Production and evaluation of fermented cassava flour preserved with ginger and garlic mixtures.

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Abstract

Fermented cassava flours were produced using different ratios of ginger and garlic mix as preservatives. The flours were divided into nine (9) groups labelled A1 (cassava flour+1.5% ginger) A2 (cassava flour+2.0% ginger), A3 (cassava flour+2.5% ginger), B1 (cassava flour 1.5% garlic), B2 (cassava flour+2.0% garlic), B3 (cassava flour+2.5% garlic), C1 (cassava flour+1.5% ginger garlic), C2 (cassava flour 2.0% ginger garlic) and C3 (cassava flour+2.5% ginger garlic) while an untreated sample served as the control for the study. The flours were given storage treatment for a period of three (3) weeks and were evaluated for their proximate composition, phytochemical properties and microbiological quality using standard analytical methods. The data obtained were subjected to statistical analysis using statistical package for social sciences (SPSS) version 21. The following range of values were obtained for moisture (9.85 to 11.60%), ash (2.45 to 2.75%), fat (0.65 to 1.20%), crude fiber (2.10 to 2.66%), protein (3.40 to 3.80%) and carbohydrate (78.75 to 80.60%). It was evident that the cassava flours had good shelf-life stabilities owing to their low concentration of moisture and fat as well as appreciable quantities of minerals owing to their relatively high ash content. The result for physiochemical properties revealed that pH (4.57 to 4.66) and total titratable acidity (0.110 to 0.146) of the cassava flours also conferred better keeping qualities on the flours. Microbiological quality of the cassava flours preserved with either ginger or garlic had lower microbial loads compared to those preserved with a combination of ginger and garlic and the microbial load of the cassava flours was observed to increase in the period of storage (increased for 0 day to three (3) weeks). It was concluded that ginger and garlic both had a preservative effect on the cassava flours owing to their antimicrobial activities. However, the bacterial loads of the preserved cassava flours were within tolerable microbiological standards and were fit for human consumption. Isolation, characterization and identification of microorganisms from the cassava flours was recommended.

Keywords: Cassava, Flour, Ginger, Garlic, Mixtures.

Introduction

Cassava (*manihot esculenta crantz*) is a tuberous roots crop grown in the tropics with low cost vegetative propagation. It belongs to the family of *euphorbiaceae* and originated from South America [1]. The root is drought resistant and capable of growing in different types of soil and seasons [2]. Cassava is one of the major staple food in the tropical and subtropical region which can provide food for a population of more than 500 million across Africa, latin America and Asia [3,4]. The root is the major edible part of the crop is rich in carbohydrate and the starch content 86.49% is higher compared to other root and tuber crops such as yam (24%), sweet potato (69.15%) and taro (11.2%) [5]. However, it is low in protein, fat, fibre as well as some vitamins and minerals [6].

Utilization of cassava root as a food source and as industrial raw materials is limited because of the rapid postharvest deterioration which starts within two days after harvest [7,3]. This situation shortens the shelf-life of the root, leading to postharvest loses, low product yield and poor market quality of fresh root and minimally processed cassava food products such as cassava flour. Fresh cassava root contains a toxic compound (hydrogen cyanide) which is harmful for human consumption and detrimental for the use of cassava in food industries [8].

However, processing techniques such as peeling, fermentation, soaking and drying can detoxify and reduce the cyanide content, improve palatability and add value to the root [9]. Converting cassava root into food forms and raw materials such as fufu, cassava flour, tapioca, flour, chips and pallets

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***Received:** 20-Jun-2023, Manuscript No. AAFMY-23-103189; **Editor assigned:** 23-Jun-2023, PreQC No. AAFMY-23-103189(PQ); **Reviewed:** 07-Jul-2023, QC No AAFMY-23-103189; **Revised:** 10-Jul-2023, AAFMY-23-103189(R); **Published:** 17-Jul-2023, DOI:10.35841/aafmy-7.4.152*

***Citation:** Okoronkwo Christopher Uche, Obinwa C.O. Production and evaluation of fermented cassava flour preserved with ginger and garlic mixtures. J Food Microbiol. 2023;7(4):152*

can extent the shelf-life, facilitate trade and promote industrial use [2].

Ginger *(Zingiber officinale*) belongs to the family *Zingiberaceae* and genus zingibers, other names of ginger are African ginger, race ginger, cochin ginger, Gon Jiang, Gegibre, Ingwer, etc [10]. Ginger is an erect perennial plant growing from one to three feet in height, it produces clusters of white and pink flower buds that bloom into yellow flower [11]. Medieval writing from many European countries indicates that ginger was a standard ingredient in recipe for the kitchen and the apothecary, herbs, medicine [12,13].

In the other hand, Garlic (*Allium sativum*) is an herb related to onion, leeks and chives. It is commonly used for conditions related to the heart and blood system. Garlic is a perennial bulb, though indigenous to central Asia, Sebia and west of the Himalayas.

Garlic have been known as a common food flavor, spices and it is one of the herbs most commonly used in modern folkloric medicine. It was an important medicine to the ancient Egyptian as listed in the medical text codex Ebars (Ca-1550 BC) especially for the working class involved in heavy labor because it was an effective remedy for many ailments such as heart problem, headache, bites, worms and tumours.

Globally, there is a notable increase in the demand and processed cereal, especially wheat which influences the prices of cereal-based products [14]. The rising cost of importing wheat flour for food in many developing countries such as Nigeria has spurred the need for research to develop suitable flour from local agricultural materials such as cassava which is cheaper and also present suitable quality attributes. Moreso, wheat flour contains a glutein which causes celiac disease especially to gluten intolerant persons [15]. Non-wheat glutein free flour developed from root and tuber crops such as cassava (*Manihot esculenta*), sweet potato (*Ipemea betatas*), potato (*solanum tuberosum*), yam (*Dioscorea spp*) etc can offer the potential to alleviate the problem of rising cost of cereals and also gluten intolerance [16]. Hence, the objective of this research work

Materials and Methods

Sources of material

Fresh cassavas were obtained from the National Root Crop Research Institute, Umudike, Abia State Nigeria, authenticated by a botanist in the Department of Botany, Abia State University, Uturu.

Sample preparation/fermentation

The fresh cassava roots (*manihot esculenta*) were processed into flour using a modified method as described by [17] and adopted by [18].

The cassava roots were washed, peeled and re-washed with clean portable water. They were steeped in water inside plastic containers and allowed to ferment for 72 hours. At the end of the steeping period, the fermented sample were rewashed with fresh water and grated into pulp using a 3.5 HP petrol engine powered grater. The fermented soft pulp were dispersed in water and sieved with a test sieve of 2.0mm aperture. The recovered sediment were packed in sacks and dewatered using a hydraulic press. The resulting cake were pulverized by hand, spread in trays and dried to 10% moisture content in a hot air oven at temperature range of 50-550C for 12hours.

The dried fermented cassava were milled into flour using a disc attrition mill and divided into ten (15) batches.

Cassava flour samples treated with ginger/gari

- ✓ Blending ratio of gari to ginger +garlic mix
- ✓ A1 Fermented cassava flour + 1.5% ginger
- ✓ A2 Fermented cassava flour + 2.0% ginger
- ✓ A3 Fermented cassava flour + 2.5% ginger
- ✓ B1 Fermented cassava flour + 1.5% garlic
- ✓ B2 Fermented cassava flour + 2.0% garlic
- ✓ B3 fermented cassava flour + 2.5% garlic
- ✓ C1 Fermented cassava flour + 1.5% garlic/ginger
- ✓ C2 fermented cassava flour + 2.0% garlic/ginger
- ✓ FCF - Untreated cassava flour

All the samples of cassava were thereafter packaged in an air-tight containers for further analysis.

Production of ginger powder

This was done following the method as described by Sukajang et al, (2010). Fresh mature Rhizome of ginger were separated, washed thoroughly, peeled and sliced (about 2mm thickness) with sharp knife and sun dried to a final moisture content of 10%. They were then ground into powder-using electric kitchen grinder. The powder was sieved with a wire mesh of 2mm aperture and stored in steril glass bottle in a clean and dry environment.

Production of garlic powder

This was done using the method described by [19]. The outer cover of the garlic cloves were removed by peeling, washed with clean water, sliced with sharp knife, and sundried to a final moisture content of 10%. The slice was then ground into powder using electric kitchen grinder. The powder was sieved with a wire mesh of 2mm aperture and stored in sterile glass bottles in a clean and dry environment.

Determination of proximate content

Moisture content was determined grammatically as described by [20]. Ash content was determined by the furnace incineration gravimetric method described by James (1995) [21]. Crude protein was determined by Kjeldahl methods described. Fat content was determined by the continuous solvent extraction method using Soxhlet apparatus. Crude fibre content was determined gravimetrically using the method described by [20] Carbohydrate content was determined by calculating the percentage difference according to [22].

Determination of Physicochemical Properties

Determination of pH

The pH values of the cassava flours were measured by dissolving 10 g of flour in 90 ml of water and immersing the pH-meter CP-505 (Elmetron, Zabrze, Poland) and taking the reading.

Determination of Total Titratable Acidity (% TTA)

Total dissolved solids were determined titrimetriccally [20].

Microbiological Analysis of Fermented Cassava Flours Preserved With Ginger and Garlic

Enumeration of total heterotrophic bacterial count

The cassava flour was diluted according to the procedure [18]. The samples (5 g) were weighed aseptically and diluted with 45ml of buffered peptone water. Thus, the first dilution of I0-1 was obtained; the other dilutions were prepared from this first 10-1 dilution to dilutions of 10-2. Then, petri dishes were labeled at the bottom of the plate according to the samples and about 1 ml of the prepared dilution sample was placed in the petri dish near a flame. Nutrient agar was prepared according to Manufacturer's instruction and sterilized by autoclaving at 121°C for 15 minutes. The nutrient agar was cooled and poured into the plates. The plates were then mixed for uniform spreading and were allowed to solidify, inverted and incubated at 37°C for 24 hrs for bacterial colony formation. At the end of the incubation, the number of viable colonies were counted and expressed as colony forming units/gram (cfu/g) for the plates that contain between 30 to 300 of bacterial growth. Each colony was isolated in a pure form by sub-culturing in a fresh nutrient agar plate for further studies and identification. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin was observed.

Enumeration of coliforms

One (1 g) of flour was serially diluted to 10^{-2} and 0.1 milliliter of each dilution was plated on (ICSMF, 1996) violet red bile agar plates and incubated at 37°C for 24hrs. The colony forming unit per milliliter sample (cfu/ml) was counted in each case with the aid of electronic colony counter.

Enumeration of fungi

Potato Dextrose Agar (PDA) was inoculated with 1 ml of the diluted flour in Petri dishes and incubated at 37°C for 3 days for fungal growth. At the end of the incubation, the number of fungal colonies were counted and expressed as colony forming units/gram (cfu/g).

Identification of bacterial isolates

Determinations of isolates were done by Gram staining and biochemical characterization of the bacteria isolates as described by Bergey's Manual of Systematic Bacteriology.

Statistical Analysis

The mean score of the laboratory analysis results were subjected to one way Analysis of Variance (ANOVA), using the Statistical Package for the Social Sciences (SPSS) version 21. The mean scores were separated using Duncan's Multiple Range Test (DMRT). Significant difference was determined at $P<0.05$ level of probability and the results were expressed as the mean ± standard deviation of duplicate determinations.

Results and Discussion

Proximate composition of cassava flours preserved with ginger and garlic

The proximate composition of cassava flour preserved with ginger and garlic were presented in Table 1. The moisture content of the cassava flours preserved with ginger and garlic showed significant ($p<0.05$) difference and ranged from 9.85 to 11.60% with sample C3 having the least value and sample B1 recording the highest value while the moisture content of the control was found to be 11.80% and was quite higher compared to the preserved flours indicating that addition of ginger and garlic as preservatives helped to lower the moisture content of the cassava flours. According to a study conducted [23], moisture content of wheat, rice, green gram and potato

Table 1. *Proximate composition (%) of cassava flour preserved with ginger and garlic.*

Sample	Moisture	Ash	Fat	Crude fiber	Protein	Carbohydrate
A1	10.80^{d} + 0.00	2.45^{d} + 0.07	0.75^{d} + 0.07	2.10^{d} + 0.14	3.40^{d} + 0.14	80.50^{d} +0.14
A2	10.60^{dc} + 0.14	2.55^{bc} + 0.07	0.95^{bc} + 0.07	2.30^{bcd} + 0.14	3.50^{cd} + 0.00	80.10^{bc} +0.14
A3	10.30^{c} + 0.14	2.60^{abc} + 0.00	1.20^{a} + 0.00	2.40^{bc} + 0.00	3.65^{abc} + 0.07	79.85^{c} + 0.21
B1	11.60^{ab} + 0.00	2.55^{bc} + 0.07	0.65^{d} + 0.07	2.25^{abc} + 0.07	3.65^{abc} + 0.07	79.30^{c} + 0.14
B2	11.40^{b} + 0.14	2.65^{ab} + 0.07	0.80^{cd} + 0.00	2.45^{ab} + 0.07	3.70^{ab} + 0.00	79.00^{dc} + 0.14
B3	11.10^{c} ± 0.14	2.70^{ab} ± 0.14	1.05^{b} ± 0.07	2.60^{a} ± 0.00	3.80^{a} ± 0.07	78.75^{e} ± 0.14
C1	10.50^{e} ± 0.14	2.60^{abc} ± 0.00	0.65^{d} ± 0.07	2.20^{de} ± 0.00	3.45^{d} ± 0.07	80.60^{a} ± 0.14
C2	10.30^{e} ± 0.14	2.75^{a} ± 0.07	0.75^{d} ± 0.07	2.35^{bcd} ± 0.07	3.55^{bcd} ± 0.07	80.30^{ab} ± 0.14
C3	9.85^{f} ± 0.21	2.70^{ab} ± 0.00	0.95^{bc} ± 0.07	2.45^{ab} ± 0.07	3.70^{ab} ± 0.00	80.35^{ab} ± 0.21
Control	11.80^{a} ± 0.00	2.30^{d} ± 0.14	0.95^{bc} ± 0.07	2.45^{ab} ± 0.07	3.70^{ab} ± 0.00	78.80^{e} ± 0.14

Values are means ± standard deviation of duplicate determinations. Two means along the same column with different superscripts are significantly ($p<0.05$) different.

Key: *Sample Al = cassava flour + 1.5 % ginger, Sample A2 = cassava flour + 2.0 % ginger, Sample A3 = cassava flour + 2.5 % ginger, Sample Bl = cassava flour + 1.5 % garlic, Sample B2 = cassava flour + 2.0 % garlic, Sample B3 = cassava flour + 2.5 % garlic, Sample Cl = cassava flour + 1.5 %;ginger/garlic, Sample C2 = cassava flour + 2.0 % ginger/garlic, Sample C3 = cassava flour + 2.5 % ginger/garlic, Control = untreated cassava flour*

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flour were 13~%,-11%, 8% and 9% respectively. [24] Also reported that cassava flour sold in the market showed moisture content in the range of 6.34 to 14.58% [25] reported that high-quality flour usually contains moisture content range from 9.0% to 12.0%; indicating that the cassava flours in this work were of high quality and low moisture content. According to [23], low moisture content in cassava flour is desirable since this would decrease hydrogen cyanide content as well as improve the palatability of cassava flour. Moisture is an important parameter in the storage of cassava flour as very high levels greater than 12% allow for microbial growth and thus low levels are favourable and give relatively longer shelf life. Hence, all the cassava flour samples had good moisture levels and hence have the potential for better shelf life.

The ash contents of the cassava flours preserved with ginger and garlic were significantly ($p<0.05$) higher than that of the control and it ranged from 2.45 to 2.75% with sample Al recording the least value and sample C2 having the highest value while the control recorded 2.30% suggesting that addition of ginger and garlic as preservatives enhanced the ash content of the cassava flours .Also, these values were higher than the range (1.21 to 1.78%) reported ash content of cassava flour by [26]. Ash content has also been reported in previous studies to range from 1.46 to 2.71% [27], 1.90 to 2.84% [28] and 1.44 to 2.35%/ [29]. The differences in reported ash contents could be attributed to differences in dry matter contents and their proximate composition. Ash content is an indicator of mineral contents and is used as measurement of quality of flours in the food industry [26]. Therefore, the high ash content of the preserved cassava flours suggests that they contain appreciable amounts of minerals which could have health-promoting benefits.

The fat content of the cassava flours preserved with ginger and garlic ranged from 0.65 to 1.20% with sample B1 and C1 having the least values and sample A3 having the highest value while that of the control flour was observed to be 0.95%. It was also observed that addition of ginger and garlic as preservatives had no clear effect on the fat content of the cassava flours. These values were higher than 0.55 and 0.68% reported for fat content of cassava flour from [26]. Fat content in flour influences paste texture by favouring a stable viscosity [30]. However, high-fat content in cassava flour is an undesirable attribute since too much fat will lead to the high possibility for rancidity and increase cloudiness in flour [31]. The crude fiber content of the cassava flours preserved with ginger and garlic ranged from 2.10% in sample Al to 2.60% in sample B3 while that of the control was recorded as 2.45%. This was not surprising as [32] reported that crude fiber in cassava flour falls between the ranges 1.66 to 4.27%. Similarly, [33] reported that fiber content of cassava ranged from 1.5 to 4% in processed products such as flour; although the content varies in different cultivars. However, there was no clear trend on the effect of addition of ginger and garlic as preservatives on the crude fiber content of the cassava flours.

Protein is an important macronutrient and a functional ingredient in food formulations. The protein content of the cassava flours preserved with ginger and garlic ranged from 3.40% in sample Al to 3.80% in sample B3 while that of the control was found to be 3.70%. These values were quite higher than 1.14%, 1.55% and 1.60% observed by [34], for 'protein content of cassava flour. Again, there was no clear trend on the effect of addition of ginger and garlic as preservatives on the protein content of the cassava flours. According to the findings of [35] and [17], the contents of protein, essential amino acids, and protein quality of cassava flour can be enhanced by the fermentation. Apart from the fermentation, fortification with protein rich sources [36] and formulation of composite flour with legume and cereal flour [37] have been proposed to improve the protein content and nutrition level of cassava flour.

The addition of ginger and garlic as preservatives improved the carbohydrate content of sample Al to A3 and sample C1 to C3 as well as sample B1 and it ranged from 78.75% for sample B3 to 80.60% for sample C1 while that of the control was observed to be 78.80%. This was expected as cassava is known to be a rich source of carbohydrate [38]. However, these values were lower than the range (83.42 to 87.35%) reported by [28] for cassava varieties and the difference could be attributed to genetic variations between the crops. Hence, the cassava flours are good sources of energy owing to their high carbohydrate content.

Physicochemical properties of cassava flours preserved with ginger and garlic

The physicochemical properties of cassava flours preserved with ginger and garlic were presented in Table 2. The pH values of the cassava flours preserved with ginger and garlic were significantly ($p<0.05$) lower than that of the control (4.89) and it ranged from 4.57 in sample B3 to 4.66 in sample Al. These values were lower than results (6.75 and 6.72) recorded for pH of cassava flour [26]. pH is one of the important attributes in order to maximize the application of cassava flour in food industries especially in the making of bakery products [16]. The addition of ginger and garlic to cassava flours in this study had lowering effect on pH values; hence this confer better keeping qualities and makes their utilization easier for industrial applications.

The Total Titratable Acidity (TTA) of the control (0.083%) was significantly ($p<0.05$) lower than the range (0.110 to 0.146%) reported for the cassava preserved with ginger and garlic flours. Sample Al had the least TTA of 0.110% while sample B3 had the highest TTA of 0.146%. These values were close to the range (0.001 to 0.125%) reported for TTA of cassava flour by [39]

Microbial load of cassava flours preserved with ginger and garlic

The microbial load of cassava preserved with ginger and garlic during storage was given in Table 3. On the first day (day 0), it was evident that the total heterotrophic bacterial count (THBC) ranged from $2.0x10^5$ cfu/g for sample A3 and B3 to $1.9x10^6$ cfu/g for sample C2 while the control was found to be $1.82x10^5$ cfu/g. However, during storage, the THBC increased ranging from $4.0x10^5$ cfu/g (sample B3) to $7.2x10^5$ cfu/g (sample C1)

Table 2. *Physicochemical properties of cassava flour preserved with ginger and garlic.*

Sample	PH	Total Titratable Acidity (%)
Al	$4.66^{b} \pm 0.02$	$0.110^{e} \pm 0.002$
A2	$4.63^{cd} \pm 0.01$	$0.120^{d} \pm 0.001$
A3	$4.62^{cde} \pm 0.00$	$0.128^{C} \pm 0.000$
B1	$4.61^{cde} \pm 0.00$	$0.134^{bc} \pm 0.001$
B3	$4.59^{ef} \pm 0.01$	$0.139^{b} \pm 0.002$
B3	$4.57^{f} \pm 0.01$	$0.146^{a} \pm 0.002$
Cl	$4.64^{bc} \pm 0.00$	$0.117^{d} \pm 0.001$
C2	$4.61^{cde} \pm 0.00$	$0.121^{d} \pm 0.003$
C3	$4.60^{de} \pm 0.00$	$0.138^{b} \pm 0.002$
Control	$4.89^{a} \pm 0.01$	$0.083^{f} \pm 0.006$

Values are means ± standard deviation of duplicate determinations. Two means along the same column with different superscripts are significantly (p<0.05) different.

Key: *Sample Al = cassava flour + 1.5 % ginger, Sample A2 = cassava flour + 2.0 % ginger, Sample A3 = cassava flour + 2.5 % ginger, Sample Bl = cassava flour + 1.5 % garlic, Sample B2 = cassava flour + 2.0 % garlic, Sample B3 = cassava flour + 2.5 % garlic, Sample Cl = cassava flour + 1.5 % ginger/garlic, Sample C2 = cassava flour + 2.0 % ginger/garlic, Sample C3 = cassava flour + 2.5 % ginger/garlic, Control = untreated cassava flour*

Table 3. *Total Heterotrophic Bacterial Count (cfu/g) of cassava preserved with ginger and garlic and given storage treatment.*

Sample	Day 0	Week1	Week 2	Week 3
Al	3.6×10^5	5.4×10^5	7.3 x 10	1.4×10^6
A2	3.7×10^5	6.1 $x10^5$	9.3×10^5	1.0×10^6
A3	2.0×10^5	4.8×10^5	7.4×10^5	8.6×10^5
B1	2.3×10^5	5.1×10^5	6.8×10^5	9.1×10^5
B2	2.1×10^5	4.3×10^5	8.1×10^5	1.02×10^6
B3	2.0×10^5	4.0×10^5	6.4×10^5	1.13×10^6
C1	5.7×10^5	7.2×10^5	1.03×10^6	1.28×10^6
C21	1.9×10^6	5.4×10^5	8.2×10^5	1.04×10^6
C3	1.8×10^5	4.3×10^5	7.0×10^5	9.3×10^5
Control	1.82×10^5	2.31×10^6	2.48×10^6	2.83×10^6

Values are means ± standard deviation of duplicate determinations. Two means along the same column with different superscripts are significantly (p<0.05) different.

Key: *Sample Al = cassava flour + 1.5 % ginger, Sample A2 = cassava flour + 2.0 % ginger, Sample A3 = cassava flour + 2.5 % ginger, Sample Bl = cassava flour + 1.5 % garlic, Sample B2 = cassava flour + 2.0 % garlic, Sample B3 = cassava flour + 2.5 % garlic, Sample C1 -cassava flour + 1.5 % ginger/garlic, Sample C2 = cassava flour + 2.0 % ginger/garlic, Sample C3 = cassava flour + 2.5 % ginger/garlic, Control = untreated cassava flour*

while the control recorded $2.31x10^5$ cfu/g for week 1, $6.4x10^5$ cfu/g (sample B3) to $1.03x10^6$ cfu/g •(sample Cl) while the control recorded $2.48x10^6$ cfu/g for week 2 and $8.6x10^5$ cfu/g (sample A3) to $1.28x10^6$ cfu/g (sample C1) while the control recorded $2.83x10^6$ cfu/g for week 3. The result showed that ginger and garlic tends to reduce the bacterial load of the samples although those samples preserved without ginger and garlic mix had relatively higher bacterial loads compared with those preserved with either ginger or garlic. The preservative effect exerted by ginger and garlic was not surprising as ginger has been reported to possess antimicrobial activity [40] while garlic also has been reported to have antimicrobial activity on both gram positive and gram negative bacteria [41]. This observed increase in THBC of the cassava flours could be attributed to contamination which occurred during packaging and storage. It has also been reported that the number of organisms will increase if equipment is not adequately cleansed and sanitized.

During the first and second week of storage, no fungal growth was observed Table 3. However, in the third week, the Total Fungal Count (TFC) ranged from $6.0x10^3$ cfu/g for sample C2 to $1.7x10^4$ cfu/g for sample C1 while the control recorded $3.8x10^4$ cfu/g and no fungal growth was observed for sample B2 and B3. In the fourth week of storage a similar trend was observed as there were no visible fungal growths for sample B2 and B3 but the TFC recorded for other samples ranged from $8.0x10^3$ cfu/g (sample C2) to $1.8x10^4$ cfu/g (sample C1) while that of the control was $4.7x10^4$ cfu/g. These observations clearly indicated that ginger and garlic worked effectively in terms of reducing the fungal growths of the samples and this could be attributed to their, antimicrobial activity Table 4.

In terms of Total Coliform Count (TCC), no visible growth was observed on the first day for the entire samples but during the first week of storage, only sample B1 had TCC of $1.2x10^4$ cfu/g and it was lower than $3.4x10^4$ cfu/g recorded for the control. In the second week of storage, only sample A3 and B3 had visible fungal growth of $1.9x10^4$ cfu/g and $1.4x10^4$ cfu/g respectively, against $3.8x10^4$ cfu/g observed for the control. Lastly, during the third week of storage, only sample Bl, B3 and C1 had visible TCC of $2.6x10^4$ cfu/g, $2.3x10^4$ cfu/g and $1.4x10^4$ cfu/g respectively while the control recorded $5.8x10^4$ cfu/g. Hence, ginger and garlic had a preservative effect on the

Table 4. *Total Fungal Count (cfu/g) of cassava preserved with ginger and garlic and given storage treatment.*

Sample	Day 0	Week 1	Week 2	Week 3
Al	NG	NG	1.3×10^4	1.7×10^4
A2	NG	NG	1.0×10^4	1.1×10^4
A3	NG	NG	NG	NG
B1	NG	NG	8.0×10^3	1.2×10^4
B2	NG	NG	NG	NG
B3	NG	NG	NG	NG
C1	NG	NG	1.7×10^4	1.8×10^4
C2	NG	NG	6.0×10^3	8.0×10^3
C3	NG	NG	NG	NG
Control	NG	NG	3.8×10^4	4.7×10^4

Values are means ± standard deviation of duplicate determinations. Two means along the same column with different superscripts are significantly ($p<0.05$) different.

Key: *Sample Al = cassava flour + 1.5 % ginger, Sample A2 = cassava flour + 2.0 % ginger, Sample A3 = cassava flour + 2.5 % ginger, Sample Bl = cassava flour + 1.5 % garlic, Sample B2 = cassava flour + 2.0 % garlic, Sample B3 = cassava flour + 2.5 % garlic, Sample Cl = cassava flour + 1.5 % ginger/garlic, Sample C2 = cassava flour + 2.0 % ginger/garlic, Sample C3 = cassava flour + 2.5 % ginger/garlic, Control = untreated cassava flour, NG = no growth*

Table 5. *Total Coliform Count (cfu/g) of cassava preserved with ginger and garlic and given storage treatment.*

Sample	Day 0	Week 1	Week 2	Week 3
Al	NG	NG	NG	NG
A2	NG	NG	NG	NG
A3	NG	NG	1.9×10^4	NG
B1	NG	1.2×10^4	NG	2.6×10^4
B2	NG	NG	NG	NG
B3	NG	NG	1.4×10^4	2.3×10^4
C1	NG	NG	NG	1.4×10^4
C2	NG	NG	NG	NG
C3	NG	NG	NG	NG
Control	NG	3.4×10^4	3.8×10^4	5.8×10^4

Values are means ± standard deviation of duplicate determinations. Two means along the same column with different superscripts are significantly ($p<0.05$) different.

Key: *Sample Al = cassava flour + 1.5 % ginger, Sample A2 = cassava flour + 2.0 % ginger, Sample A3 = cassava flour + 2.5 % ginger, Sample Bl = cassava flour + 1.5 % garlic, Sample B2 = cassava flour + 2.0 % garlic, Sample B3 = cassava flour + 2.5 % garlic, Sample Cl = cassava flour + 1.5 % ginger/garlic, Sample C2 = cassava flour + 2.0 % ginger/garlic, Sample C3 = cassava flour + 2.5 % ginger/garlic, Control = untreated cassava flour, NG - no growth.*

cassava flours due to their antimicrobial activity.

According to the International Commission on Microbiological Specifications for Foods [42], ready to eat food with plate count of <103 cfu/g is acceptable, between 10^4 to 10^5 cfu/g is tolerable and $>10^6$ cfu/g is unacceptable. Hence, the cassava flours in this study were within tolerable microbiological limits during the second week of storage and unacceptable microbiological limits in the third week of storage and may not be fit for human consumption Table 5 [43].

Microbial isolates from cassava flours preserved with ginger and garlic

The species of microorganisms detected and isolated from the cassava flours preserved with ginger and garlic were presented in Table 6a and b. The bacterial isolates were *Staphylococcus aureus, Bacillus sp., Enterobacter sp. and Micrococcus sp*. The fungal isolates were *Trichoderma sp., Penicillium sp. and Rhizopus sp*. The isolation of *Staphylococcus aureus and Bacillus sp.* agreed with the findings of [34,45] which reported that these microorganisms were implicated in ready-to-eat foods. The presence of molds *Rhizopus sp*. and *Trichoderma sp*. in the cassava flours may have resulted from the dust and soil within the environment of production as these molds have the ability to form spores which is abundant in the environment [46].

The occurrence of *Bacillus sp*. in the cassava flours is not desirable as it has been reported to be associated with the production of toxin and diarrhea [47]. The bacteria could be found in the soil, dust and raw food and can survive normal cooking conditions due to its formation of heat-resistant spores [48].

The presence of *Staphylococcus aureus* in the cassava flours was mainly due to human contact which suggests poor hygienic practices during the processing of cassava since the organism is a normal flora of the skin and nasal passage [49].

In this study, the presence of molds *Rhizopus sp., Trichoderma sp. and Aspergillus sp.* in the cassava flours is of serious public

Table 6a. *Colonial morphology and biochemical characterization of the bacterial isolates.*

Colonial morphology	Gram reaction	Cell shape	Indole reaction	Citrate utilization	H2S production	Catalase	Probable organism
Large smooth yellow colonies on mannitol salt agar plates	+ve	Cocci in clusters	-ve	+ve	-ve	+ve	*Staphylococcus aureus*
Creamy coloured, irregular-shaped colones on Nutrient agar plates	+ve	Rod	-ve	+ve	-ve	-ve	*Bacillus sp.*
Pink colonies on Macconkey agar plates	-ve	Rod	-ve	+ve	-ve	+ve	*Enterobacter sp.*
Tiny yellow, irregular-shaped colonies on Nutrient agar plates	+ve	Cocci	-ve	-ve	-ve	-ve	*Micrococcus sp.*

Table 6b. *Macroscopic and microscopic features of isolated fungi.*

Macroscopic features	Microscopic features	Fungi isolated
White cottony mycelia covering the entire plate with age	Non-septate hyphae with erect and simple sporangiophores	*Rhizopus sp.*
Rapidly-growing white colonies turning blue-green with age	Septate hyphae with conidia borne on large branched conidiophores	*Trichoderma sp*
Bright green colonies with white edges	Ellipsoidal conidia borne on smooth-walled conidiophores with septate hyphae	*Penicillium sp*

health concern as also suggested by [50] which reported that these organisms have been implicated with the production of mycotoxin.

In addition to the high microbial counts of cassava flours, the isolation of *Bacillus sp., Staphylococcus aureus* and *Rhizopus sp. d*emonstrates a potential health risk as these organisms are pathogenic and have been implicated in food borne diseases [48]. Hence, it is important for food to be free from contaminations as much as possible [51-57].

References

1. Nhassico D, Muquingue H, Cliff J, et al. Rising African cassava production, diseases due to high cyanide intake and control measures. J Sci Food Agric. 2008;88(12):2043-9.
2. Taiwo KA. Utilization potentials of cassava in Nigeria: The domestic and industrial products. Food Rev Int. 2006;22(1):29-42.
3. Opara LU. CIGR Handbook of Agricultural Engineering, Volume IV Agro Processing Engineering, Chapter 2 Root Crops, Part 2.4 Cassava Storage.
4. Montagnac JA, Davis CR, Tanumihardjo SA. Processing techniques to reduce toxicity and antinutrients of cassava for use as a staple food. Compr Rev Food Sci Food Saf. 2009;8(1):17-27.
5. Jennings D. Tropical Root and Tuber Crops. Cassava, Sweet Potato, Yams and Aroids. By V. Lebot. Wallingford, UK: CABI (2009), pp. 413,£ 37.50. ISBN 978-1-84593-424-8. Exp Agric. 2009;45(3):382-.
6. Okoronkwo, CU, Oganezi NC, Unegbu VN. Production and Shelf-Life Extension of Grape Juice Sweetened with Honey and Preserved with Ginger. Ann Food Process Preserv. 2022;6(1):1032.
7. Sanchez T, Chavez AL, Ceballos H, et al. Reduction or delay of post-harvest physiological deterioration in cassava roots with higher carotenoid content. J Sci Food Agric. 2006;86(4):634-9.
8. Iglesias CA, Sanchez T, Yeoh HH. Cyanogens and linamarase activities in storage roots of cassava plants from breeding program. J Food Compos Anal. 2002;15(4):379-87.
9. Ezeama CF. Food Microbiology Fundamentals and Applications. Nat Prints Lim. 2007.
10. Indiarto R, Subroto E, Angeline S. Ginger rhizomes (Zingiber officinale) functionality in food and health perspective: A review. Food Res. 2021;5:497-505.
11. Langner E, Greifenberg S, Gruenwald J. Ginger: History and use. Adv Ther. 1998;15(1):25-44.
12. Widmaier, W. Flanzenheilkunde WBV Brolonisch-mediziniche Verlag.s society. Schondorf. 1986.
13. Wong, S. The flavor of ginger. 2000.
14. FAO. Food and agricultural organization of the United Nations Statistical Division, FAO, 2013.
15. Briani C, Samaroo D, Alaedini A. Celiac disease: From gluten to autoimmunity. Autoimmun Rev. 2008;7(8):644-50.
16. Aryee FN, Oduro I, Ellis WO, et al. The physicochemical properties of flour samples from the roots of 31 varieties of cassava. Food control. 2006;17(11):916-22.
17. Christopher UO, Valentine U. Processing and shelf-life extension of garri using chitosan as a preservative. J Basic Appl Res Int. 2021;27(10):23-31.
18. Douglas M, Heyes J, Smallfield B. Herbs, spices and essential oils: post-harvest operations in developing countries. UNIDO and FAO. 2005;61:1-8.
19. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis of the AOAC. 18th Edition. Washington DC. 2006; 20-22.
20. AOAC. Official Method of Analysis of the Association of Official Analytical Chemists. AOAC, Arlington. 1990.
21. Pearson D. The chemical analysis of foods. 7th Edition. Longman Group Ltd. 1976.
22. Cumbana A, Mirione E, Cliff J, et al. Reduction of cyanide content of cassava flour in Mozambique by the wetting method. Food Chem. 2007;101(3):894-7.
23. Apea-Bah FB, Oduro I, Ellis WO, et al. Factor analysis and age at harvest effect on the quality of flour from four cassava varieties. World J Dairy Food Sci. 2011;6(1):43-54.

Citation: *Okoronkwo Christopher Uche, Obinwa C.O. Production and evaluation of fermented cassava flour preserved with ginger and garlic mixtures. J Food Microbiol. 2023;7(4):152*

24. Hsu CL, Chen W, Weng YM, et al. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chem. 2003;83(1):85-92.

25. Chisenga SM, Workneh TS, Bultosa G, et al. Proximate composition, cyanide contents, and particle size distribution of cassava flour from cassava varieties in Zambia. AIMS Agri Food. 2019;4(4).

26. Rojas CC, Nair B, Herbas A, et al. Proximal composition and mineral contents of six varieties of cassava (Mannihot Esculenta, Crantz), from Bolivia. Bol J Chem. 2007;24(1):71-7.

27. Emmanuel OA, Clement A, Agnes SB, et al. Chemical composition and cyanogenic potential of traditional and high yielding CMD resistant cassava (Manihot esculenta Crantz) varieties. Int Food Res J. 2012;19(1):175-81.

28. Eleazu CO, Eleazu KC, Awa E, et al. Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. J Biotechnol Pharm Res. 2012;3(2):42-6.

29. Moorthy SN. Physicochemical and functional properties of tropical tuber starches: A review. Starch-Starke. 2002;54(12):559-92.

30. Mishra S, Rai T. Morphology and functional properties of corn, potato and tapioca starches. Food Hydrocoll. 2006;20(5):557-66.

31. Fakir MS, Jannat M, Mostafa MG, et al. Starch and flour extraction and nutrient composition of tuber in seven cassava accessions. J Bangladesh Agric Univ. 2012;10(452-2016-35665):217-22.

32. Gil JL, Buitrago AJ. Cassava in animal feed. Cassava in the third millennium: Modern production, processing, utilization and marketing. Int Center Trop Agri. 2002:527-69.

33. Tambo Tene S, Klang JM, Ndomou Houketchang SC, et al. Characterization of corn, cassava, and commercial flours: Use of amylase-rich flours of germinated corn and sweet potato in the reduction of the consistency of the gruels made from these flours-Influence on the nutritional and energy value. Food Sci Nutr. 2019;7(4):1190-206.

34. Oyeyinka SA, Ayinla SO, Sanusi CT, et al. Chemical and physicochemical properties of fermented flour from refrigerated cassava root and sensory properties of its cooked paste. J Food Process Preserv. 2020;44(9):e14684.

35. Otondi EA, Nduko JM, Omwamba M. Physico-chemical properties of extruded cassava-chia seed instant flour. J Agric Food Res. 2020;2:100058.

36. Onyango C, Luvitaa SK, Unbehend G, et al. Nutrient composition, sensory attributes and starch digestibility of cassava porridge modified with hydrothermally-treated finger millet. J Agric Food Res. 2020;2:100021.

37. Adebowale AA, Sanni LO, Awonorin SO. Effect of texture modifiers on the physicochemical and sensory properties of dried fufu. Food Sci Technol Int. 2005;11(5):373-82.

38. Akama O. Physiochemical properties of dried cassava flour from balls and chunks. Int J Sci Technol Res. 2013;2:63-70.

39. Olaniran AF, Abiose SH, Adeniran AH. Biopreservative Effect of Ginger (Z ingiber officinale) and Garlic Powder (A llium sativum) on Tomato Paste. J Food Safety. 2015;35(4):440-52.

40. Udu-Ibiam OE, Ogbu O, Ibiam UA, et al. Phytochemical and antioxidant analyses of selected edible mushrooms, ginger and garlic from Ebonyi State, Nigeria. IOSR J Pharm Biol Sci. 2014;9(3):86-91.

41. International Commission on Microbiological Specifications for Foods. Microorganisms in foods 5: Characteristics of microbial pathogens. Springer Sci Business Media. 1996.

42. Nichols GL, Little CL, Mithani V, et al. The microbiological quality of cooked rice from restaurants and take-away premises in the United Kingdom. J Food Prot. 1999;62(8):877-82. `

43. Mensah P, Yeboah-Manu D, Owusu-Darko K, et al. Street foods in Accra, Ghana: How safe are they? Bulletin of the World Health Organization. 2002;80(7):546-54.

44. Apinis AE. Mycological aspects of stored grain. Biodeterior Mater. 1972;2:493-8.

45. Rajkowski KT, Bennett RW. International Handbook of foodborne Pathogens. M.D. Miliotis and J.W. Bier (ed). 2003;40-51.

46. Akindele P, Ibrahim K. Microbiological analysis of ready-to-eat-foods obtained from Bukaterian within the Ekiti state University and environment, Ado-Ekiti, Nigeria. J Adv microbiol. 2016;1(2):1-8.

47. Garrett ES. Microbiological standards, guidelines, and specifications and inspection of seafood products. Food Technology. 1988;42(3):90.

48. Husaini AM, Gbodi TA, Akanya HO, et al. Health implications of toxigenic fungi found in two Nigerian staples: Guinea corn and rice. 2009;3(9):250-256

49. Wagner AB. Epidemiology of food poisoning outbreaks in Singapore, 2001-2005. Epidemiological News Bulletin, J Microbiol. 2009;31:68-72.

50. Association of Official Analytical Chemists, Association of Official Agricultural Chemists (US). Official methods of analysis of the Association of Official Analytical Chemists. The Association; 1925.

51. Burns AE, Gleadow RM, Zacarias AM, et al. Variations in the chemical composition of cassava (Manihot esculenta Crantz) leaves and roots as affected by genotypic and environmental variation. J Agric Food Chem. 2012;60(19):4946-56.

Citation: *Okoronkwo Christopher Uche, Obinwa C.O. Production and evaluation of fermented cassava flour preserved with ginger and garlic mixtures. J Food Microbiol. 2023;7(4):152*

52. Cardoso AP, Mirione E, Ernesto M, et al. Processing of cassava roots to remove cyanogens. J Food Compos Anal. 2005;18(5):451-60.

53. Charles AL, Sriroth K, Huang TC. Proximate composition, mineral contents, hydrogen cyanide and phytic acid of 5 cassava genotypes. Food chem. 2005;92(4):615-20.

54. Oyeleke A, Manga SB. Essential of laboratory practice. 3rd ed. Minna, Niger state, Nigeria: Tobest Publi. 2008:12-29.

55. Samson RA, Varga J. Aspergillus systematics in the genomic era. Utrecht: CBS Fungal Biodivers Centre. 2007:306

56. James, C.S. Analytical chemistry of food. Chapman and Hall, London, 1995;64-65

57. Sukajang N, Jongpanyalert B, Chantachum S, et al. Effects of sodium ascorbate and drying temperature on active protease of dried ginger. Asian J Food Agro-Ind. 2010;3(1):52-8.

Citation: *Okoronkwo Christopher Uche, Obinwa C.O. Production and evaluation of fermented cassava flour preserved with ginger and garlic mixtures. J Food Microbiol. 2023;7(4):152*