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Full Length Research Paper

Shelf stability of agidi produced from maize (*Zea mays*) and the effects of sodium benzoate treatment in combination with low temperature storage

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The shelf stability of agidi produced from maize (*Zea mays*) during ambient temperature (30.0±1.5°C) storage and the effects of sodium benzoate treatment in combination with low temperature storage (12-14°C) was evaluated for eight weeks. Results indicates high total aerobic bacterial count ($1.05x10^{10}$ cfu/g) and fungi count ($4.6x10^{9}$ cfu/g) at the 12th day of storage and thereafter, decrease gradually till the end of the storage period. Treatment with 0.15% sodium benzoate and refrigeration at 12-14°C drastically retarded microbial growth up till the 21st and 28th day of storage. Seven bacteria genera (*Bacillus, Staphylococcus, Streptococcus, Lactobacillus, Leuconostoc, Pseudomonas* and *Alcalegene*) and seven fungi genera (*Aspergillus, Penicillium, Alternaria, Fusarium, Rhizopus, Mucor* and *Geotrichum*) were detected and isolated. The pH decreased from 4.15±0.01 to 3.10±0.02 at the end of storage period while the titratable acidity increased from 0.002±0.001 to 0.005±0.001. However, the pH and titratable acidity were fairly stable in samples treated with sodium benzoate and refrigeration of 12-14°C. An increase of 37.94% and 32.19% were recorded for the moisture and fibre contents, respectively. Conversely, a decrease of 12.92, 45, 81.32 and 44.95% were detected and recorded for the carbohydrates, lipid, protein and ash contents. However, treatment with 0.15% sodium benzoate and refrigeration at 12-14°C kept these parameters fairly stable all through the storage period. Overall sensory evaluation shows that sodium benzoate treated and refrigerated samples were highly acceptable even though freshly prepared samples were preferred.

Key words: Shelf sability, agidi, sodium benzoate and low temperature**.**

INTRODUCTION

Agidi is a gel-like traditional fermented starchy food item produced from maize (Z*ea mays*), although millet and sorghum can also serve as raw materials. Its colour depends on the cereal used. It is cream to glassy white from maize, light brown from sorghum and grey to greenish colour from millet. It is know by different names in different localities such as eko (Yoruba), akasan (Benin), komu (Hausa) and agidi (Ibo).

It is becoming very popular, with acceptability cutting across the various multi-ethnic groups and socio-economic classes. The ease of consumption, alone or with soup, stew, beans cake (akara), moi-moi, as light meal especially amongst post operative patients and other patients in the hospitals makes it very popular. Agidi has economic potentials especially now that emphasis is on development of local foods.

Production of agidi is laborious, cumbersome and time consuming. Currently, it varies from one locality to another resulting in a non-uniform product, non-specified quality indices, unknown shelf life and lack of safety indices (thus limiting product acceptability to immediate locality). Furthermore, agidi deteriorates rapidly in storage (2-3 days), warranting repetition of the cumbersome and time consuming production cycles in order to keep product available. All these suggest the need to develop methods of extending the shelf life while

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maintaining the economic, safety, nutritional and total quality in order to meet the increasing demand.

In line with the foregoing, this work was designed to investigate the post process shelf stability of agidi during storage and the effects of combination of preservative factors with the aim of developing measurable and reliable data and indices for safe processing and shelf stability of agidi.

MATERIALS AND METHODS

Preparation of agidi

Maize grains (*Z. mays*) were obtained from New Benin main market, Benin City, Edo State, Nigeria and processed according to the conventional traditional method with slight modifications. Briefly, the maize grains were sorted and cleaned, washed with clean water, soaked in clean water for 24-48 h, drained and wet milled using a commercial milling machine. This was followed by wet sieving with aid of a clean muslin cloth. The mixture was allowed to settle and the ogi slurry supernatant decanted, and boiled and cooked with occasional stirring at 78.5±2.5°C (Figure 1).

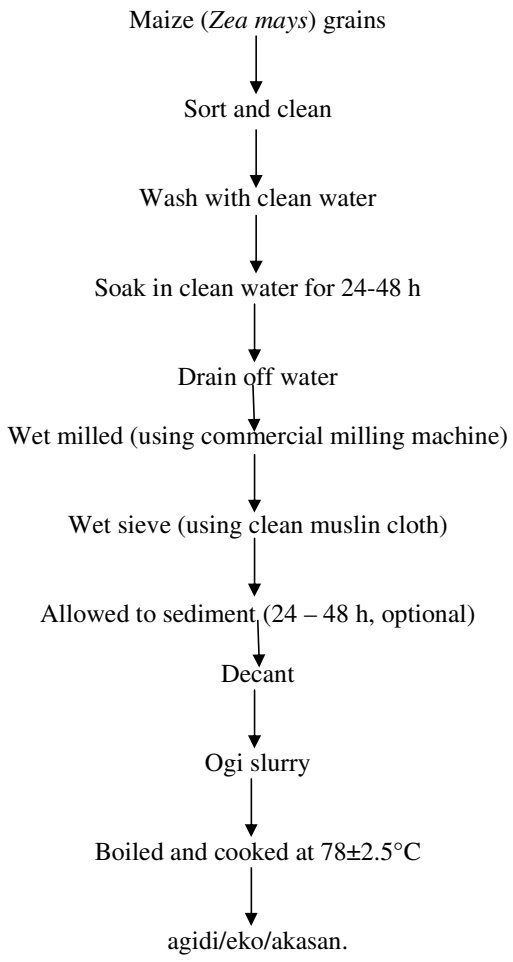

Figure 1. Traditional method of processing agidi.

Sample treatment

The agidi produced was subdivided into two batches and treated according to the scheme developed by Ogiehor et al. (2003) as follows:

Batch 1: 78.5±2.5°C (conventional batch/control)
Batch 2: 78.5±2.5°C + 0.15% sodium benzoate (BDH, Ltd, Poole, England)

After the treatment with sodium benzoate, each batch was wrapped (approximately 250g/wrap) with high density polythene (HDPE) previously sterilized (UV sterilizer, model UV-2500, RIV, Italy) and thereafter stored at ambient (laboratory) temperature (30.0±1.5°C). However batch 2 was subdivided into two subgroups. One group was held at 12-14°C in a commercial cold room system and the second batch at 30.0±1.5°C.

Microbiological assay

The various groups, types and numbers of microorganisms associated with the various samples were analysed, enumerated and quantified according to the methods described by Harrigan and McCance (1976). 25 g of agidi was aseptically removed from each samples and homogenized in 225 ml of 0.1% (w/v) sterile peptone water for three (3) minutes in a Colworth stomacher (A.J Seward and Co. London). Ten fold serial dilutions were subsequently prepared by transferring 1 ml of the homogenate into 0.1% (w/v) peptone water as diluent. Further serial dilutions were carried out. Following these, 1 ml of appropriate dilutions was asceptically plated using the pour plate technique for total viable bacterial count on nutrient agar (Biotech) and total viable fungi count on potato dextrose agar (Biotech) supplemented with chloramphenicol. The media used were prepared and incubated according to the manufacturer's instructions. At the end of the incubation period, the colonies were enumerated and expressed as colony forming unit per gram (cfu g^{-1}) according to Vanderzannt and Splisttoesser (1992). Isolation, characterization and identification of the associated microorganisms, were carried out for qualitative determination using colonial, morphological and biochemical characteristics (Harrigan and McCance, 1976). The fungal isolates were identified based on examination of the colonial heads, phialides, Conidiophores and presence or absence of foot cells or rhizoids (Samson and Reneen-Hoekstra, 1993).

Biochemical assays

pH was determined by homogenizing 10 g of the various samples in 20 ml of distilled water and using a referenced glass electrode pH metre (Jenway, 3020, England). Titratable acidity was carried out by titrating 0.1 N sodium hydroxide against 10 ml of sample using phenolphthalein indicator as previously reported by AOAC (1990). Moisture content was determined by the oven methods as described by AOAC (1990). Available carbohydrates, crude protein, lipid, ash and fibre were also determined according to the methods described by AOAC (1990).

Sensory evaluation

Quality attributes such as appearance, aroma, taste texture and mouth feel were evaluated for acceptability with the freshly prepared samples and samples stored (28 days). Using a nine point hedonic scale (1=9…..9=1, Watts et al., 1989), a ten member panel was used to assess the various quality attributes for overall acceptability (Watts et al. 1989).

Statistical Analysis

The various data obtained were subjected to statistical analysis of mean, standard deviation and analysis of variance. The significant value was determined by the t-distribution test using appropriate computer package.

RESULTS

Results of the quality changes associated with agidi during storage and the effects of sodium benzoate treatment alone or in combination with low temperature (12-14°C) storage are shown in Tables 1-4. Boiling, cooking and processing of raw ogi into agidi at 78.5±2.5°C drastically reduced the total viable bacterial count by 8.85 folds and total viable fungi count by 9.66 folds. Following this, steady increase was observed up to the 12th day of storage with 10.02 and 9.66 folds increase in the total viable bacteria and fungi counts respectively (Table 1). No microbial growth was detected in samples treated with 0.15% sodium benzoate up to the 21st day of storage. Similarly, no viable microbial count was recorded up to the 28th day of storage for samples treated with 0.15% sodium benzoate and stored at 12-14°C (Tables 2-3). Seven bacteria genera (*Bacillus, Staphylococcus, Streptococcus, Lactobacillus, Leuconostoc, Pseudomonas* and *Alcalegene*) and seven fungi genera (*Aspergillus, Penicillium, Alternaria, Fusarium, Rhizopus, Mucor* and *Geotrichum*) were detected and isolated.

Following boiling and cooking, the pH increased from 3.45±0.05 to 4.15±0.01 and thereafter decreased gradually all through the storage period to 3.10±0.02 at the end of the storage period. Conversely the titratable acidity decreased from 0.04±0.01 to 0.002±0.001 following processing and thereafter increased gradually to 0.005±0.002 at the end of storage period. However, the pH of sodium benzoate treated samples and samples stored at 12-14°C increased to 4.36±0.02 after processing and thereafter remained fairly stable and terminated at 4.01±0.01 and 4.30±0.02, respectively, at the end of the storage period.

An increase of 37.94% and 32.19% were recorded for moisture and fibre contents, respectively, in the non-treated samples. Conversely, a decrease of 12.92, 45, 81.32 and 44.95% were detected and recorded for carbohydrates, lipid, protein and ash contents, respectively, in the non-treated samples (Table1). However, these parameters were fairly stable all through the storage period in samples treated with 0.15% sodium benzoate and samples stored at 12-14°C.

Overall sensory evaluation shows that the various quality attributes such as appearance, texture, aroma/odour, mouthfeel and taste were significant at various levels ($p<0.001$, 0.01, 0.05) amongst the various samples evaluated (Table 5). Overall acceptability were found to be in the order freshly prepared samples > sodium benzoate treated sample + refrigeration at 12-14°C > sodium benzoate treated samples + ambient temperature storage.

DISCUSSION

The initial drastic reduction (8.85 and 9.66 folds) recorded in the total viable bacteria and total viable fungi counts (Table 1) may be attributed to the effects of processing (boiling, stirring, cooking at 78±2.5°C). While the subsequent rapid microbial proliferation during storage may be due to nutrient availability and favourable microenvironment with resultant recovery of homeostatic inbalance (Ogbulie et al., 1993; Ogiehor, 2002). However lack of growth observed and recorded up to the 21st day of storage in samples treated with 0.15% sodium benzoate may be associated with the antimicrobial effects of sodium benzoate. Whereas, the longer period (28th days) of no microbial growth observed in samples treated with sodium benzoate and held at 12-14°C may be related to the combine effects of sodium benzoate and low temperature storage on the associated microorganisms, leading to extensive physiological and homeostatic disturbance. These findings support previous reports on the use of combination of preservatives for the stabilization of traditional and novel foods (Leistner, 1992, 1994; Gould, 1988; Ogiehor et al., 1999; Ogiehor et al., 2003)

Volatization of some of the organic compound and organic acids during boiling, cooking and processing may account for the initial increase in pH while the subsequent decrease observed all through the storage period may be associated with the extensive microbial activities which help to break down carbohydrates for the production of organic acids. This may be responsible for gradual increase recorded in the titratable acidity. Similar findings for other food items have been documented (Okafor, 1977; Eka, 1986; Ogiehor et al., 2003)

The significant and noticeable degree of deterioration observed and recorded in the carbohydrate, protein, lipid and ash content of agidi during ambient temperature storage may be traced to the extensive microbial growth and the associated activities (Table 1). However, the stability recorded in samples treated with sodium benzoate and samples held at 12-14°C may be attributed to the antimicrobial effects of sodium benzoate and the partial growth retardation by the low temperature storage condition. The use of sodium benzoate and low temperature storage to control microbial growth in foods and enhanced food stability has been previously reported (Jay, 1976; Ogiehor, 2002; Ogiehor et al., 2003).

The high degree of acceptability recorded in the various attributes of appearance, texture, aroma, mouth feel and taste in the sodium benazoate treated samples and samples held at 12-14°C may be associated with quality stability recorded, essentially by the seemingly lack of microbial growth and associated activities. Overall

Table 1. Microbiological, biochemical and physico-chemical quality changes in agidi during storage at 30±1.5°C.

Parameter	FUB	0	2	4	6	8	10	12	14	16	18	20
TVC(bacteria)	$1.30x10^6$	$0.5x10^1$	$4.5x10^1$	$8.4x10^3$	1.20x10	$1.50x10^7$	$1.10x10^8$	$1.05x10^{10}$	$8.4x10^9$	$7.6x10^7$	$6.4x10^6$	$5.2x10^5$
TVC(fungi)[1]	$1.6x10^5$	ND	$0.4x10^1$	$1.2x10^2$	$3.8x10^4$	$2.6x10^6$	$3.4x10^7$	$4.6x10^9$	$6.44x10^8$	$4.1x10^6$	$3.6x10^4$	$2.1x10^4$
pH	3.45±0.5	4.15±0.1	4.10±0-.02	4.10±0.01	3.96±0.1	3.74±0.2	3.65±0.04	3.46±0.03	3.38±0.02	3.34±0.03	3.21±05	3.10±0.02
TA	0.004±0.1	0.002±01	0.002±0.1	0.002±0.1	0.03±0.1	0.003±0.1	0.003±0.1	0.004±0.1	0.004±0.1	0.004±0.1	0.005±0.001	0.005±0.01
MC	44.20±13	29.65±10	30.02±0.5	31.30±0.6	33.40±00	35±65±6	37.40±0.9	38.60±0.8	39.7±0.12	40.20±1.0	40.60±0	40.90±0.5
CHO	47.30±15	61.10±13	ND	ND	ND	ND	ND	ND	ND	ND	ND	53.20±1.78
LIPID	1.05±0.3	1.01±0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.55±0.001
PROTN	1.35±0.1	2.41±0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.45±0.002
ASH	0.902±0.2	1.09±0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.60±0.001
FIBRE	1.04.±.01	2.05±0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.71±0.02

TA = Titratable acidity; MC= Moisture content; CHO= Carbohydrates; TVC = Total Viable Count; Na = No Growth; ND = Not determined; FUB = fresh unboiled.

Table 2. Microbiological, biochemical and physico-chemical quality changes in agidi treated with 0.15% sodium benzoate at 30.5±1.5°C.

Storage of agidi (days)												
Parameters	**0HR**	**2**	**4**	**7**	**10**	**14**	**21**	**28**	**35**	**42**	**49**	**56**
TVC Bacteria(cfu/g)	$0.5x10^1$	NG	NG	NG	NG	NG	NG	$0.4X10^1$	$0.3X10^1$	$1.2X10^2$	$1.6X10^3$	$2.0X10^3$
TVCFungi(cfu)	ND	NG	NG	NG	NG	NG	NG	$0.2X10^1$	$0.5X10^1$	$0.9X10^1$	$1.1X10^2$	$1.8X10^2$
pH	4.15±0.01	4.36±0.02	4.36±0.01	4.36±0.01	4.33±0.01	4.31±0.01	4.30±0.02	4.29±0.03	4.25±0.01	4.15±0.01	4.10±0.02	4.02±0.02
TA	0.002±0.1	0.002±0.1	0.002±0.01	0.002±0.01	0.002±0.01	0.002±0.01	0.002±0.01	0.002±0.0	0.002±0.0	0.002±0.1	0.002±0.01	0.002±0.01
MC	29.65±1.0	29.65±1.0	29.65±0.6	29.65±0.8	29.68±0.5	29.89±0.8	29.96±0.4	30.05±0.6	30.05±0.6	31.66±0.7	32.20±0.3	32.60±0.5
CHO	61.10±1.3	61.10±1.5					ND					59.65±0.6
Lipid	1.01±0.01	1.01±0.01					ND					0.88±0.001
Protein	2.41±0.02	2.41±0.02					ND					2.26±0.01
Ash	1.09±0.02	1.09±0.02					ND					1.01±0.001
Fibre	2.05±0.01	2.05±0.01					ND					2.5±0.02

TA = Titratable acidity; MC= Moisture content; CHO= Carbohydrates; TVC = Total Viable Count; Na = No Growth; ND = Not determined.

Table 3. Microbiological, biochemical and physico-chemical quality changes in agidi treated with 0.15% sodium benzoate stored at 12-14°C.

Parameter	Storage of Agidi (Days)											
	0 h	2	4	7	10	14	21	28	35	42	49	56
TVCbacterial (cfu g^{-1})	NG	NG	NG	NG	NG	NG	NG	NG	$0.4X10^{1}$	$0.9X10^{1}$	$1.6x10^{1}$	$2.6x10^{1}$
TVC(fungi) cfu g^{-1}	NG	NG	NG	NG	NG	NG	NG	NG	NG	$0.3X10^{1}$	$0.8x10^{1}$	$1.4x10^{1}$
pH	4.36±0.012	4.36±0.02	4.34±0.01	4.33±0.01	4.33±0.01	4.33±0.01	4.32±0.01	4.32±0.02	4.31±0.02	4.30±0.01	4.28±0.002	4.25±0.002
TA	0.002±0.00	0.002±0.00	0.002±0.00	0.002±0.00	0.002±0.00	0.002±0.00	0.002±0.00	0.002±0.0	0.002±0.001	0.002±0.0	0.002±0.001	3.50±0.6
MC	29.65±1.0	29.65±1.0	29.68	29.89±0.6	29.66±0.8	29.7±0.10	29.83±0.5	29.94±0.6	30.05±0.7	30.20±0.8	30.45±0.4	3.50±0.6
CHO	61.10±1.5											59.10±0.7
Lipid	1.01±0.001				ND							0.98±0.001
Protein	2.41±0.02				ND							2.30±0.01
Ash	1.09±0.02				ND							1.05±0.001
Fibre	2.05±0.01				ND							2.46±0.5

NB: TA = Titratable acidity; MC = Moisture content; CHO = Carbohydrates; TVC = Total Viable count; ND = Not Determined.

Table 4. Sensory parameters of the various agidi samples.

Samples	Appearance	Texture	Aroma	Mouth feel	Taste	Overall score
Fresh sample	6.94±0.60	7.14±0.401	6.33±0.7	$6.45±0.20^{q}$	6.15±0.50	6.60±0.47***
SB treatment/ ambient storage[i]	$5.65±0.50^{a}$	$5.04±0.25^{d}$	4.96±0.34	5.14±0.60	$5.09±0.72^{b}$	5.18±0.39*
SB treatment/ refrigeration [ii]	$5.71±0.15^{a}$	$6.15±0.80^{d}$	5.04±0.01	$6.25±0.22^{q}$	$5.65±0.50^{b}$	5.76±0.46**

Value with the same superscript in a column are not significantly different.
SB = sodium benzoate
[i] 30.5±1.5°C
[ii] 12-14°C
*** = Preferred sample
** = Highly acceptable
* = Acceptable

acceptability scores shows that sample treated with sodium benzoate and held at 12-14℃ were highly acceptable even though, freshly prepared samples without any treatment were most preferred.

In summary, this study have shown the effectiveness of the antimicrobial properties of sodium benzoate and the benefits of low temperature storage in keeping the nutritional, sensory, economic and the total quality of agidi during storage. Furthermore, data obtained can be harnessed and used as spring board for industrialization.

REFERENCES

AOAC (1990). Official methods of analysis. 11th edition Association of official analytical chemists. Wahsington DC.

Eka OU (1986). Studies on the fermentation of cassava. Some enzymes of micro organisms in the fermentation liquor and the effect of fermentation on nutrient status of cassava products. West Afr. J. Biol. Chem. 31 (1-4): 3-12.

Gould GW (1988). Interference with homeostasis. In: Homeostatistic mechanisms in microorganisms. Whittenbury R, Gould GW, Banks JE, Board JF (Eds). FEM Symposium 170(44): 220-228.

Harrigan WF, McCance ME (1976). Laboratory methods in Foods and Dairy microbiology. Academic press, London. p. 416.

Jay JM (1978). Modern food microbiology. (2nd ed.) D Von Nostrand company, New York. p. 479.

Leistner L (1992). Food preservation by combined methods. Food Res. Int. 25: 151-158.

Leistner L (1994). Further developments in the utilization of hurdle technology for food preservation. J. Food Engr. 22: 411-422.

Ogbulie JA, Ogiehor IS, Isu NR (1993). Effects of Chemical and Physical treatment on the shelf life of fermented African oil bean seed (Ugba). Nig. J. Biotechnol. 11: 168-176.

Ogiehor IS, Ohenhen RE, Okwu IG, Agbonlahor FE (1999). Production of food Condiment (sauce) from African Oil Bean (*Pentaclethru macrophylla*-Bentham) and preservation by combined application of temperature and sodium chloride. Nig. J. Microbiol. 13: 87-94.

Ogiehor IS (2002). Extension of shelf life of Garri by combination of preservative factors. Ph.D.Thesis Uni.of Benin. p. 189.

Ogiehor IS, Iyamu MI, Ize-Iyamu KO (2003). Biodeterioration of akpu produced from cassava (*Manihot esculenta* – Crantz) and the effects of sodium benzoate alone or in combination with ascorbic acid advances in Nat. Appl. Sci. Res. 1(1): 1-19.

Okafor N (1977). Microorganisms associated with cassava fermentation for garri production. J. Appl. Bacteriol. 42(2): 279-284.

Samson RA, Reenen-Hoekstra ES Van (1988). Introduction to foodborne fungi, 2nd ed. Central bureau voor schimmel cultures, Boarn.

Vanderzannt C, Splittstoesser DF (1992). Compendium of methods for the microbiological examination of foods, 3rd ed. American public health association, Washington DC.

Watts BM, Yumaki CL, Jeffery LE, Elais LG (1989). Basic sensory methods for food evaluation. The International Development Research Centre, Ottawa, Canada. p. 159.