory evaluation of stored cassava bread samples in a future experimentation. This will assist in having a full quality assurance package for cassava bread producers, consumers and marketers.

4. CONCLUSION

The results from this study shows the use of calcium propionate and ascorbic acid as legal food additives in the recipe for preparation of bread from 10%, 20% and 40% inclusions of HQCF can assist in shelf-stability of the product for a maximum of 4days at room temperature. However, as the 40% HQCF bread was not acceptable to the consumers, there is therefore the need to modify the recipe for preparation of bread at this level of substitution in order to increase its acceptability by consumers.

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