Identification And Characterization Of Compounds Responsible For Aromas In Fermented Cassava Leaves "Ntoba Mbodi"

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Summary

In Congo, "the ntoba mbodi" is produced by traditional manufacturing. This is a food constituted by fermented cassava leaves much appreciated by its taste and flavor. During the fermentation, the non-lactic flora, specialy *Bacillus*, supplants the lactic flora and the pH 7 at the beginning of the fermentation becomes alkaline (pH 8.5). The dynamics of this microbial combination explains the production of aromas responsible partly for the organoleptic quality of the "ntoba mbodi".

At the end of the fermentation, after the extraction of aromas the analysis reveals that most of the compounds are terpenes 87.5% of which the most abundant is limonene with a percentage of 68.5621% of the identified compounds.

Note that certain compounds have aromatic properties that retained the attention of the aromas industry, still others have interesting pharmacological properties.

Key words: leaves, cassava, aromas, fermentation

Introduction

The fermentation is a natural process in which the microorganisms presents (bacteria, yeasts, molds) on and / or in the raw materials transform them into feeding.

The man has mastered this natural process that applies mainly to the food where he has a double interest, first in food preservation by inhibiting the unwanted microbial flora with fermentations products (acids, alcohols) and also in food transformation by modifying the organoleptic properties (texture, taste, aroma ...)

More than 3,500 traditional fermented foods are counted in the world. In Africa, in Congo particularly at the family level or small-scale production units, it is prepared a food made from fermented cassava leaves (*Manihot esculenta Crantz*) called "ntoba mbodi" much appreciated by the locals populations.

The cassava leaves fermentation is alkaline (pH = 8.5); this is due to the action of microbial enzymes such as proteases, deaminases and decarboxylases that will generate amines. This pH alkalization explains the kinetics of the microbial population where non lactic flora, more particularly *Bacillus*, supplants the lactic flora.

The activity of pectinolytic enzymes produced by *Bacillus* such as *B. pumilus*, *B. subtilis*, *B. brevis*, *B. circulans* and *B. marcerans*, *Staphylococcus xylosus* and *Erwinia spp* is maximal after 48 hours of fermentation (Mokemiabeka et al. , 2011; Sanni et al, 2002) (Kobawila et al, 2003;.. Yao et al, 2009). This is translated, firstly, by cassava leaves softening and browning which mark the end of fermentation and, secondly, by the maximum output of organic acids (8 g / l for the lactic acid) in alkaline medium (Kobawila et al., 2003; Yao et al, 2009).

The softening corresponding to the rupture of the cell walls allows the enzymes to enter into contact with their substrates in other cell compartments. This is the case of the vegetal linamarase and polyphenol oxidase. Linamarase contributes to the detoxification of fermented cassava leaves (*Manihot esculenta Crantz*) by elimination of cyanogenic glucosides giving cyanohydrins, unstable under alkaline conditions. Indeed, the alkaline pH favors the spontaneous hydrolysis of cyanohydrins in acetone and hydrocyanic acid. Hydrogen cyanide can then solubilize or volatilize. The cassava leaves fermentation is a process that contributes to the elimination of cyanogenic glucosides in more than 70% (Louembe et al., 2003; Mokemiabeka and al., 2011).

As for the polyphenol oxidase by oxidation of phenolic compounds it leads to browning, which causes the color change of cassava leaves from green to dark green after fermentation.

At the end of fermentation, the 4th day, we note a very characteristic smell due to maximum production of aromas. Among the attributes of the organoleptic quality of the food, the aroma is one among key elements for the consumer choice from which the desire to undertake this qualitative and quantitative study of the production of aromas at the end of fermented cassava leaves fermentation.

Material and Methods

Plant Material

The " *ntoba mbodi* " was produced from cassava leaves (*Manihot esculenta Crantz*) aged 2 to 3 months. It comes from a homemade production workshop in the south of Brazzaville.

Traditional manufacturing of "*ntoba mbodi*"

The traditional manufacturing of "*ntoba mbodi* " is a process of several steps of which the most important is the fermentation phase that lasts 4 days. Farther, there occurs a significant alteration of " *ntoba mbodi* " (Mokemiabeka et al ., 2011).

As in most empirical processes, the fermentations take place under the action of endogenous microorganisms presents on the leaves and responsible of products, though valued of large organoleptic and hygienic variability (Louembe et al., 2003).

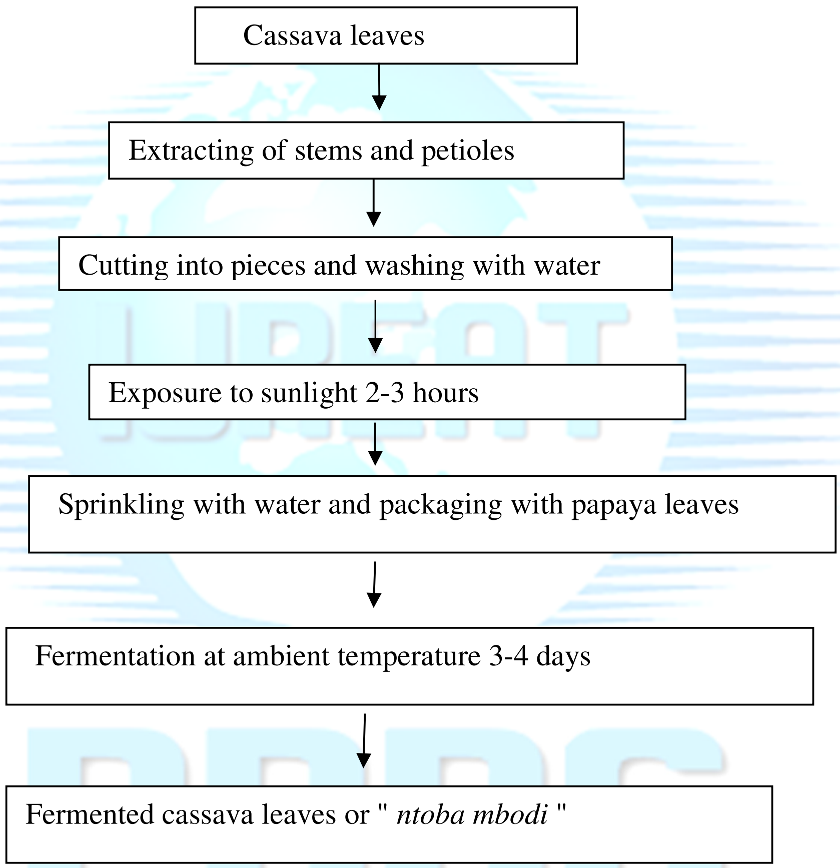

Diagram 1: Production of fermented cassava leaves "*ntoba mbodi*"

Sample preparations

Samples of cassava leaves at different times of fermentation are placed in tubes with as indication (F0, F1, F2, F3, and N), corresponding respectively to the leaves before packaging with papaya leaves, 24 hours, 48 hours and 72 hours after packaging and fermented cassava leaves (tube N, finished product).

For each sample of leaves, pounding is done with the Waring Blendor; 15 g of obtained paste were weighed and placed in a test tube. The tubes are rated TF0, TF1, TF2, TF3 and TN. For each sample 3 test tubes are prepared.

Preparation and Analysis of aromatic extracts

Solubilization is carried out by maceration. Under a laminar flow hood, to the first sample tube are added 20 ml of ethyl ether (solvent 1) and 20 ml of cyclohexane (solvent 2) were added in the last two tubes. Then, all the tubes were in vortex for sedimentation during 3 min and the supernatants were collected in screw tubes which are placed in the refrigerator.

The sample analysis was carried out by gas chromatography (GC) coupled with mass spectrometry (GC / MS). The apparatus used was a Hewlett Packard 5973/6890 equipped with an injector (280 ° C) and an HP-5 column (25m x 2.25 mm, 0.25µm film thickness). The temperature programme was 50 ° C (5 min), the temperature was increased to 300 ° C at a rate of 5 ° C / min. The carrier gas was helium at a flow rate of 1.1 ml / min. The volume injected was 1 µl of the sample diluted to 10% (v / v) with acetone.

Retention indices of aroma compounds were determined according to Van Den Dool approach (Van Den Dool and Kratz, 1963). [4] The identification of compounds was performed by comparing their mass spectra with those presented by Mc Lafferty (Mc Lafferty and Stauffer, 1989), Adams (Adams, 2001) and Joulain (Joulain and Konig, 1998).

Results and discussion

The results of the identification and quantification of "*ntoba mbodi*" showed 8 compounds including 87.5 % of terpenes. These are: β -pinene, myrcene, α -terpinene, limonene, γ -terpinene , menthone and estragole. We also note the presence of diethyl phthalate as non terpene compound.

Table 2: Compounds identified in the extract from the " *ntoba mbodi* "

Chemical constituents	%
β-pinene	1,9014
Myrcene	2,6015
α-terpinene	3,0887
Limonene	68,5621
γ-terpinene	1,4542

Menthone	0,9241
Estragol	20,2391
Di ethyl phthalate	0,1664

The identification and quantification of compounds responsible for aromas in fermented cassava leaves "*ntoba mbodi*" reveal the predominance of terpene hydrocarbons, the most important is limonene. These results are comparable to those found with the cassava tubers retted "*bikedi*" (Dhellot et al., 2014). It is noted that on the 8 compounds of "*ntoba mbodi*" 6 are found in the "*bikedi*".

Among the products identified in the "*bikedi*", terpene compounds represent 41.19%. In the "*ntoba mbodi*" limonene represents 68.5621% against only 23.9185% in the "*bikedi*" (Dhellot et al., 2014). It could come from the degradation of the sugars in which there is a production of mevalonic acid that leads to the formation of geranyl pyrophosphate (GPP), limonene precursor (Mann et al., 1994) (Turner G et al., 1999). The used matrix may be the glucose coming from the cellulose degradation thanks to the cellulose enzyme system (Mokemiabeka et al., 2011).

In the "*ntoba mbodi*" the estragol represents 20.2391% and the myrcene 2.6015% against respectively 33.8179% and 2.4749% in the "*bikedi*".

At the end of the cassava leaves fermentation in "*ntoba mbodi* ", the presence of the aromas compounds in "*ntoba mbodi*" is the result of microbial dynamics. This type of microbial association is also observed in certain fermentations of food and West Africa starchy beverages (Yao et al., 2009); it is the case of "ogi" fermented food made from corn consumed in Benin and Ghana. Lactic acid bacteria can be found associated with *Corynebacterium*, *Aerobacter*, *Candida mycoderma*, *Saccharomyces cerevisiae*, *Rhodoturula*, *Cephalosporium*, *Fusarium*, *Aspergillus*, *Penicillium* (Sanni et al., 2002).

Limonene is the predominant compound in many essential oils, particularly essential oils of lemon, sweet orange, celery and green tangerine. At room temperature it is a colorless liquid with specious smell fresh and proper of orange characteristic to citrus. Among others, it is used in the food and pharmaceutical industry to flavor bitter alkaloids. It also has herbal virtues because it is recognized as an anticancer agent (Crowell, 1999) with a potential value as a dietary anti-cancer tool in humans (Tsuda et al., 2004).

With a percentage of 20.23 % of total compounds, the estragol is the most abundant compound after limonene. It is colorless or light yellow sometimes with aniseed odor of tarragon, where it represents up to 80 % of its essential oil. It is also found in the essential oil

of basil. The estragol is suspected to be carcinogenic and genotoxic (Cestac P et al., 2005), (Vigushin D.M. et al., 1998).
Myrcene , monoterpene hydrocarbon, found in fermented foods like " *bikedi* " palm wine " *nsamba* " and " *ntoba mbodi* " is a key molecule for the synthesis of compounds such as vitamins A and E , geraniol and its derivatives. It also reduces sensitivity to pain by increasing endogenous morphinopeptides (Tressl et al ., 1975).

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