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Physico-Chemical and Sensory Characteristics of Fermented *Manihot esculenta Crantz* and *Manihot glaziovii Leaves (Ntoba mbodi)* in Congo, Brazzaville

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Abstract:- The objective of this study is to characterize and optimize the flavor of *Ntoba mbodi* in order to contribute to the basic knowledge needed to master the sensory properties of the product. In 2018, a study was conducted on 2-week to 3-month-old fermented leaves of *Manihot esculenta Crantz* and *Manihot glaziovii* (*Ntoba mbodi*) cassava. We determined protein content, titratable acidity, water content, ash content and hydrogen potential with 50 samples after further work. The sensory evaluation was carried out by a panel of 10 assessors for the tasting of *Ntoba mbodi*. On 3 samples (E1, E2 and E3), 3 parameters were determined namely : color, bitter taste and flavour. The physicochemical analysis showed that the protein content varies between 17 and 24 %, the titratable acidity varies between 0.75 and 4 g/L and the hydrogen potential between 6 and 8. The sensory evaluation showed that the averages of the treatments of the color varied between 2.0 and 3.5 of the bitter taste between 2.2 and 2.7 and of the flavor 3. The results by category of the hedonic test show that the dark green color of E1 is different from E2 and E3 and that there is no significant difference between the three samples on the other 2 aspects, P< 0.05. All in all, Ntoba mbodi is an alkaline pH fermented food containing beneficial lactic acid bacteria for the digestive tract.

Keywords: *Titratable Acidity, Ph, Proteins, Chikwangue, Cassava, Lactic Acid Bacteria.*

I. INTRODUCTION

Cassava leaves, which are widely used as vegetables, provide protein, vitamins, and minerals to the population. *Ntoba mbodi* is a fermented food made from cassava leaves in various packaging (papaya leaves, plastic sheeting, etc.). It is produced in the Congo and northwestern Angola. It is a customary product highly prized for its particular natural taste and aroma. The rate of proteins (17 and 34 %) and the presence of probiotics contained in this foodstuff make *Ntoba-mbodi* a very interesting and beneficial food for the body. The fermentation of cassava leaves being spontaneous, cannot be controlled, one can imagine the diversity of the final products by their organoleptic characteristics and also the sanitary risks that this can represent.

Indeed, during the fermentation, the total microflora increases ; on the other hand, the population of lactic bacteria stops growing after 24 hours of fermentation and decreases thereafter. The proportion of lactic flora to total flora decreases from 65% to 4%, during fermentation while that of non-lactic flora increases from 34.97 % to 95.92 % [1]. The variations in the intensity of the attributes that constitute the flavor of *Ntoba mbodi* can be explained mainly by the fluctuation, by the variations of its volatile composition and, to a lesser extent, by the variations of its non-volatile components, such as acidity and protein content and sensory attributes.

Sensory evaluation as a tool for daily progress is a way to highlight particularities, similarities and differences. But it is necessary to form a sensory evaluation group. Flavor is the quality that is perceived by the sense of taste. Natural flavors are mostly of plant origin.

Volatile molecules can reach the olfactory epithelium by direct route.

Moreover, the flavor corresponds to the combination of at least 3 sensory systems : olfactory, gustatory and tactile, to which it is sometimes necessary to add the thermal system [2]. Also, the choice of methods is made according to the objectives. From the hedonic test, which is linked to pleasure, the tasters chose between the three samples (E1, E2 and E3). They judged according to the levels that were converted into numbers for the analysis of variance for the hedonic test in the document entitled Senso Bab [3]. But knowing how to use sensory evaluation, allows to valorize

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products under official quality signs. Cassava leaves contain hydrocyanic acid (HCN), a powerful poison for the body, highly volatile and soluble in water. But the fermentation of cassava leaves eliminates it considerably [4, 5, 6, 7]. Further research will lead to these goals.

II. MATERIALS AND METHODS

➢ *Biological Materials*

The biological material is fermented cassava leaves or *Ntoba mbodi*.

The leaves of *Manihot esculenta Crantz* and *Manihot glaziovii* commonly called rubber, aged from 2 weeks to 3 months.

➢ *Methods*

- *Titratable Acidity (AOAC, 1975)*

This is an analysis to determine the acids contained in a product. This method is carried out by neutralizing the total acidity with a soda solution (0.1 N).

We prepared a solution of soda to 0,1 N ; we pour 30 milliliters of the distillate (5 g of product + 25 mL of distilled water) contained in a beaker in an erlen meyer; we added then 3 to 4 drops of phenolphthalein in the solution (distillate); then, the soda solution contained in the burette is made to flow drop by drop into the meyer erlen while stirring until the appearance of a purple tint and the reading of the volume of the turn is made on the burette.

Titratable acidity (g/L of H2SO4) = number of moles of soda ((NaoH) × 0.4

- *The pH*

The pH is determined by using the buffer solution, in a beaker, we mixed 5 grams (g) of fermented cassava leaves or Ntoba mbodi with 20 milliliters (mL) of distilled water, then we stirred to homogenize. Next, we prepared a pH 4 buffer solution at a temperature of 25 °C with 40 milliliters of distilled water, where a digital pH meter is then plunged and the buffer pH values ranging from 3.7 to 4 are obtained. Once the pH meter is immersed in the filtrate, a few seconds later, it shows the pH value of the product. The same operation is repeated with the rest of the samples.

- *Protein Content (AOAC, 1975)*

The protein content was determined after determination of total nitrogen according to the Kjeldahl method (VELP SCIENTIFICA DK 6) after sulfuric mineralization in the presence of selenium catalyst. The nitrogen content value obtained was multiplied by 6.25 to quantify the protein content.

➢ *Sensory Analysis*

The tasting of the Ntoba mbodi by the panelists or tasters capable, that is to say initiated, each one had samples of cooked Ntoba mbodi to taste in order to detect the organoleptic characteristics contained in the fermented leaves of cassava.

We carried out the hedonic test with a 5-level category scale with a panel of 10 subjects aged between 17 and 25 years (students). The aim is to measure the appreciation of the products. The tasters will choose for each sample the category that corresponds to their appreciation after having detected the organoleptic characteristics contained in the fermented cassava leaves.

➢ *Data Analysis*

At the end of the data analysis, the categories are converted into numerical ratings, which allowed us to have the results of the hedonic test of a scale ranging from 1 to 5, where 1 corresponds to "do not like at all" and 5 "like a lot". The ratings of each sample are presented in tables, results by category and calculated using the Analysis of Variance (ANOVA) for the hedonic test to determine if there are significant differences in the average degree of appreciation between samples with a probability less than or equal to 0.05. With the hedonic test, categories are assigned to specific levels (numbers). The tables are drawn up after calculating the averages of the treatments.

III. RESULTS AND INTERPRETATION

The physicochemical characterization of *Ntoba mbodi* on titratable acidity, protein content and pH is represented in the following tables:

Table 1 Titratable Acidity of Fermented Leaves of *Manihot esculenta Crantz* and *Manihot glaziovii* or *Ntoba mbodi*

Samples	Titratable Acidity (g/L)	
	Manihot esculenta Crantz	***Manihot glaziovii***
ECH $_1$	3,74	0,75
ECH $_2$	2,91	3,72
ECH $_3$	2,20	4,16
ECH $_4$	2,71	1,03
ECH $_5$	3,4	2,45
ECH $_6$	2,91	0,77
ECH 7	2,20	3,42
ECH $_8$	2,71	4,10
ECH $_9$	4	1,08

ECH $_{10}$	2,91	2,40
ECH $_{11}$	2,21	0,76
ECH $_{12}$	3,31	3,73
ECH $_{13}$	3,74	4,14
ECH $_{14}$	2,91	1,11
ECH $_{15}$	2,22	2,44
ECH $_{16}$	2,71	0,79
ECH $_{17}$	3,74	3,72
ECH $_{18}$	2,92	4,15
ECH $_{19}$	2,23	1,05
ECH $_{20}$	2,71	2,43
ECH $_{21}$	3,71	0,76
ECH $_{22}$	2,91	3,74
ECH $_{23}$	2,20	4,16
ECH $_{24}$	2,74	1,03
ECH $_{25}$	4	2,45

From Table 1, the titratable acidity of fermented leaves of *Manihot esculenta Crantz* ranges from 2.20 to 4 g/L.

Sample 3 (fermented leaves of *Manihot glaziovii* with 6 % salt) has the highest titratable acidity 4.16 g/L.

The titratable acidity of two leaf varieties ranges from 0.75 to 4.16 g/L.

Table 2 Protein Content of Fermented Leaves of *Manihot esculenta Crantz* and *Manihot glaziovii* or *Ntoba mbodi*

Samples	**Protein Content (%)**	
	Manihot esculenta Crantz	***Manihot glaziovii***
ECH $_{1}$	19	18
ECH $_{2}$	20	22
ECH $_{3}$	21	24
ECH $_{4}$	21	18
ECH $_{5}$	17	23
ECH $_{6}$	20	19
ECH 7	19	22
ECH $_{8}$	20	23
ECH $_{9}$	19	18
ECH $_{10}$	17	23
ECH $_{11}$	18	19
ECH $_{12}$	19	21
ECH $_{13}$	20	23
ECH $_{14}$	21	19
ECH $_{15}$	18	22
ECH $_{16}$	18	19
ECH $_{17}$	21	21
ECH $_{18}$	20	22
ECH $_{19}$	20	19
ECH $_{20}$	19	21
ECH $_{21}$	18	21
ECH $_{22}$	21	21
ECH $_{23}$	20	23
ECH $_{24}$	20	19
ECH $_{25}$	19	22

The protein content of fermented leaves of *Manihot esculenta Crantz* (*Ntoba mbodi*) ranges from 17 to 21 %. The highest protein content is 21 %, that of samples 3, 4, 14 and 22. While, that of *Manihot glaziovii* (*Ntoba mbodi*) is between 18 and 24 %. The highest protein content, 24 %, with sample 3 fermented leaves of *Manihot glaziovii* with 6 % salt.

ISSN No:-2456-2165

Table 3 pH of Fermented Leaves of *Manihot Esculenta Crantz* and *Manihot Glaziovii* or *Ntoba mbodi*

Samples	pH	
	Manihot esculenta Crantz	***Manihot glaziovii***
ECH $_1$	7,1	6,5
ECH $_2$	7,1	7,0
ECH $_3$	7,1	7,0
ECH $_4$	7,1	7,2
ECH $_5$	7,6	7,5
ECH $_6$	7,1	6,6
ECH 7	7,1	7,1
ECH $_8$	7,2	7,1
ECH $_9$	7,1	7,1
ECH $_{10}$	7,5	7,6
ECH $_{11}$	7,1	6,5
ECH $_{12}$	7,1	7,0
ECH $_{13}$	7,2	7,0
ECH $_{14}$	7,2	7,2
ECH $_{15}$	7,2	7,3
ECH $_{16}$	7,1	6,6
ECH $_{17}$	7,1	7,3
ECH $_{18}$	7,2	7,2
ECH $_{19}$	7,1	7,2
ECH $_{20}$	7,5	7,4
ECH $_{21}$	7,1	6,6
ECH $_{22}$	7,1	7,2
ECH $_{23}$	7,1	7,0
ECH $_{24}$	7,2	7,1
ECH $_{25}$	7,5	7,4

The hydrogen potential (pH) of *Manihot esculenta Crantz* leaves ranges from 7.1 to 7.6.

From Table 3, the pH of the fermented leaves of *Manihot glaziovii* varies between 6.5 and 7.5. We note that only sample 1 has a slightly acidic pH 6.5 but close to neutrality; on the other hand, the other samples have a pH value of 7 and tend towards alkalinity.

Thus, the salt used during the fermentation of cassava leaves did not have too much influence on the pH change.

Table 4 Results by Category (Dark Green)

Tasters	E1	E2	E3	Total Tasters	Taster Averages
1	2	5	3	10	3,33
2	2	4	2	8	2,66
3	3	2	2	7	2,33
4	1	3	3	7	2,33
5	4	2	5	11	3,66
6	1	3	3	7	2,33
7	2	4	2	8	2,66
8	3	5	3	11	3,66
9	1	2	4	7	2,33
10	1	5	5	11	3,66
Total treatment	**20**	**35**	**32**	-	-
Big total	-	-	-	**87**	-
Average treatment	**2,0**	**3,5**	**3,2**	-	-

According to Table 4, the average value of the dark green color, is between 2 and 3.5 on a scale ranging from 1 to 5 levels, of the dark green color aspect; for the control sample (E1) less dark than the other two samples.

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Table 5 Results by Category (Bitter Taste)

Tasters	E1	E2	E3	Total Tasters	Taster Averages
1	3	2	4	7	2,33
2	2	2	4	8	2,66
3	1	1	2	4	1,33
4	2	1	4	7	2,33
5	5	4	3	12	4
6	2	3	1	6	2
7	2	2	2	6	2
8	4	2	2	8	2,66
9	1	3	3	7	2,33
10	2	2	2	6	2
Total treatment	**24**	**22**	**27**	**-**	**-**
Big total	**-**	**-**	**-**	**71**	**-**
Average treatment	**2,4**	**2,2**	**2,7**	**-**	**-**

According to Table 5, the results obtained from the tasting of the three (03) samples show that the average bitter taste is between 2.2 and 2.7; the products are less bitter.

Table 6 Results by Category (Flavour)

Dégustateurs	E1	E2	E3	Total Des Dégustateurs	Moyennes De Dégustateurs
1	4	2	3	9	3
2	3	3	2	8	2,66
3	2	4	4	10	3,33
4	2	4	3	9	3
5	4	3	4	11	3,66
6	2	4	4	10	3,33
7	4	3	4	11	3,66
8	4	2	2	8	2,66
9	3	4	3	10	3,33
10	3	2	4	9	3
Total treatment	**31**	**31**	**33**	**-**	**-**
Big total	**-**	**-**	**-**	**95**	**-**
Average treatment	**3,1**	**3,1**	**3,3**	**-**	**-**

According to this table, the results obtained from the tasting of the three (03) samples show that the average flavor is between 3.1 and 3.3; the products were moderately appreciated.

IV. DISCUSSION

We conducted surveys among 50 women producers of *Ntoba mbodi* whose age varies between 40 and 60 years with a secondary or primary level of education, including illiterates.

The questions asked to the sure producers: The fermentation time of the fermented cassava leaves varied between 3 and 5 days, but some women gave reasons for the 5-day fermentation time.

Indeed, during the dry season, the duration of fermentation often reaches 5 days due to the coolness. On the other hand, during the rainy season, fermentation often lasts 4 days; it is the presence of heat that contributes to the softening of the cassava leaves and to the reduction of the fermentation time.

After 5 days of fermentation, the products are altered, as many authors have mentioned [8]. The types of packaging used for the sale of *Ntoba mbodi* are the leaves of *biloria or Lasimorpha* (*Sénegalesissis*), taros (*Colocasia esculenta*), a source of polyphenols and vitamin c two anti-oxidant substances that help reduce free radicals and prevent the aging process and the risk of disease, and the leaves of *Amaranthaceae* or plant leaves (often used in the packaging of chikwangue) that are used to sell the product (*Ntoba mbodi*). These leaves also play a protective role and extend the life of the product.

Among the different methods of production of *Ntoba mbodi* concerning 50 female producers surveyed, we have the fermentation in the leaves of papaya (*Carica papaya*), facilitate digestion, calm inflammation, protect the digestive system and treat gastric and duodenal insufficiency. Papaya leaves also contain papain, which is an enzyme used to cut hydrogen bonds.

The production of fermented cassava leaves or *Ntoba mbodi* includes: the softening or change in texture of the cassava leaves during fermentation.

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It is after four (4) days that there is an effective softening of the leaves.

Indeed, cassava leaves that underwent fermentation with the addition of salt in the proportions of 2 and 4 % and those of the control sample softened slightly after two days and were completely softened in four days. These results are similar to those found by Kobawila et al [9] who stated that after four days the cassava leaves were completely softened. For total softening after three days of fermentation with proportions of 6 and 8 % salt added in these samples resulted in *Ntoba mbodi* after three days. Thus, salt is a softening agent for cassava leaves. Therefore, it helps to reduce the fermentation time.

At the end of three days of fermentation there is the presence of a rather characteristic odor for the cassava leaves fermented with salt of proportion 6 and 8 % and a rather characteristic odor for the sample without salt or control.

The production of fermented cassava leaves or *Ntoba mbodi* (with two varieties of cassava leaves) reveals that salt facilitates the softening of cassava leaves, it slows down the smell and reduces the duration of fermentation to a some concentration.

Finally, the color change was considerable on the third day in the leaves fermented with 6 and 8 % salt and a remarkable total color change after four days of fermentation for the cassava leaves fermented with 2 and 4% salt and the control. The cassava leaves change color from green to dark green : this is the browning resulting from the oxidation of phenolic compounds under the action of the enzyme polyphenol oxidase [9]. This is an interesting phenomenon because it improves the sensory properties of the fermented product (*Ntoba mbodi*) [8]. The pH of the fermented cassava leaves is between 7 and 8, sample 1 (*Manihot glaziovii* leaves fermented with 2 % salt) has the lowest pH (6.5) and sample 25 (*Manihot esculenta Crantz* leaves fermented without salt or control sample) has the highest pH (7, 6). The pH of *Ntoba mbodi* tends towards alkalinity. This means that the salt used during fermentation does not influence the pH (hydrogen potential), hence the alkaline pH. These results are similar to those found by Kobawila et al [9] showing that the pH reaches the maximum value of 7.95 in 72 hours. Dhellot [1] mentioned that fermented cassava leaves are basic (8.5) at the end of fermentation. The production of Ntoba mbodi is accompanied by a significant alkanization pH 8.6 of the fermentation medium. This characteristic is found in other fermented products such as ugba (pH 8.6), ogiri (pH 7.9), natta (pH 8.4) and soumbala (pH 9.0) [9]. The alkalinity of the pH is an important factor in microbial competition : it determines the sequence of the microbial flora.

The titratable acidity varies between 0.75 and 4 g/L of H2SO4. Sample 3 has the highest titratable acidity, 4.16. On the other hand, sample 1 has the lowest acidity of 0.75. The protein content of the two varieties of fermented cassava leaves is between 17 and 24 %. It is a product, rich in protein, macronutrient or substance essential for the body, defense against infections, maintenance and manufacture of antibodies (15). The preparation of the panelists for the recognition of odors (aromas) and flavors of products in relation to age reveals that all panelists in the age range between 17 and 25 years were able to recognize what was contained in the samples; this age group did not experience difficulties regarding the tasting. Perhaps it is with this age group that tasting should be initiated. The first point to emphasize is the level of school education; the other points come after.

For example, Magnusson et al [10] have pointed out the effect of the level of education on the consumption of food products. Muller et al [11] studied the influence of the level of literacy when performing descriptive sensory tests. They observed that a high level of literacy reduces the training time. However, this factor does not influence the level of agreement between panelists.

The second point to emphasize is training. Indeed, the repeatability is better for flavors and flavor. Training also contributes to increase the level of repeatability according to Robert et al [12]. Another point to note is age: according to the results on panelist preparation by age, all assessors perceived almost the same thing. We find that the effect of age in tasting the products does not have too much influence, because most often these taste tests are practiced with adult subjects and not with children.

The results of Thomas-Danguin, Rouby et al [13] confirm, in the realization of several olfactory tests on 121 healthy elderly people, that they do not observe any effect of age or sex, during the supraliminal detection and discrimination tests.

On the other hand, Pineau [14] pointed out that age, gender, level of experience in sensory analysis and level of education are the four variables that characterize the panelist. The effect of sensory ability in particular: school education and training that may help the panelist to better perform the test.

The results of the tasting tests seem to be difficult with uninformed subjects.

The dark green color appearance perceived in samples E1 (2.0), E2 (3.4) and E3 (3.2) is significantly different, the color of sample E1 is slightly dark green different from that of E2 and E3 which are totally dark green. The bitter taste 2.4, 2.2 and 2.7 respectively of samples E1, E2 and E3 is not significantly different as well as the flavor E1 (3.1), E2 (3.1) and E3 (3.3) ; hence, the samples are not significantly different bitter taste and flavor. The flavour of the products was favourably appreciated but, with an average value per sample higher than 3, on a scale from 1 to 5.

The tasters have significantly preferred all varieties of fermented leaves.

ISSN No:-2456-2165

Thus, we can successfully ferment cassava leaves according to the above proposed production line.

V. CONCLUSION

All women producers, regardless of their level of education, are able to transform cassava leaves into Ntoba mbodi or fermented cassava leaves. From these surveys, we realized that experience in the production of Ntoba mbodi does not depend on intellectual level. However, they observe the fermentation of cassava leaves for a period of 3 to 5 days, exceeding this period, one witnesses the alteration of the product.

Control sample. The pH of the fermented cassava leaves varies between 7 and 8 and the titratable acidity is between 0.75g/L H2S04 and 4.16 g/L H2S04.

The protein content was between 17 % and 24 %.

The sensory evaluation allowed us to understand that all the panelists in the age group of 17 to 25 years did not experience difficulties in tasting and describing organoleptic characteristics.

In addition, the analysis of variance (ANOVA) for the hedonic test shows us that there is a significant difference between the means of the hedonic results for the three samples, in terms of color. According to the treatment average (results by category), the three samples are not significantly different from the point of view of bitter taste and flavour.

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