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Multivariate Analysis of Intrinsic Parameters of Garri for the Quantitative Prediction of its Microbial Loads

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Abstract: The quantitative prediction of microbial load of garri is essential for deciphering its population dynamics and as well as its microbiological. Current techniques for microbiological quality determination of food relies on the cumbersome laboratory procedures which are not suitable for rapid quantitative measurements of microbial loads. In view of this, this study was carried out to determine the multivariate statistical analysis of intrinsic parameters of garri for the quantitative prediction of its microbial loads. The pH and titrable acidity of the food were measured using pH meter and colorimetric acidity titration method respectively while the moisture content was determined using the recommended standard method by A.O.A.C. Results obtained from this study revealed a direct relationship between moisture content, titrable acidity and total viable bacterial count of the garri samples while the total viable fungal count of garri was largely dependent on the pH values of the garri samples. Furthermore, an inverse relationship exist between the mean pH values and the titrable acidity of the garri samples. In conclusion, the results from this study showed that titrable acidity, pH and moisture content could be conveniently used for predicting the total viable bacterial count while only pH and titrable acidity had a statistical significant relation for predicting the total viable fungal counts.

Key words: Total viable bacterial counts · Total viable fungal counts · Intrinsic factors · Garri

INTRODUCTION

Garri is a roasted granule of cassava that is widely consumed in both rural and urban areas [1]. It is by far the most popular form in which cassava is consumed in West Africa [2] and indeed in Africa [3]. The recent increase in the consumption of garri as food has been ascribed to the possibility of consuming it directly with cold water and/or the possibility of quickly reconstituting it with hot water to form dough with soup [3]. The production of garri begins with the sorting of cassava without rot, peeling, washing, grating, fermentation, sifting, frying, cooling, sieving and packaging [4]. However, the practices associated with the production, processing and post processing handling of garri such as spreading on the floor, mats, display in open bowl in the markets and sales points and use of various packaging materials to haul finished products from rural to urban areas may exacerbate microbial contamination [5]. According to Hildrum *et al.* [6], the analysis of food quality has been characterized in the past by single univariate measurements of single parameters, which may have several draw backs. In microbiological analysis for instance, the principal drawback to standard plating methods for determining the numbers and type of microorganisms in a food product include the time required for pre-enrichment and incubation of the product and the plates respectively. Therefore, there is a need for developing of rapid and multivariate techniques for rapid assessment of microbiological quality of food products [7]. Such innovation in food analysis will require more knowledge on how the intrinsic

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factors of the food together affect the viable counts of microorganism in it. Hence, this study was carried out to give a scientific report on how pH, titrable acidity and moisture contents of garri circulating in Ogun State, Nigeria can be related to the viable microbial counts of this food.

MATERIALS AND METHODS

Collection of Garri Samples: Yellow and white garri samples were purchased from the four geopolitical zones of Ogun State, Nigeria. Laboratory size samples suitable for intrinsic factors and microbiological analyses were obtained according to the method described by Pomeranz and Meloan [8].

Intrinsic Factors Analysis: The intrinsic factors analyzed were pH, moisture content and the titrable acidity. The pH of each dispensed garri samples suspension of 2% strength was determined at room temperature (29±2°C) using electrodes of a pH meter (Hanna instruments) placed directly into each suspension. The pH meter with accuracy of 0.1 was first standardized using buffer solution of pH 4 and 9. The determination was performed in duplicate to find the mean pH of the sample. The titrable acidity was determined as described by Friedrich [9] using colorimetric acidity titration as follows: Equal parts of deionized distilled water (ddH_2o) were added to the solid samples and macerated in a blenders at 100rpm for 2minutes before centrifuging for 5minutes at 2500 rpm at room temperature. To 10ml of the supernatant sample solution in a clean Erlenmeyer flask were added 5 drops of 1% phenolphthalein indicator solution and a magnetic stir bar before stirring on a magnetic stir plate. Then 0.1N NaOH was carefully titrated against the sample solution to the end point of pH 8.2 until a faint but definite pink colour, which was stable for 5 to 10 seconds was obtained. The titrable acidity was calculated using the equation:

$$TA(g/100ml) = \frac{(V)(N)(meq.wt.)(w)}{(1000)(Vs)}$$

where v is volume (ml) of NaoH solution used for titration N = Normality of NaoH solution, meq.wt is milli-equivalent weights of acid: lactic acid = 90, Vs = sample volume = 10ml. The analysis was performed in triplicate to find the mean titrable acidity of each sample. The moisture contents of the garri samples were determined by the Oven drying method at 105°C [10].

Enumeration of Total Viable Bacterial and Fungal Counts: The viable bacterial and fungal count analyses were performed on samples of the garri following the procedures of the International Commission for specification for food [11]. A ten fold serial dilutions up to 10^{-10} for each sample were prepared in 0.1% peptone water and 1% glucose broth and subsequently plated onto standard plate count Agar (PCA) and Saboraud dextrose Agar for viable bacterial and fungal counts respectively. The PCA plates were incubated at 37°C for 24h while the SDA plates were incubated at 27°C for 72h. The colony forming unit (CFU) were counted on plates having between 30 and 300 colonies using Quebec colony counter. The enumeration of viable microbial count was carried out in duplicate on each sample and the isolated organisms were identified using standard methods [12].

Statistical Analysis: Results were subjected to student t test analysis and linear multiple regression using Statistical Package for Social Sciences (SPSS) version 15.0. The significant differences between means were determined at $P<0.05$.

RESULTS

The mean viable bacterial count of 4.55±0.55 in white garri was significantly higher than the mean count of 3.01±0.29 obtained in yellow garri ($t=2.48$, $p<0.05$) while no significant difference was found between the mean fungal count of 6.26±0.61 in white garri and 7.46±0.30 in yellow garri ($t=1.78$, $p>0.05$).Also, an inverse relationship was observed between the mean value of pH and titrable acidity of dried cassava powder (garri). A significantly higher moisture content and titrable acidity of 30.2±0.90 and 0.41±0.01g/100ml in white garri was more than the 26.3±2.09 and 0.35±0.01g/100ml in yellow garri. However, the pH of yellow garri seem to be higher than that of white garri but this was found not to be statistically significant ($t=1.46$, $P>0.05$) Table 2. Table 3 showed that the moisture content, pH and titrable acidity have a statistical relation ($p<0.05$) with the viable bacterial count when the combined effects of moisture, pH and titrable acidity were treated as independent variables. It thus appeared that viable bacteria count could be conveniently predicted statistically from the linear equation given below:

Table 1: Bacterial and fungal counts of dried cassava powder (garri) in Ogun State, Nigeria

	Garri samples Log(cfu/g) (Mean ± SEM)			
Parameters	Yellow garri	White garri	t value	p value
Total viable bacterial counts	3.01±0.29	4.55±0.55	2.48	<0.05
Total viable fungal counts	7.46±0.30	6.26±0.61	1.78	>0.05

Table 2: Intrinsic factors of dried cassava powder (garri)

	Garri samples Log(CFU/g) (Mean ±SEM)			
Intrinsic factors	Yellow garri	White garri	tvalue	pvalue
Moisture content	26.3±2.09	30.2±0.90	2.69	<0.05
pH	5.97±0.33	5.5±0.45	1.46	>0.05
Titrable acidity	0.35±0.01	0.41±0.01	7.35	<0.0

Table 3: Relationship between total viable bacterial counts and the intrinsic parameters of garri

Intrinsic factors of garri	Co-efficient (β)	S.E	tvalue	pvalue
Constant (α)	$6x10^{-7}$	$6x10^{-5}$	-11.70	0.001
pH	$4.5x10^{-6}$	3859699	11.58	0.001
Moisture content	3893450	727268.4	5.35	0.033
Titrable acidity	$3.9x10^{-5}$	2894433	12.10	0.029

Model, $y= \alpha+\beta_1x_1 + \beta_2x_2 + \beta_3x_3$.

Table 4: Relationship between total viable fungal counts and the intrinsic parameters of garri

Instrinsic factors	coefficient(β)	S.E	tvalue	Pvalue
Constant (α)	$5x10^{-8}$	$1x10^{-8}$	-5.5	90.031
pH	$4.5x10^{-7}$	7449511	6.059	0.026
Moisture content	3115648	1403683	2.20	0.157
Titrable acidity	$3.9x10^{3}$	3331144	7.123	0.012

Model, $A= \alpha +\beta_1x_1 + \beta_3x_3$.

$Y= \alpha + \beta_1x_1 + \beta_2x_2 + . \beta_3x_3$.

Where
Y = total viable bacterial counts
α = constant
β_1 = coefficient of regression of bacterial count on moisture content
x_1 = moisture content
β_2 = coefficient of regression of bacteria count on pH
x_2 = pH values
β_3 = coefficient of regression of bacteria on titrable acidity
x_3. = tittrable acidity values.

Table 4 depicts that only pH and tittrable acidity has a statistical relation ($p<0.05$) with the fungal counts when the combined effect of moisture, pH and titrable acidity of garri were treated as independent variables. This means that the viable fungal count can only be predicted conveniently from the linear equation below

$A = \alpha +\beta_2x_2 + \beta_3x_3$, where
A = Total viable fungal count
α = constant
β_2 = coefficient of regression of fungal count on pH
x_2 = pH values
β_3 = coefficient of regression of fungal count on the titrable acidity
x_3 = titrable acidity values

DISCUSSION

The significant difference obtained in the mean bacterial count of white and yellow garri is not surprising because it has been postulated that addition of palm oil to any food prevent the penetration of oxygen and therefore provide an unconducive environment for the growth and survival of aerobic bacteria [13,14], but this is not so for the mean viable fungal count as there exist no significant difference in the count of both yellow and white garri

samples. This observation seem to be directly associated with the pH values of the two garri samples. This is because the range of pH values obtained in our study incidentally favors the growth of fungi in food [15]. Also, inverse relationship observed between the mean pH values and titrable acidity of garri may be due to the activities of lactic and acetic acid bacteria [7]. These bacteria are known to develop in succession during cassava fermentation for production of garri [16].The lactic acid bacteria convert glucose in the cassava samples to lactic acid during fermentation [17]. A significantly higher moisture content observed in white garri than in yellow garri may be due to the higher hygroscopic nature of white garri than yellow garri [18] as addition of palmoil to yellow garri automatically limit the amount of water that can be absorb by this type of garri. In relating the microbiological quality of garri samples to their intrinsic properties, a direct relationship was observed between the mean moisture content, the mean titrable acidity and the mean bacterial count in garri samples. This findings is consistent with that of Olugbuyiro *et al*. [7] who also observed similar result for cocoa powders. Similarly, direct relationship was also found between the mean pH values and the mean viable fungal counts of garri samples. Garri samples can be described as a complex ecosystem consisting of itself as the abiotic environment and the microbes that live in it as the biotic factors. When microbes are placed in environment below or above neutrality, their ability to proliferate depends upon their ability to bring the environmental pH to a more optimum range [19]. When placed in acidic environment, the cells must either keep H^+ ions from entering as rapidly as they enter. Such key cellular compounds as DNA and ATP require neutrality [20]. Also, when most microbes grow in acidic media, their metabolic activities result in the media or substrates becoming less acidic while those that grow in high pH environments tend to effect a lowering of pH [7]. In conclusion, the results of this study showed that titrable acidity, pH and moisture content could be conveniently used for predicting the total viable bacterial count while the pH and titrable acidity was found having a statistical significant relationship with the total viable fungal counts.

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