

## Behavior of Grains in the Course of the Smothering Phase of the Traditional Process of Malting of Corn (*Zea mays* sp.) in the Production of Lotoko, a Brandy of the Basin of Congo

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### ABSTRACT

#### Keywords

Smothering stage of malting process, Diver grains, Cell density of yeast, Quality of malt searched.

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In the pursuit of our work on the traditional process of malting of corn, study of smothering phase consists to appreciate the evolution of degree of softening of grains of the malt in relationship with the cell density of yeast. In the course of steeping process the humidified corn acidify oneself spontaneously (initial acidity: 0.5 ml of 0.1 N NaOH solution and final acidity: 1.2 ml of NaOH) and sustains a germination process which the stopping is assured by smothering the germinated grains. This supplementary phase of malting process unrolling in the anaerobic conditions and hot (from 35°C at 50°C) spontaneously, killing the germ of the grains, is observed at the end of germination stage. The results of the test at the water reveal a good degree of softening of the grains of malt when the corn used in the malting originates from a long period of post-harvest stocking (from 4.2 at 8 months). The cell density of population of developed yeast in the heart of grains malt of corn increases with the duration of the post-harvest stocking of corn grains of whom they originate (250x10<sup>6</sup> CFU cells of yeast by gram of dry malt for 3 months of stocking of corn in comparison with 510x10<sup>6</sup> CFU cells of yeast/gram for 8 months).

### Introduction

The traditional know-how of malting process of corn in the manufacture of *lotoko*, a brandy currently proceeded in the countries of the Basin of Congo (Delaude *et al.*, 1993; Maidou and Kamayen, 1994; Mwesigye and Okurut, 1995; Diakabana *et al.*, 2007; Aloys and Angeline, 2009), includes four main stages:

steeping, germination, smothering and draying.

The steeping prepares the grains of corn at the germination process which is visualized by the formation of organs of growing: plantule, ridicule and radicules. For the technological

reasons, the development of organs of growing may be limited in order to avoid the casualties of matters and apparition of undesirable aroma and tastes (Diakabana *et al.*, 2013-20141a).

In order to limit vegetating process of grain, a complementary phase of malting, unrolling in the anaerobic condition, is operated for stopping the germination by killing the germ and leading to the softening of the grains.

This stage promotes the enrichment of enzymatic activities of amylases (Aniche and Palmer, 1990; Nwaga *et al.*, 2009; Diakabana *et al.*, 2013-2014a) and fermentative of the traditional malt (Diakabana *et al.*, 2007 and 2014b; Rakotosaona *et al.*, 2015).

This softening process of the grains of maize happening in the course of the suffocating is owed to the degradation of material which the degree is appreciated by the number of diver grains obtained by using the method of test at the water.

A good index of material degradation of the malt must be lower at 30% of diver grains (De Clerck, 1963).

This study aims to evaluate the degree of the softening of grains of malt of corn and their level of impregnating of a flora of yeast, a factor searched after by the traditional producers of *lotoko* for appreciation of the technological quality of the malt.

## **Materials and Methods**

### **Vegetable material**

The corn (*Zea mays* sp.) used is a local variety named *accros*, a yellow cultivar which the duration of post-harvest stocking of different samples tested fluctuates from 2 at 8 months.

### **Method of malting process of corn**

The operation of malting process is effected in four stages: steeping, germination, smothering and drying (Figure 1).

### **Smothering of germinated corn**

After the operation of germinating, the grains of germinated corn are compressed in a bag of humidified polystyrene and doubled with another bag of polystyrene in order to create the anaerobic conditions: the thickness of coat is about 25 cm.

### **Drying of the malt of corn**

At the end of stage of smothering, the grains of green malt are displayed on the rack (thickness of the coat: about 2 cm) of the solar drier (inclined type; area of drying: 5.13 m<sup>2</sup>) and exposed at sunlight during 2 at 3 days (Diakabana, 2006). The natural ventilation of this system being manually adjusted, the maximum temperature of drying is limited at 50°C.

### **Analytical methods**

Determination of physical-chemical parameters during the smothering process

#### **Titrateable acidity**

The titrateable acidity is appreciated by titrimetry (Diakabana *et al.*, 2007 and 2013). Being given the buffering capacity could influence the essay; the knowledge of the titrateable acidity is used to compare the evolution of the liquid of steeping of corn.

#### **Temperature measurement**

During the development of the smothering process of germinated grains, the measure of the temperature in surface, in the dead and

bottom of the layer of grains is carried out by means of thermometer at diving (25 cm).

### **Rate of humidity and dry matter content**

The humidity and dry matter are determined according to method by drying-room of 2 at 5 grams of samples of dried grains at 105°C during 3 hours as far as the mass become constant (Diakabana *et al.*, 2013-2014a).

### **Evaluation of number of diver grains**

The aim of the technique is to evaluate the number of diver grains not having come under the disintegrating process. The modified method of try of diver or test at water (Diakabana *et al.*, 2013-2014a) is used. The grains of disintegrated malt sufficiently float on the surface when they are again plunged in the water.

Hundred (100) grains of the malt levied from a given lot are steeped in 125 ml of distilled water (try in triple). After agitation, each sample is leaved at rest for 3 and 10 minutes.

The number of diver grains (submerged grains) after three minutes for the first try and ten minutes for the other try is counted. The mean of the three tests are taken as percentage of diver grains.

### **Tendency of the number of diver grains**

The tendency of the number of diver grains of each sample of malt tested is the percentage taken as mean of essays effected after 3 minutes and 10 minutes. Malt well disintegrated does not count more of 30% of diver grains.

### **Counting of cells of yeast population**

Ten (10) grams of malt are milled at Waring Blenderhomogenizator. The milling is placed

in suspension into 90 ml of peptone water. The PDA (Potato Dextrose Agar) medium is used for isolation and counting of cells of yeast (Ampe *et al.*, 1999; Take and Kharat, 2012; Diakabana *et al.*, 2014c).

This culture medium is seeded with 0.1 ml of distinct dilutions at the rate of three box of Petri for each dilution. The culture occurred at 30-37°C during 3 at 5 days in the bacterial incubator.

The counting realized after culture corresponds at the mean of colonies developed (between 10 and 100 isolates by box of Petri). Results are expressed in CFU/gram of dry malt (Colony Forming Unit/g).

### **Statistical analysis results**

For evaluate the number of diver grains in touch with the index of disintegrating process of samples tested, then the counting of the cells of yeast population developed in the heart of grains of malt obtained at the end of malting process, following statistical values are considered: mean, standard deviation and confidence interval [mean  $\pm$  standard deviation]. The tendency of the number of diver grains is the value of the confidence interval of the essays of diver grains tested after 3 minutes and 10 minutes. The statistical method modified based on the law of Gauss-Laplace in bell is used (Larrieu, 1988).

## **Results and Discussion**

### **Evolution of titratable acidity**

Titratable acidity of the steeping increases spontaneously during the humidifying process of grains of corn (Figure 2).

The evolution of this titratable acidity is cleanly more raised with the grains

originating from a more long duration of post-harvest stocking (1.15 ml of solution of 1.1N NaOH during 28 hours of steeping for the grains coming out of a stocking of 3 months in comparison with 0.9 ml of NaOH for that originating of post-harvest stocking of 2 months).

### **Evolution of the germination process of grains of corn**

After to have absorbed sufficiently the water during the steeping stage, the humidified grains step spontaneously in germination stage. In the course of the germination process, the organs of growing of the grain expand and conduct at the formation of germinated grains into the mass (Figure 3).

### **Evolution of the temperature value of the coat of the germinated grains during the smothering stage**

To stop the germination stage, germinated grains are subjected to physical conditions relative to suffocation process.

In the course of the smothering process, the grains are getting hot spontaneous (Table 1). The temperature value of the coat of the green malt increases more quickly on surface in comparison with in the dead and the bottom of the layer during the 71 first hours of the process (at 25 hours of smothering, the temperature value is 47.3°C, 44.3°C and 39.3°C respectively on surface, in the dead and the bottom of the coat).

At every level of the layer of green malt in the course of the smothering stage, the values of temperature increases gradually as far as to attain a maximum (49°C, then 48.5°C at 71 h of smothering and 43°C at 94 h respectively on surface, in the dead and the bottom of the coat. Those values diminish in the end of process (43°C on surface, 39.5°C in the dead

and 36.5°C in the bottom of layer at 118 hours).

### **Behavior of grains of malt**

At the end of the stage of smothering process, the death of the germ conducts to stop of vegetating process of germinated grains having leaded, according to conditions of physical treatment of the mass of germinated grains, to withering of the organs of growing (Figure 4).

### **Appreciation of the degree of softening of malt**

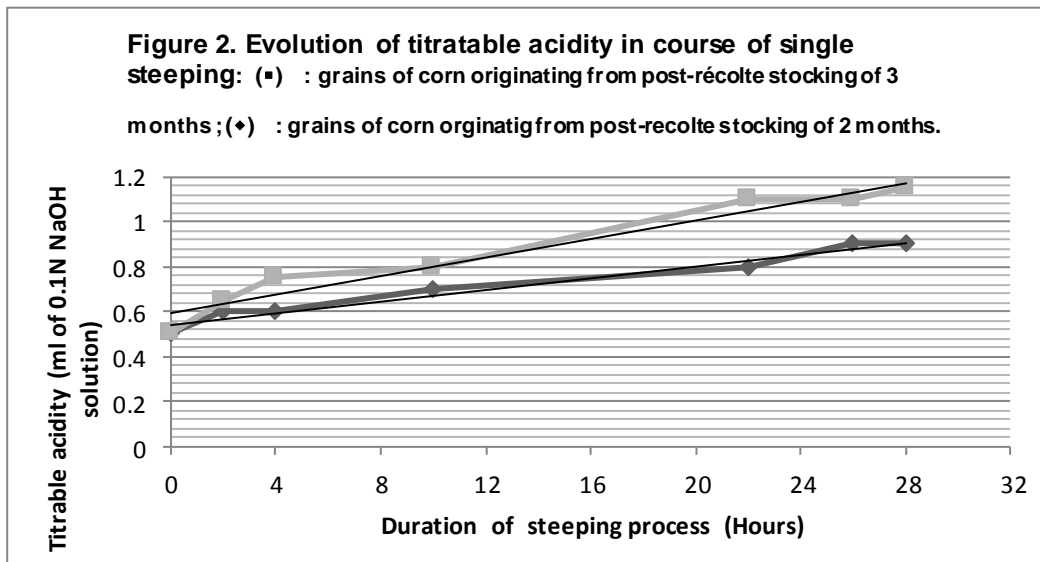
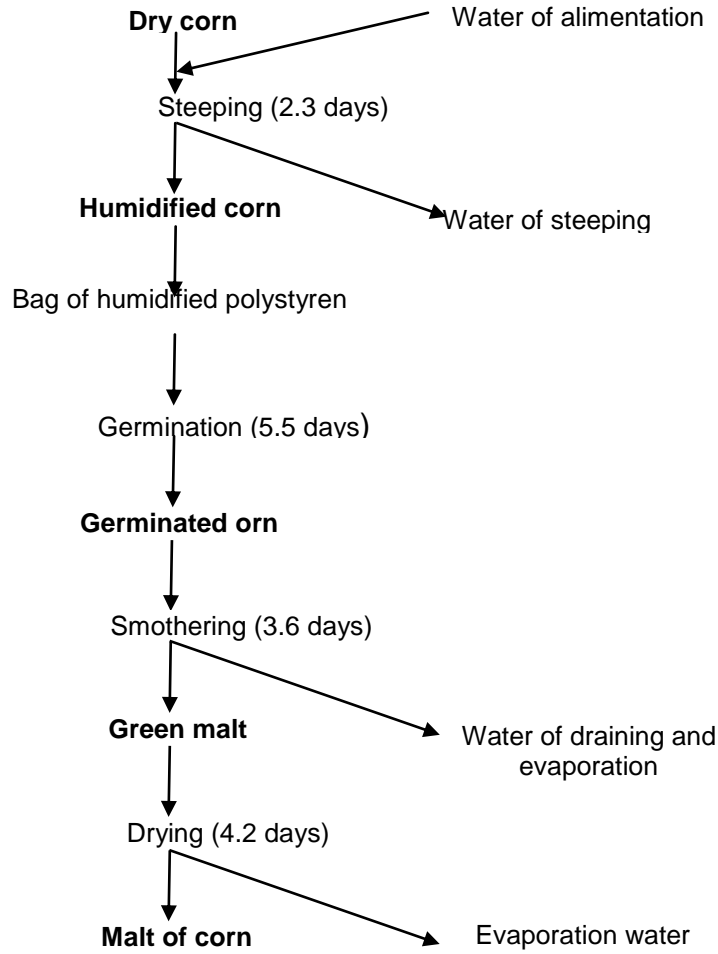
In the course of the stage of smothering process, the grain of green malt grows soft progressively as far as a desired index value.

The end of smothering stage is appreciated when the grain became fragile in consequence of degradation of the tissues. Then, the value of degree of degradation of malt is appreciated according to the measure of the number of diver grains, no disintegrated (Table 2).

Excepted for the first essay (I) of smothering, the softening of grains of the malt from single steeping is more advanced (20.75% of diver grains for the single steeping according to the third test of smothering III) that of the grains originating of the sequential steeping (27% of diver grains).

The efficacy of the smothering process for the disintegration of the grains of malt is linked at the duration of post-harvest stocking of the corn. The number of diver grains is more weak when the duration of stocking is more long (18.5% of diver grains of malt, no disintegrated, originating of the post-harvest stocking of 6 months and 9.75% of diver grains of malt for the corn stocked during 8 months).

**Fig.1** Diagram of traditional process of malting of corn (mean of duration by cycle: 15.5 days)





**Fig.3** Formation of organs of growing at the end of germination stage



**Fig.4** Withering of organs of growing in end of the smothering stage



**Table.1** Evolution of the temperature of the layer of the germinated grains of corn during the smothering process

Duration (h)	Temperature in surface (°C)			Temperature in the dead(°C)			Temperature in the botton (°C)		
	M	Σ	IC	M	Σ	IC	M	Σ	IC
0	31.70	1.40	[31.7 ± 1.4]	31.80	1.44	[31.8 ± 1.44]	31.70	1.39	[31.7 ± 1.39]
2.5	40.50	1.00	[40.5± 1]	39.5	0.80	[39.5 ± 0.8]	35.50	0.50	[35.5 ± 0.5]
25	47.30	2.62	[47.3 ± 2.62]	44.30	2.24	[44.3 ± 2.24]	39.30	2.24	[39.3 ± 2.24]
46	46.00	3.53	[46 ± 3.53]	45.00	4.21	[45 ± 4.21]	40.70	4.60	[40.7 ± 4.6]
71	49.00	2.24	[49 ± 2.24]	48.50	2.24	[48.5 ± 2.24]	40.80	334	[40.8 ± 3.34]
94	47.50	1.73	[47.5 ± 1.73]	48.50	1.73	[48.5 ± 1.73]	43.00	1.73	[43 ± 1.73]
118	43.00	1.00	[43± 1]	39.50	2.50	[39.5 ± 2.5]	36.50	2.50	[36.5 ± 2.5]

M: Arithmetic mean of 16 cycles of malting process; Σ: standard deviation; IC: Confidence interval.

**Table.2** Appreciation of the disintegration of malt of corn according to the method of test at the water: (Grains of corn across variety: duration of post-harvest stocking variable from 2 at 8 months)

Test of smothering	Duration of stocking corn (months)	Mode of steeping of corn	Number of diver grains by 100 after three (3) et ten (10) minutes		
			After 3 min (%)	After 10 min (%)	Tendancy (%)
I	2.0	Tu	40	49	44.5 ± 4.5
	2.0	Ts	42	43.5	42.75 ± 0.75
II	4.2	Tu	23	21	22 ± 1.0
	4.2	Ts	27	29.5	28,25 ± 1,25
III	4.8	Tu	25	16.5	20.75 ± 4.25
	4.8	Ts	28	26	27 ± 1.0
IV	6.0	Tu	14.5	22.5	18.5 ± 4.0
V	8.0	Tu	11.5	8	9.75 ± 1.75

Tu: single steeping; Ts: sequential steeping.

**Table.3** Evaluation of the cell density of the yeast population of the corn malt: (across variety corn: duration of post-harvest stocking variable from 2 at 8 months)

Test of smothering N°	Duration of stocking corn (months)	Mode of steeping of corn	Cell density of yeast (10 <sup>6</sup> CFU/g dry matter of malt)
I	2.0	Tu	116 ±4
	2.0	Ts	116 ±2
II	3.0	Tu	250 ±5
	3.0	Ts	200 ±3
III	4.2	Tu	310 ±5.4
	4.2	Ts	300 ±4
IV	4.8	Tu	400 ±5
	4.8	Ts	390 ±6
V	6.0	Tu	490 ±4
VI	8.0	Tu	510±3

Tu: single steeping; Ts: sequential steeping.

### **Cell density of the flora of yeast developed in the heart of grains of the malt of corn**

In the course of the smothering phase, a microbial flora expands spontaneously in the heart of germinated grains of corn.

At the end of the march of smothering phase of germinated grains, the cell density of the population of yeast is evaluated (Table 3). The value of cell density of the population of yeast of the malt increases when the duration of post-harvest stocking of the corn having served at the malting process is more long; for the test with corn coming out a post-harvest stocking during three months  $250 \times 10^6$  CFU of yeast are formed by gram of dry malt in comparison with  $510 \times 10^6$  CFU of yeast formed by gram of malt for the case of corn coming out height months of stocking.

The grain becoming feverish, in the course of smothering stage, loses the vitality of its germ and dies; it come under a tissue disintegrating visualized by a softening like is the case for malting process of other cereals used in production of special malts (Derdelinckx, 1990).

The keeping of the temperature value at 43-49°C, during several days, in the course of this smothering stage should favor the enzymatic proteolysis activity that should conduct at the absence of the activity of  $\alpha$ -glucosidase of the malt permitting the liberation of glucose in the must of *lotoko* from maltose and maltotriose (Diakabana *et al.*, 2014d and 2016).

The cell development of the population of yeast observed in the end of smothering is in touch with the phenomenon of spontaneous acidification of biological descent established in the course of steeping process of corn grain (Van Beek and Priest, 2002; Louembé *et al.*, 2004).

In the procedure of malting process the use of the corn originating from a long duration of post-harvest stocking contributes at the enrichment of the flora of yeast (Diakabana *et al.*, 2014c).

The amylases formed in the course of germination process of the corn grain (Diakabana *et al.*, 2013-2014a) solubilise the starch by liberating the glucose and oligosaccharides (maltose, maltotriose) (Aniche and Palmer, 1990) that the yeasts consume for their cell growing (Diakabana *et al.*, 2014b).

In comparison with preceding stages of the malting process, the softening of the grain is obtained according to the operational conditions of the smothering. It takes place right side out of the coat of floury albumen in consequence of enzymatic degradation of the  $\beta$ -glucans and pentosans of the cell wall (Home, 1991; Leclercq *et al.*, 2000). That justified well the conditions of suffocation, high temperature and keeping of humidity assured right side out of the coat of germinated and compressed grains in order to a well disintegration of the malt.

The combined action of a spontaneous culture of yeasts (Diakabana *et al.*, 2014b) and enzymatic activities in the course of the smothering process (Diakabana *et al.*, 2013-2014a) conducts at the formation of the natural flavors linked at the presence of aromatic substances especially of the amylic alcohols, desired for the well organoleptic quality of *lotoko* (Diakabana *et al.*, 2007).

However this stage unrolling in the conditions becoming anaerobic (Diakabana *et al.*, 2008) progressively, is more long than in the case of the malting process of barley.

The optimization of the procedure of smothering process, for the biological



producing of the flavours, can contribute to improve the organoleptic quality of foods and beverages improved based of corn malt (Mosha and Svanberg, 1993; Diakabana *et al.*, 2013).

The isolation of yeast from malt of corn (Diakabana *et al.*, 2014b) and their fine identification coupled at the knowledge of the sanitary aspects could conduct to develop a proceeding of production of yeast strains generally recognized as safe "GRAS" (Holzapel, 1997; FAO/WHO, 2002) and interesting for enrichment in natural flavours of food and beverages (Zanou-Tchoko *et al.*, 2011; Diakabana *et al.*, 2013).

In the course of the march of smothering process of corn grain, a connection narrow exists between development of enzymatic activities of saccharification of the malt of corn (Diakabana *et al.*, 2013-2014b) and cells growing of yeast. The oligosaccharides then liberated "in situ" enrich the cell density of yeast of the malt (Diakabana *et al.*, 2014b) searched by the traditional distillators.

In conclusion, for employment of corn malt as ingredients used in improved distillery, the evaluation of the amylolytic activities of enzymes and ethylic fermentation is necessary in order to an eventual correcting relative at the quantitative and/or qualitative irregularities observed in the context of well conduct of the fermentation process.

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