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Sensory Evaluation During In-Process Optimization of 'Itugha' Production

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Abstract

The Fermentation of *irvingia gabonensis* seeds into itugha, considered more nutritious than the raw seeds from which it is produced, is a traditional technology. The quality of Itugha is usually measured by four key parameters (drawiness, taste, aroma and flavor). This study aimed at using sensory evaluation for product quality assessment during optimization, standardization and design of the fermentation flow process, with the view to ease production process and validate process reproducibility. The in-process monitoring was carried out under controlled conditions; pH, temperature, % titratable acidity and total organic acids throughout the production process. Results indicate that early stage of fermentation was initiated by *Bacillus spp* (pH 6-7, 30°C and 1.8% acidity of extract); intermediate stage fermentation was caused by *Micrococcus* and *Streptococcus spp*, at pH 5.6, 35-38°C and 4.4% acidity of extract. Late stage fermentation was effected by *Candida tropicalis* DMB 321, giving final product quality at pH 4.5-5.1, tempt 70°C as 5.4% acidity of extract, citric acid 2.4% DM, glycolic acid 1.22% DM and oxalic acid 2.98%. Sensory analysis on 9-point Hedonic Scale was used. Overall acceptability for Like Extremely was 7.5; Like very much 8.8; Like moderately 9.00 and overall acceptability ranking 72. Population t-test analysis-value was 21.18 and F-value 12.25. Progress of flavor development showed no flavour in early stage fermentation, alcoholic aroma by 3rd day (intermediate stage) and stringent spicy aroma by 6th day which became prominent after application of heat (final stage). Optimization gave desired sensory attributes that impacted positively on product quality and consumer acceptability.

1. Introduction

Irvingia gabonensis is a non-timber forest plant. Its fruit is made up of a fleshy mesocarp, an inner nut consisting of a hard shell and kernel of two cotyledons coated with a hull which make up the seed. *Irvingia gabonensis* belong to the Family: *Simarombaceae*; Sub-family: *Irvingiaceae*; Genus: *Irvingia*; Species: *Irvingia gabonensis* [1] Varieties: *Var gabonensis* and *Var excelsa* [2]. The local name is *kuwing*, (Agoi Ibami) [3]. *Var gabonensis* succulent fleshy mesocarp is sweet and known to whiten human teeth when chewing the fruit. *Irvingia* seed is mainly used as soup thickener in Nigeria. The seed is known to have short shelf life and as an oil seed, is predisposed to easy contamination by moulds and possibly aflatoxins. In other West African countries, the seeds are used as, ingredient in dika bread and as substitute for

cocoa butter in chocolate [4], or a cheese-like substance used in cooking fish and meat [5], or roasted and used as flavouring agent in salads etc. Itugha is a low standard spread used by natives as a compliment to eating yam, plantain, cocoyam, and water yam. It is also highly valued in family circles. The high premium placed on itugha in traditional circles makes it very prominent in the peoples' dietary. Due to urbanization, this food product is getting extinct while borrowed foods with lesser nutritional value, is entering the peoples dietary. In a report [3] documenting traditional processing of *irvingia var gabonensis* seeds into *itugha*, it was shown that this traditional technology involves size reduction, repeated pounding, fermentation and heat treatment.

In an industrial fermentation for commercial production, process medium and process conditions play a critical role because they affect the formation, concentration and yield of the fermentation end-product [6]. Several optimization techniques are available for optimization of fermentation medium and fermentation process conditions. Some of these are borrowing, component swapping, biological mimicry, one-factor-at-a-time, factorial design, central composite design etc. [7]. In fermentation process optimization, different combinations and sequence of processes conditions and medium components needs investigation to determine growth condition that produces the biomass with the physiological state best constituted for product formation [8].

Fermentation, which is the slow decomposition of organic compounds induced by micro-organisms such as yeast, bacteria and moulds considered beneficial to the process, has been implicated in the production of *itugha* from fresh *irvingia gabonensis* seeds [9]. This traditional preparation of *itugha* was however optimized by first identifying the specific unit processes involved, *itugha* production process carried out using modern technologies for each of the identified unit processes and the microbial succession monitored under controlled conditions by measuring pH, temperature and titratable acidity. Identification of micro-organisms and organic acids involved in the solid state alcoholic fermentation is also documented in this study.

Itugha, a native spread of the Agoi people of Nigeria, produced from the fermentation of dehulled fresh *irvingia gabonensis* seeds through a production process involving size reduction, pulverization, fermentation and heat treatment, is projected for commercialization. This study aims at highlighting the unit processes, optimized microbial fermentation process, sensory evaluation, and standardization of the process flow chart, which would ease process validation and reproducibility for product development, conformity assessment and commercialization. This is meant to provide a livelihood option for people living in deforested lands and/or encroaching into protected critical zones of Cross River State-Nigeria forestry reserves, for income generating activities. Commercialization of *itugha* can encourage *irvingia* propagation and domestication that would lead to promoting agroforestry for sustainable agriculture in

communities where deforestation is endangering biodiversity. Domestication of *irvingia* may also protect the ozone layer and reduce global warming. This study also, aims at evaluating organoleptic changes during the optimized production process of *itugha*, thus giving understanding of critical control points for quality standards and safety.

2. Materials and Methods

Dehulled 120g fresh *Irvingia* seeds was milled into a fine paste and stored away for six (6) days. A second sample was subjected to 45 minutes size reduction daily for six days. On the seventh (7th) day heat treatment was applied to both samples for 6hrs. Sensory analysis, pH, temperature, titratable acidity, organic acids and presumptive micro-organisms, were determined on daily basis for both samples. For sensory analysis, the 9-point Hedonic Scale [10] for acceptance and preference tests was used. Organoleptic changes including texture, aroma and taste were also monitored during the production process. In assessing the role of identified micro-organisms, three samples were used. These included the fresh seed, and the two samples of milled products. Enumeration of aerobic heterotrophic bacteria was by Method [11] and enumeration of aerobic heterotrophic fungi was by method [12]. Bacterial isolates characterization was by Method [13] and fungi screening by an Identification Scheme [14].

Organic acid content determination in *Irvingia* seed and the ferment (*itugha*), was by Gas chromatography–Mass Spectrometry, [15] with some modification. 1g of sample was pulverized with 1ml of distilled water, acidified with 1ml 1M HCl to pH 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 0.100ml of BSTFA-TMCS at 65°C for 10 minutes, diluted with 0.400ml of hexane/ethyl acetate (50% v/v) and 1 µL was injected into the GC-MS and analyzed. Gas chromatographic- mass spectral and data analysis was on Carlo Erba gas chromatograph 5160 Mega Series, equipped with a Shimadzu data Processor C-R3A: Sample was analyzed by GC-MS by injecting 1 µL of the sample in splitless 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°C. The carrier gas was hydrogen, with a flow-rate of 1ml/min. The GC oven was held at 90°C for 4min, then raised at 8°C/min. The peaks were identified by reference to a mass spectral library. In determining titratable acidity, 10g sample was added into 100ml of distilled water from which 10ml was titrated with standardized 0.1M NaOH using phenolphthalein indicator. Titre values were recorded and used to calculate the per cent titratable acidity [16]. Daily pH recording was carried out using a Phillip digital meter (Dye Unicamp pHL 442 K London, UK). Buffer tablets (BDH Chemicals) were used to prepare buffer solutions as specified by [17]. The buffer solution was dispensed into a 50ml beaker and used to set the

pH meter at pH 6.5- 7.0 before inserting the electrodes into a solution of the sample for pH measurement. The resetting of pH meter to pH 6.8- 7.0 was repeated every day before taking the pH values and temperatures were taken alongside.

Organoleptic scores for taste, texture, flavor, appearances and overall acceptability were evaluated. Two sensory evaluations were conducted, namely; acceptance and preference tests. Two tasting panels consisting of untrained sensory assessors were employed. Choice of panelist was determined mainly by their ability to verbalise and communicate perceptions. The assessors described their

opinions by answering the questions in a proforma. For paired comparison test, panelists were asked if there was a difference between the two samples of itugha obtained during optimization process and traditional methods. Responses were analysed in terms of statistical significance levels calculated using fixed levels of significance i.e 5%. A 9-point hedonic scale was designed to measure liking in terms of acceptability of the product. [18, 10].

3. Results

Table 1. Microbiological Identification and Prevailing Conditions of Treatment Materials.

Day	PH	Temp (°C)	Acidity	Microorganism
1	7.0 ± 2.6	36	0.5 ± 1.50	Micrococcus, streptococcus
2	6.4 ± 1.1	36	1.4 ± 0.40	Micrococcus, streptococcus, bacillus
3	6.0 ± 1.7	36	1.8 ± 0.10	-do-
4	5.6 ± 1.4	36	2.8 ± 0.11	Micrococcus, streptococcus, bacillus, Candida tropicalis, DMB 321
5	5.1 ± 2.2	36	4.4 ± 0.30	-do-
6	4.7 ± 1.1	36	5.0 ± 0.21	-do-
7	4.5 ± 1.1	70	5.4 ± 0.11	Micrococcus, streptococcus

Mean ± SEM, (n=3)

Table 2. Role of Micro-organisms on organoleptic Attributes.

Days	Predominant Microorganism	Viable Count (cfu/ml)	Temp (°C)	Organoleptic Changes	
				Texture	Aroma
1	Bacteria	Too numerous to count	36	Drawy mash	No smell
2	Bacteria	Too numerous to count	36	Drawy mash	No smell
3	Bacteria	>300	36	Drawy mash	No smell
4	Yeast	87	36	Drawy mash	No smell
5	Bacteria	>300	36	Drawiness reduced	Alcohol smell
6	Yeast	260	36	Drawiness ceases	Alcoholic smell persist
7	Yeast	300	36	Drawiness ceases	Alcoholic smell persist
8	Bacteria	100	70	Spreadable & oily	Spicy aroma developed

Table 3. Organic acid content of seeds and Itugha ferment.

Organic Acid	Sample (% DM)	
	Irvingia Seed	Ferment
Citric Acid	16.00 ± 1.13	2.40 ± 1.10
Glycolic Acid	1.26 ± 0.01	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

Mean ± SEM, (n=3)

Table 4. Sensory Evaluation of Itugha.

Rating	Sensory Attributes				Overall Acceptability
	Taste	Flavour	Texture	Odour	
Like Extremely	8.0 ± 0.1	7.1±3.0	9.1±0.1	7.7±1.4	7.5±0.8
Like very Much	7.9 ±1.1	7.8 ±4.1	8.4 ±1.4	6.8 ±1.7	8.8 ±1.2
Like Moderately	8.3 ±2.0	7.0 ±1.3	7.6 ±2.0	9.1 ±4.0	9.3 ±1.1
Like Slightly	8.1 ± 1.1	6.9 ±2.3	7.7 ± 1.3	7.4 ± 2.1	7.1 ± 1.3
Neither Like nor Dislike	8.4 ± 0.1	7.3 ±1.1	8.1 ± 1.1	7.9 ±1.3	6.0 ± 1.0
Dislike Slightly	-	-	-	-	-
Dislike Moderately	-	-	-	-	-
Dislike very much	-	-	-	-	-
Dislike Extremely	-	-	-	-	-

In all determinations, the number (n) of assessors =96, Present ability =7.0±1.1, Ranking =72.6±0.4.

4. Discussion

4.1. Itugha Production and Component Unit Operation

Each unit operation required its unique working conditions in order to obtain the desired product quality. At each stage product quality was measured by the organoleptic evaluation of taste, aroma and flavor. Parameters optimized included cleaning and drying, size reduction, Mixing and Forming, Fermentation and Heat Treatment.

4.1.1. Cleaning and Drying

Dehulled fresh Irvingia seeds were cleaned using washed but dried hands. Each time hands became dirty, they were re-washed throughout the period of cleansing. To reduce the level of contamination with extraneous materials, fresh irvingia fruits were washed and allowed to drain water sufficiently before splitting using clean surfaces and sharp knives, to obtain the cotyledons. Before being subjected to the next unit process, seed cotyledons were de-hulled.

4.1.2. Size Reduction

In this operation, average size of the cotyledons were reduced to particles by pounding in cleaned mortars which involved application of compression or impact force such as is the case in comminution. Moisture content significantly ($p < 0.05$) affects both the degree of size reduction and mechanism of seed components breakdown. Therefore, the working environment as a matter of fact must be dry. Repeated pounding over 5 days resulted in disruption of cells, increased surface area to promote oxidative deterioration and higher rate of microbiological and enzyme activity. Finally, a fine consistent paste obtained on the sixth (6th) day of repeated pounding/milling gave the desired product (Itugha).

4.1.3. Mixing and Forming

The repeated pounding on its own is a unit operation in which, uniform mixture of all the components was obtained by dispersing one or the other within the paste. Secondly, the paste obtained gave a dough-like texture which allowed itugha to be moulded into desired shape and size.

4.1.4. Fermentation

In food fermentation, the controlled action of selected micro-organisms is used to alter the texture and preserve foods by production of acids or alcohol either in liquid-submerged state or solid-state. Since the paste/mash obtained from milling *irvingia* seeds are stored away in closed container, the fermentation occurs in the absence of air (anaerobic) and is solid-state fermentation. According to [19], fermentation can also result in the oxidation of carbohydrates in the absence of atmospheric oxygen, a process that yields alcohol as an end product. In the first

three days of preparation of itugha, the mash was odourless and tasteless, the micro-flora predominantly bacterial and by the fourth day, alcoholic aroma became very prominent, indicating yield of alcohol. Appearance and growth of yeast identified as *Candida tropicalis* DMB 321 yeast in the fermenting substrate was accompanied by reduction in sliminess of mash, which eventually ceased by the sixth day invariably classifies this solid-state fermentation, an alcohol fermentation by *Candida tropicalis* DMD 321. All through the fermentation process and within the production period, temperature was always 30°C. Traditionally, native gourds (*lagenaria sicereria*) made ideal fermentation vessels and *ansumsum* leaves (*Piper umbellatum* leaves) made suitable wrappers [3]. Knowledge of moisture content of mash and relative humidity of container indicated that, the use of a clean, dry sealable fermentation vessel that does not regurgitate water/ sweat was the required to store Itugha.

4.1.5. Heat Treatment

Heat has important influence in food processing in many respects. It is the most convenient way of extending the shelf-life of foods by destroying enzymes and microbiological activity or removing water to inhibit deterioration. In this process, irvingia biomass sample was subjected to repeated size reduction, daily for six days and on the seventh (7th) day; the mash (now without gum) was molded into desired shape and sizes. Drying the product was done using oven set at 70°C or built-in fire place with provision for slight heating under a plate or mud surface makes it possibility for autolysis and subsequent release of microbial enzymes. Autolysis consists of plasmolysis followed by proteolysis. Half of yeast cells dry matter consists of protein which gets degraded by microbial proteases to autolysates [20]. Plasmolysis in the case of itugha production was initiated by hot air treatments and since each autolysate has its characteristic taste and odour [21], *itugha* flavor, synthesized by fermenting micro-flora was absent in the samples that failed to produce the desired products. Application of heat elicited spicy aroma, browning and oil drip after about 12 hours of low temperature heating. Therefore, the unit operations described above are linked together to form the integrated processes necessary for itugha production.

4.2. Itugha Microbiology

Numerous fermented foods owe their production and characteristics to the activity of micro-organisms. These micro-flora acts in various combinations to cause biochemical and/or chemical changes in the food systems. Fresh *irvingia gabonensis* seed surface swabbed contain *streptococcus spp* and *micrococcus spp*. Its deep seed tissues have been found to have *bacillus spp*, *streptococcus spp* and *micrococcus spp* [9]. Therefore, specific micro-organisms associated with itugha production are bacteria: *Streptococcus spp*, *Micrococcus spp*, *Bacillus spp* and fungi: *Candida tropicalis* DMB 321. The first three days of itugha

production is dominated with bacteria spp namely *Micrococcus spp*, *Streptococcus spp* and *Bacillus spp*. *Candida tropicalis* (yeast) appears on the substrate on the fourth day. *Streptococcus spp* associated with *irvingia gabonensis* seed fermentation poses a yeast-like alcohol dehydrogenase implicated by the presence of alcoholic aroma on the 3rd day of production. Within the 2-6 days of itugha production, pH decreased and titrateable acidity increased. This change in pH and titrateable acidity values is attributed to microbiological activities. For instance, *bacilli* bacteria are known to utilize both carbohydrates and protein to produce acids [22]. Yeast is an enzyme and co-enzyme source susceptible to autolysis at higher temperature [23] as each yeast strain has its own characteristic flavor before and after autolysis. Working conditions i.e. temperature below 45°C and pH 4.5-7.0 help keep yeast cells alive thereby not losing valuable constituents or enzyme activity. It takes seven (7) days to complete the fermentation of *irvingia* seeds in itugha production.

4.3. Fermentation In-Process Optimization

Fermentation, a metabolic process that converts starch, sugars to alcohol or acids and gases, is broadly, referred to as bulk growth of micro-organisms (bacteria and yeast) on a growth medium [24]. Optimization procedure therefore, involves small number of experiments under varied conditions and accumulating information about the process. In fermentation process optimization, different combinations and sequence of processes' conditions are monitored during experimentation to establish growth conditions that produce desired itugha product. Pulverizing *irvingia* seeds in one day and storing it away in a fermenting vessel, and repeated pounding of exposed *irvingia* biomass does not give itugha by the seventh day. The equipment required for pulverization is that which reduces size particles to a degree of fineness termed medium fine to very fine [25]. On the other hand repeated pounding apart, from being a size reduction operation also serves a mixing function. The considerable number of variables that operates during itugha processing, makes it almost impossible to predict precisely the effect of a particular processing unit operation on a nutrient component of the resulting food product. However, proper and adequate amount of mechanical work is required to obtain the porous mash and the cohesive force between particles required for mixing and forming. A product that meets conformity assessment is obtained under the listed conditions in table 1.

Isolation of micro-organisms meant to determine their identity and population size, indicates that during the first 3 days bacteria only were present in the mash and fermentation beyond 4 days increased the acidity of the biomass from 0.5% to 5.4% on the 7th day. The acids which could be produced from non-total oxidation of sugars, as well as the deamination of amino acids, ascorbic acid and polyphenol acids presumably gave the prevailing pH and possibly the precursor for flavour and aroma. This fermentation process is presumably initiated by bacterial activity because, both seed surface swab and deep tissue micro-flora were entirely

bacterial with candida appearing only on the fourth day. Furthermore, end-product of secondary metabolites of bacteria during fermentation could have provided the enabling environment for fungi growth. Yeast was identified on the 4th day. Appearance and continuous increase in yeast growth implies, acidic environment due to fermentation, promoted its growth. However, based on the micro-flora, possible microbial enzymes involved in *irvingia* fermentation might be both amylolytic and lactolytic enzymes [26].

Candida tropicalis DBM321, identified in *irvingia gabonensis* ferment is a food yeast, known to provide protein and impart flavor. It also has high ability for the utilization of high carbohydrate sources (substrates) for their growth [27]. Therefore, they could produce several carbohydrases including amylases, isomerases, alpha-glucose amylases as microbial enzymes [28]. The loss of elastomeric property of *irvingia* mass is indicative of the presence of hemi-cellulase which reduces the viscosity of several plant gums by conversion of D-xylans to xylo-oligosaccharides, D-xylose and L-arabinose. Repeated pounding as employed in the processing of *irvingia gabonensis* seeds into itugha, makes for the secondary metabolites to be available for microbial activity. It is important to also note that in itugha production the fermenting vessel remains closed during storage throughout its production period, implying none release of carbon dioxide for a period and when pounding is done in exposed condition this, allows for free interaction of the biomass constituents with atmospheric air.

4.4. Product and Process Characterization

In *irvingia gabonensis* biomass components degradation, natural microbial strains are involved in enzymic hydrolysis of the mash, in solid-state fermentation production process. The process requires bacteria, fungi, enzyme, 2.1±0.8% moisture and solid matrix in basic mineral medium containing 55.0±0.2% manganese, 14.28±0.8% zinc, 9.48±0% iron, 3.40±0.5% phosphorus and 12.48±0.2% vanadium to give a product with high rating as reported in previous work [29]. The operating system is functional within pH 4.5-6.4 and medium acidity 5.4-1.4%. Milling fresh *irvingia* seeds into a fine consistency in one day and keeping in a fermentation vessel for six days does not give the desired product. This suggests that repeated milling/pounding under specified conditions is a necessity for itugha production process. Thus, the shearing stresses coupled with stress relaxation time scale of minutes in pulverization to days in storage, defined conditions for measurable rheological properties.

The rheological property relevant for this product is consistency, particle size and extensibility. As in semi-moist foods, the disruption of cells allowed enzymes and substrates to become more intimately mixed thereby accelerating the availability of cellular materials (suitable substrates) for microbial growth, biochemical reactions and enzyme activity which might have caused: (a) loss of elastomeric property of the mash. (b) development of aroma. Development of flavours followed the patterns in confectionery industry

where materials are made tastier by being reduced to a small size. [30]. Pungency represents an important component of itugha flavor when dried at 70°C for 30-40 minutes, as smoothness confers spread ability because of good particle size and consistency.

4.5. Progress of Flavour Development

The development of alcoholic aroma was accompanied by

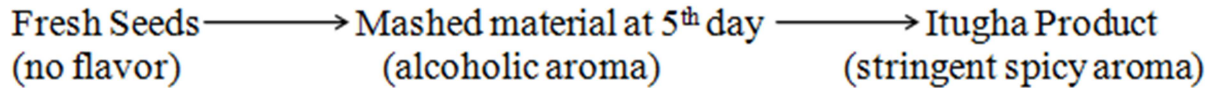


Figure 1. Progressive flavor development during Itugha production.

Within 2-6 days of processing itugha, pH decreased, while titratable acidity values increased (table 1). This change in acidity of extract is again attributed to microbiological activities. *Bacilli* bacteria are known to utilize both carbohydrates and proteins to produce acids and dextrinization as well as lipogenesis also increase acidity [22]. Five organic acids found in *irvingia* seeds are citric acid, glycolic acid, oxalic acid, malic acid and tartaric acid. The presence of alcoholic aroma on the 5th day of processing itugha and the appearance of *Candida tropicalis* DBM321 on the 4th day, is suggestive that streptococcus spp associated with *irvingia* fermentation poses a yeast-like alcohol dehydrogenase. Organic acids influence pH that determines microbial growth. They also serve as preservatives and influence formation, type and rate of thermally produced flavor [32]. Therefore, the seed microbiology, identified organic acids and progression of flavor development opens up a research need to establish specific enzymes, identification of gum biomolecules and biochemical pathways involved in this itugha production process. The five organic acids having been identified and quantified in the seed and the ferment, indicates that citric acid is the most abundant and that all organic acids recorded varying degrees of decreases (table iii) implying catabolism of organic acids during solid-state-fermentation. Citric acid pleasant taste, high solubility and flavour-enhancing properties has been reported [33], thus its implication in flavour and aroma development.

Optimization of this fermentation process would eventually lead to Itugha product development and commercialization. This would promote development of indigenous technologies required for innovation and creativity necessary for revolutionary adoption of nutritious home grown products that are fast getting extinct. *Irvingia* seed is tasteless and odourless, rheological property relevant for itugha production was consistency, particle size and

size reduction and subsequent disappearance of the elastomeric property of *irvingia gabonensis* mash. Bacterial growth suppression and primary metabolism trigger biosynthesis of secondary metabolites in cell cultures [31]. Therefore, it is quite possible that gradual yeast growth slowed respiratory rate of *irvingia* seed bacterial culture while heat during drying, facilitated flavor biosynthesis.

extensibility. Therefore *itugha* production involved a combination of processes carried out in sequence to achieve the intended changes to the raw material. This Optimization study has defined three stages in the production of itugha by indicating in each, the required pH, acidity, temperature, micro-organisms and sequence of flavor development (see flow chart). Sensory evaluation involved taking measurements based on sight, smell, mouth feel and flavor. A consumer panel was also used. Itugha was highly appreciated as its overall impression and acceptability were significant. Pungency which represents an important attribute of flavor in many respects was considered in the appreciation of this foodstuff. Pungency of itugha was given optimum attention when considering the sensory basis of its acceptability. The intensity and quality of pungency increased with increase in temperature. The carrier used was boiled yam, as the food item is usually taken with highly carbohydrate staple. The measurement hierarchy used was nominal and scale used in testing, is the category scaling i.e. hedonic scale. Since the situation was optimizing the production process, the panelist was also to confirm any overall differences between products from optimization process and traditional process. For the Sensory analysis, 9-point Hedonic Scale was used. Overall acceptability for Like Extremely=7.5; Like very much=8.8; Like moderately=9.00. Overall ranking was 72. Population t-test analysis, t-value was 21.18 and F-value 12.25. Progression of flavor development showed, no flavour in the early stage, alcoholic aroma by the 3rd day (intermediate stage) and stringent spicy aroma by 6th day which became prominent after application of heat (final stage). Optimization gave the desired sensory attributes at the prevailing conditions that impacted on product quality. Sensory evaluation indicated significant overall acceptability of the product, therefore commercialization of itugha production process is an indigenous technology awaiting industrial uptake.

Itugha Production Process Flow Chart Showing Mechanical Activities, Microbes and Stages of Production Processes.

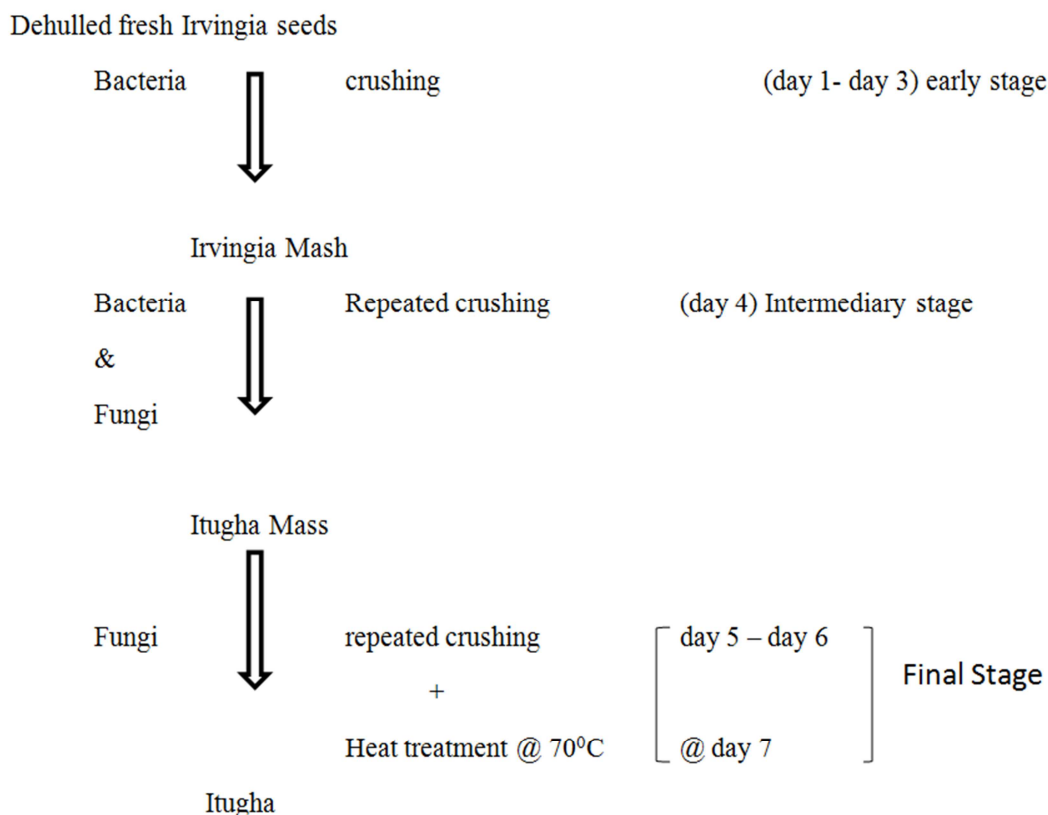


Figure 2. Optimization process flow chart for the production of Itugha.

5. Conclusion

The early stage of fermentation was initiated by bacteria. Since *streptococcus* and *micrococcus spp* are intrinsic to the *Irvingia* seed 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 would have initiated the fermentation (Table 2). Thus the early stage of fermentation was caused by *Bacillus spp* at pH 6-7, Temperature 30°–35°C (Ambient) and 1.8% acidity of extract.

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, and pH 5.6, Temp 35°C and 4.4% acidity of extract. Late and final stage micro-organism was *Candida tropicalis* DMB 321 at 70°C and 5.4% acidity of extract.

The sequence of unit operations determined the quality of product. For instance, obtaining an appropriate texture on the first day and keeping it on a fermentation vessel for 7 days did not give the required product, even though there was loss of elastomeric property of the *irvingia* mash. The implication could be that, repeated size reduction process (pounding) was crucial for itugha quality. Disruption of cell tissues allowed

enzymes and substrates to become more intimately mixed which accelerated the availability of cellular materials necessary for microbial growth. The microbial enzyme activities then resulted in loss of elastomeric property and development of aroma. In the case of aroma development, the compound produced must be volatile enough to reach the olfactory epithelium and stimulate odour receptors [34, 35]. Therefore, there was the need for heat application to elicit the aroma production which is one attribute that informs *itugha* consumer acceptability.

There was also need for controlled fermentation because, samples of the mash exposed unnecessarily to air did not give itugha product. Unfermented fresh *irvingia gabonensis* seed is odourless while itugha has a highly appreciable odour and flavor. Consequently, it appears that the flavor was synthesized by fermenting micro-flora, as unfermented product did not produce flavour. It is quite possible that gradual yeast growth suppressed respiratory rate of the *irvingia* seed bacterial cultures activity and triggered biosynthesis of secondary metabolites from primary metabolites [31], which defined some operating biochemical pathways. Another possibility is that *Candida tropicalis* may have oxidized the organic acids to carbon (IV) oxide [CO₂] [36].

This optimization technique used in *Itugha* production and

the number and parameters analysed were not fixed. Parameters were selected during in-process monitoring of the production process and fermentation, thus, making it an open-ended system. However, two samples were used to compare the outcome of one type of fermenting medium under different parameters in the first instance. Hereafter a closed-ended system shall be used to further the research. In-process *Itugha* production optimization method in-process is a combination of borrowing and component replacement techniques [37, 38, 39], and parameters that were finally critical in sensory evaluation and by extension quality of the product were microbes, pH, temperature, fermenting medium, acidity, texture and aroma.

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