Optimization Of Microbial Activity In Irvingia Gabonensis Seeds Fermentation During 'Itugha' Production

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ABSTRACT : Optimization of this process is meant to develop the design and unit processes of fermenting *irvingia gabonensis* seeds, in the production of a product considered more nutritious than the raw material from which it is produced. This study assesses the optimal conditions in which the microbes thrive and give the desired product quality. The parameters: pH, temperature, acidity of fermenting medium and the different organic acids produced during the fermentation process, were monitored on a daily basis. Bacterial isolates included *Bacillus spp, Micrococcus spp, and streptococcus spp.* Principally only one fungi *Candida tropicalis* DMB321 was involved in the entire process. Three stages were categorized in the process flow chart. The early stage fermentation caused by *Bacillus spp,* at pH 6-7, 30^{O}C and 1.8% acidity of extract; the intermediary stage micro-organism, *Micrococcus spp, and Streptococcus,* at pH 5.6, 35-38^{O}C and 4.4% acidity of extract while the late stage showed drastic decrease in bacterial load and prolific increase in growth of *Candida tropicallis,* at pH 4.5-5.1, 70^{O}C and 5.4% acidity of extract. Organic acids in the fermenting substrate included citric acid 2.4% DM, glycolic acid 1.22% DM and oxalic acid 2.98% were quantified. Optimization of this fermentation process would enable itugha product development and commercialization. Thereby expanding the frontiers of *irvingia gabonensis* utilization.

Keywords: fermenting conditions, *gabonensis*, Itugha, in- process, *Irvingia,* microbes, optimization.

1. INTRODUCTION

Bush Mango *(Irvingia gabonensis*) is essential to household economies and personal incomes. It grows wild and naturally, both in the sub-guinea and guinea savannah vegetation of West and Central Africa. Since its importance in human nutrition was discovered, and the high demand for the cotyledons for local soup preparation, the plant has been domesticated and cultivated. The seed is ground and used as soup thickeners in Nigeria. The dried seeds are known to have short shelf life, which predisposes them to easy contamination by moulds and aflatoxins (Williams et al 2015). In many West African countries, the dried and powdered seed is added to Dika bread to swell the loaf, and it is also added as substitute to cocoa powder in chocolate production to thicken the product (FIRRO Tech. Memo 1960). The powdered dried seed is cheese-like and used in cooking fish and meat to impact an appetizing and characteristic flavor and taste (Irvin1981). The seed can be roasted and used as flavouring agent in the preparation of local salads (Irvin, 1981). Among the Agoi people of Yakurr ethnic nationality in Cross River state of Nigeria, the seeds of Irvingia gabonensis is fermented and processed into a spread known as Itugha (native cheese or butter). In processing *irvingia* seeds into itugha, natives employ a traditional technology involving a sequence of operations: size reduction, pulverization, fermentation and heat treatment. Each unit operations in the process requires a unique working condition in order to obtain the desired product quality which is measured by taste, aroma and flavor (Ekpe, Umoh and Eka 2007). Itugha is highly valued in family circles and its high nutritive value been reported (Ekpe and Igile .2013). Fermentation, which is the slow decomposition of organic matter induced by micro-organisms such as yeast, bacteria and moulds has also been implicated in the production of itugha from fresh *irvingia gabonensis* seeds, thereby classifying itugha as a fermented food (Ekpe, 2009). In the traditional preparation method, mashed fresh *irvingia* gabonensis seeds are covered and kept away for that day, this is repeated every day until the *irvingia* mash loses its elastomeric property. Without this protocol the final product fails and itugha is not obtained. The implication being that the fermentation process has to be controlled. The production process abhors water as both cleaning, pounding and storage has to be in dry environment. Thus, this is a solid state fermentation in which microorganisms intrinsic to *Irvingia gabonensis* are involved in initiating the process and the *Irvingia mass* providing the solid support on which successive microorganisms grow depending on the metabolites generated. The fermenting solid medium comprises both the substrate and the solid support(mash) on which the fermentation takes place (Capalbo et al 2001). This, process optimization, considers monitoring set parameters for each unit operation for entire process flow. Identification of the enzymes and enzymatic complexes able to breakdown the mucilage in *irvingia gabonensis* during itugha production is a research need. However, to optimize the microbial processes involved in itugha production, the process was carried out using modern technologies, for each of the identified unit process and production process carried out under controlled environment by measuring prevailing pH, temperatures, titratable acid and identification of organic acids produced, for the process that gave an acceptable product.. This study aims at optimizing the microbial fermentation process in order to standardize the design and the process flow chart so as to ease production process validation and reproducibility for product development and commercialization.

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2. MATERIALS AND METHODOLOGY

Itugha was produced using modern technologies for each of the defined and specific unit operations under controlled environment. Dehulled 120g of fresh Irvingia seeds were milled to a fine consistent paste and stored for six (6) days. A second sample was subjected to repeated size reduction

daily for six days. On the seventh (7^{th}) day heat treatment was applied for 6hrs. pH, temperature, titratable acidity were determined daily in both samples. Presumptive micro-organisms on the mash and fresh seeds. were identified daily for the six (6) days production period. Organoleptic changes including texture, aroma and taste were also monitored during the production process. In assessing the role of identified micro-organisms, enumeration of aerobic heterotrophic bacteria was by Method of Holt,1982 and enumeration of aerobic heterotrophic fungi by Hunter and Bennet,1973 method. Bacterial isolates characterization was by Method of Gerhardt et al 1981 and fungi by Biomerieux API (1989) Identification Schemes. Organic acid content was determined in *Irvingia* seed and the ferment called itugha, by Gas chromatography – Mass Spectrometry, Bengtsson and Lehotay method (1996) with some modification. 1g sample was pulverized with 1ml of distilled water, acidified with 1ml 1M HCl to a pH of about 1.0, saturated with NaCl, then extracted with 3ml of ethyl acetate and 3ml of diethyl ether. The organic phases were combined and evaporated to dryness under nitrogen. The sample was derivatised with 0.1ml of BSTFA-TMCS at 65^0C for 10 min, diluted with 0.4ml of hexane/ethyl acetate (50% v/v) and 1 µ1 was injected into the GC-MS and analysed. Gas chromatographic, mass spectral and data analysis on Carlo Erba gas chromatograh 5160 Mega Series, equipped with a Shimadzu data Processor C-R3A: Sample was analysed by GC-MS by injecting 1 u1 of the sample in spliteless mode onto an open tubular glass capillary column 25m x 0.32mm i.d coated with SE 52, and the injector was kept at 250^0C. The carrier gas was hydrogen, with a flow-rate of 1ml/min. The GC oven was held at 90^0C for 4min, then raised at 8^0C/min. The peaks were identified by reference to a mass spectral library.

3. RESULTS AND DISCUSSION

Table I: *Microbiological status of treatment material*

Seed	Presumptive identification
Surface swabbed seed	Streptococcus spp, micrococcus spp
Seed deep tissues	Bacillus spp, Streptococcus spp, micrococcus spp

Table II: *Daily Quality Parameters and Characteristics*

Microbes of Treatment Materials

Days	pH	Temperature	Acidity	Micro-organisms
1	7.0 ± 2.6	Ambient	0.5±1.50	Micrococcus, streptococcus
2	6.4 ± 1.1		1.4 ± 0.40	Micrococcus, streptococcus, bacillus
3	6.0 ± 1.7	-do-	1.8 ± 0.10	-do-
4	5.6 ± 1.4	-do-	2.8 ± 0.11	Micrococcus, streptococcus, bacillus, candida tropicalis, DMB 321
5	5.1 ± 2.2	-do-	4.4 ± 0.30	-do-
6	4.7 ± 1.1	-do-	5.0 ± 0.21	-do-
7	4.5 ± 1.1	70^0C	5.4 ± 0.11	Micrococcus, streptococcus

Mean ± SEM (n=3)

Table III: Role of Micro-organisms

Days	Predominant micro-organisms	Viable Count (cfu/mg)	Tempt (^{o}C)	Organoleptic changes (Texture)	Organoleptic changes (Aroma)
1	Bacteria	$>10^6$	32±1.50	Viscous jelly mash	No smell
2	Bacteria	$>10^6$	32±1.50	-do-	-do-
3	-do-	>300	32±1.50	-do-	-do-
4	Yeast	<90	32±1.50	-do-	-do-
5	Bacteria	>300	32±1.50	Reduced jelly mash	Alcohol smell
6	Yeast	<260	32±1.50	Drawiness ceases	Alcoholic smell persist
7	Yeast	<300	32±1.50	-do-	-do-
8	Bacteria	<100	32±1.50	Spreadable & oily	Spicy aroma developed

Table IV Organic acid content

Organic Acid	SAMPLES (% DM)	
	Irvingia Seed	Ferment
Citric Acid	16.00± 1.13	2.40± 1.10
Glycolic Acid	1.26 ± o.01	1.22 ± 0.01
Oxalic Acid	6.59± 1.20	2. 98± 0.08
Malic Acid	6.28± 1.40	0.11± 0.00
Tartaric Acid	1.44± 0.02	0.19± 0.01

The early stage of fermentation was caused by bacteria. Since streptococcus and micrococcus spp are intrinsic to the Irvingia seed (Table I) and the sample that was crushed to a fine texture on day (1) did not produce Itugha, it can be deduced that the entrant of Bacillus into the micro flora of the mash on day 2 , would have initiated the fermentation (Table II). Thus the early stage fermentation was facilitated by Bacillus spp at pH 6-7, temperature 30^0C – 35^0C (Ambient) and 1.8% acidity of extract.

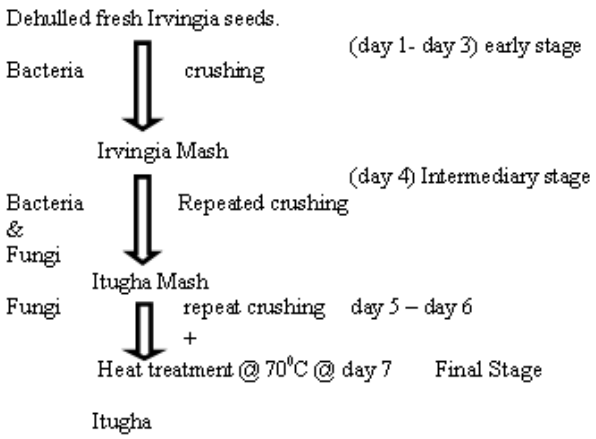

Fig. 1: *Itugha production process flow chart*

The intermediary stage micro-organisms were both bacteria and fungi. These are micrococcus spp, streptococcus spp and candida tropical as this, was detected at that stage, at pH 5.6, temp. 35^{0}C and 4.4% acidity of extract. Late and final stage micro-organisms was candida tropicalis DMB 321 at 70^{0}C and 5.4% acidity of extract. Organic acids influence pH that determines microbial growth and also serve as preservatives. The marked decrease in levels of organic acids in the process e.g for citric acid (16.00% in seed to 2.40% in the ferment) and malic acid(6.28% seed to 0.11% ferment) Table IV ,can be attributed to formation of thermally produced flavour(Maga,1994).

4. CONCLUSION

Since crop production as per yield per hectare and annual output has substantially been addressed in third world countries like Nigerian's Agricultural Sector, attention need now be directed towards documenting information that would promote value addition and marketing of agricultural produce. A process flow chart for itugha production has been established and three stages in the process identified. For each stage, prevailing pH, temperatures and acidity defined. Thus, optimization of this fermentation process has set a platform that could lead to Itugha product development and commercialization. This would aid development of indigenous technologies required for innovations and creativity necessary for revolutionary value addition in agriculture.

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