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Full Length Research Paper

Studying the ethylic fermentation process of the mucilage juice of cacao by *Saccharomyces cerevisiae* yeast

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The aim of the study was to optimize the ethylic fermentation process of the mucilage juice of cacao by *Saccharomyces cerevisiae* yeast. The juice fermentation process was effected by inoculating *S. cerevisiae* on 0.2 g of activated yeast/liter of juice for essay E1, and on 0.5 g of activated yeast/L for essay E2. Results reveal that increasing the pH value of the process effected in the acidic zone raised the rate of the essay inoculated with 0.5 g of yeast/liter than that inoculated with 0.2 g/L yeast/liter of juice. The amounts of the soluble extract transformed and yield of the transformed soluble extract were greater (90.60%) for E1 compared to those of E2 (84.41%). The efficacy of yeast utilized in the transformed soluble extract/100 g of the mucilage juice of cacao/gram was higher for essay E1 (81.95) compared to that of essay E2 (30.54).

Key words: Mucilage juice of cacao, ethylic fermentation, rate of yeast, soluble extract, yield of transformation, efficacy.

INTRODUCTION

Cacao is the third foodstuff that is highly merchandized in the whole world. Having three million tons of cacao per year, African continent has become the world leader in cacao production. Cacao tree (*Theobroma cacao* L) is one of the most important plants in the tropical agro-forester. This tree known as cacao tree belongs to the family of Sterculiaceae, Theobroma kind. It is esteemed that in plantation it must keep pace from twenty-five to thirty years. Theobroma kind has 22 species of which *T. cacao* is the sole one that is cultivated commercially.

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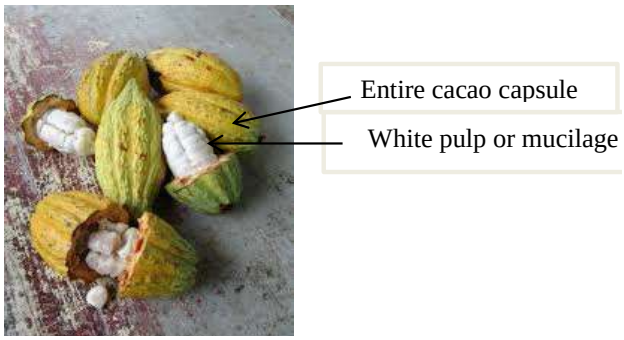

Photo 1. Entire cocoa capsules and by cutting transversal showing surrounded seeds of the white pulp or mucilage.
Source: Authors

Indeed, it produces seeds used to prepare chocolate or to extract cacao butter (Zhang et al., 2011).

Cacao tree is a bush cane of 5 to 7 m height on average. Its fruits, cocoa capsules (Photo 1), are berries that are grossly lengthened like American football.

Cacao capsules contain numerous seeds (between 25 and 75) which are regrouped by splice and named cacao beans rich in starch, fats, and alkaloids. A bean of cacao, obtained after undergoing different stages of post-harvest treatment, weighs about 1 g. It is enveloped with resistant thin dandruff named husk. Every mulberry seed is surrounded by a pulp named mucilage. It is white, aqueous, and sweetened. The post-harvest treatments of cacao depend on its over-production.

During the stage of indenting, a great quantity of pulp is obtained, which ferments after some time (Hamdouche, 2015). At this stage, more than 300 million liters of mucilage juice, commonly called water of cacao, produced every year are abandoned in the fields. Yet, it is a good drink that is appreciated by agricultural workers and their children.

Due to its high level of sugar, the mucilage juice of cacao is very fermentable and could serve as raw material for the production of ethanol (Stewart, 2016), vinegar, and other derivative products.

In Congo, except for the production of beans for exportation, the mucilage juice and cacao capsules that are not mature enough are not used. They are considered as industrial wastes.

The principal objective of this work was to contribute to the valorization of the mucilage juice of cacao via fermentation, notably to determine the physico-chemical and technological parameters relative to the process of fermenting the mucilage juice of cacao.

MATERIALS AND METHODS

Raw material

The raw material used for the study is cacao fruit. It was obtained from cacao tree from the fields located at *Pokola* village in the Administrative Department of Sangha, situated at 642 km of Brazzaville. A total volume of the reaction mixture of 6 L constituted the mucilage juice of cacao at 17% Brix of soluble extract standardized with sugarcane.

This mucilage juice of cacao fruit was standardized on ground, in the month of August 2022. It was pasteurized at 85°C for 20 min, and then placed in bottles of 1 L. It was immediately corked and sent to Brazzaville for it to undergo the process of ethylic fermentation in the laboratory of Food Microbiology of the National Institute of Engineer Sciences Research, Innovation and Technology.

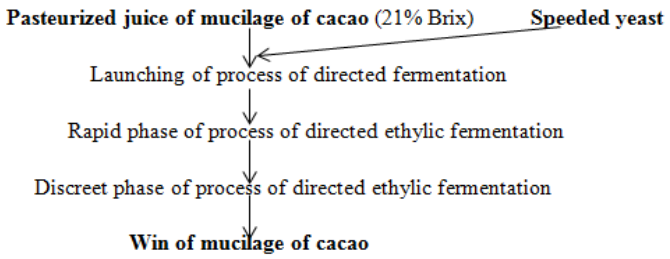

Figure 1. Diagram of process of ethylic fermentation of juice of mucilage of cacao into wine of mucilage of cacao.
Source: Authors

Biological yeast

The biological yeast used for the seeding of the samples of mucilage juice to direct the fermentation process was an industrial strain commercialized at Brazzaville under Saf-instant label (S.I. Lesafre, France).

This reactional medium is separately sown with activated yeast via ethylic fermentation; notably 0.2 g of yeast/L was used for essay E1 and 0.5 g of yeast/liter was for essay E2, to optimally seed the biological yeast (Diakabana et al., 2016).

Operatory material

The operatory material with a total voluminal capacity of 1.5 L was used for the experimentation; it is a cylinder-conical fermenter that allows one to take the samples for analysis, and also allows the evacuation of fermentation gas.

The combined volume of the reaction mixture for each one of the two tested essays done in triplicate was 1 L.

Methods

The ethylic fermentation process of the mucilage juice of cacao was in two phases: one, consumption of soluble extract spontaneously and the other is discreet phase (Figure 1).

Activation of the yeast

Before the mucilage juice of cacao was seeded, 0.2 g of *Saccharomyces cerevisiae* yeast and 0.5 g of yeast were first activated in 10 mL of the mucilage juice of cacao, for 30 min. They were incubated at ambient temperature. This was the mother-suspension. The cell density of the inoculum was then evaluated based on Heineken method adapted by Diakabana et al. (2013):

(1) 0.1 mL of the mother-suspension of the yeast was taken and

diluted in 0.9 mL of sterilized distilled water;
(2) after this diluted suspension was homogenized, a drop was taken and then deposited on Malassez cell. Then, it was covered with a special slide and observed with a microscope with objective X40. The rectangular (Faurie, 2019) cell of Malassez was squared;
(3) the number of the yeast cells was counted in each one of the 5 zones A, B, C, D, and E;
(4) the number of yeast was determined as follows: Number of yeast cells = (A + B + C +D + E) × k; with k = 0.5 × 10^6 cells/mL (coefficient given by Heineken).

Seeding the mucilage juice of cacao

For the two samples tested for triple times, ethylic fermentation was done using the active yeast (initial biomass) as follows: 13.4 × 10^6 cells/mL of cacao for essay E1 and 33.5 × 10^6 cells/mL for essay E2.

Determination of the technological parameters

The determination of the soluble extract was done with two methods: using refractometer and pycnometer. In the two tested cases, the work was started via preliminary cooking, filtration, and homogenization.

Using refract meter

In measuring the soluble extract using refractometer, the value obtained was expressed in % Brix at 20°C.

Using pycnometer

The method of pycnometer described by EBC and adapted by Diakabana et al. (2013) was employed (Dowdy and Mosher, 2022) to determine the soluble extract and the ethanol content of the mucilage of cacao.

In a balloon of 500 mL, 100 ± 0.1 g was weighed and filtered inside the mucilage. After collecting 85 mL of the distillate, this quantity (100 ± 0.1 g) was poured into the distilled water and weighed. After homogenization, the density of the distillate was determined using a Gay-Lussac pycnometer at 20°C.

After measuring the density, the alcohometeric titer was evaluated based on the table of conversion of Goldiner and Klemann (De Clerck, 1963).

This technique permits us to follow the evolution of the ethylic fermentation of the mucilage juice of cacao, notably the yield of the transformed sugars (% in weight) and the coefficient of yield relative to the conversion of soluble extract into ethanol (% in weight) (Diakabana et al., 2013):

$$Rdt = \frac{St}{S0} \times 100;$$

where St = quantity of soluble extract transformed and So = initial quantity of soluble extract.

The formula described by Leveau and Bouix, adapted by Diakabana et al. (2013) was used to calculate the coefficient of yield relative to the conversion of soluble extract into ethanol (% in weight):

$$Y_{Et/St} = \frac{Et}{St} \times 100;$$

Where Et = Global quantity of formed ethanol and St = transformed soluble extract.

Calculation of daily consumption of soluble extract

Daily consumption of the soluble extract was calculated in the linear zone using the kinetic curve of the process as follows:

Ei-Ef;

Where Ei= initial value and Ef=following value.

Determining the efficacy of the yeast used

The efficacy of the yeast used in the process of ethylic fermentation was determined by the relation of the quantity of transformed soluble extract St/quantity of yeast used.

Statistical analysis

For the analysis of the technological parameters relative to the process of fermentation, the method based on the law of bell of Gauss-Laplace (Larrieu, 1988) was used to determine the repeatability of measures of analysis and operations. The average values of pH, soluble extract in the mucilage juice, speed of the fermentation process of the tests done three times, standard deviation, and confidence intervals were determined for a coefficient of variation inferior or equal to 0.1%. The average efficacy of the yeast used the yield of relative transformation of soluble extract, and the coefficient of relative yield to the conversion of soluble extract into ethanol were appreciated for a coefficient of variation inferior to or equal to 0.1%.

RESULTS AND DISCUSSION

The physico-chemical and technological parameters relative to the fermentation process of the mucilage-juice of cocoa were examined.

Evolution of the consumption of soluble extract and the pH during the fermentation process of the mucilage juice of cacao

The evolution of the consumption of the soluble extract and the pH during the fermentation process of the mucilage juice of cacao is visualized by the profile of the soluble extract, the rise of the pH, and technological parameters of every essay tested.

Evolution of soluble extract

During the ethylic fermentation process, the consumption profile of the soluble extract of the mucilage juice of cacao by *S. cerevisiae* decreases (Table 1).

Starting from the initial value of the soluble extract evaluated at 21% Brix, essay E_1 (with 0.2 g of yeast/liter) went down more rapidly (3.35% Brix/day), during the 4

Table 1. Evolution of the soluble extract during the progress of the process of ethylic fermentation of the must of cacao in function of the rate of seeding of *S. cerevisiae* yeast employed.

Period (day)	Essay 1 (E1)		Essay 2 (E2)	
	Soluble extract (% Brix)	Consumed Soluble extract by day (% Brix/day)	Soluble extract (% Brix)	Consumed Soluble extract by day (% Brix/day)
0	21	**0**	21	0
1	17.1	3.9	18.3	2.7
2	13.5	3.6	14.7	3.6
3	10.2	3.3	11.4	3.3
4	7.6	2.6	8.3	3.1
5	7.4	0.2	7.8	0.5
6	7	0.4	7.5	0.3
Average value between 0 and 4 days	/	3.35	/	3.175

E1: Seeding with 0.2 g of yeast/liter of must (13.4 × 10^6cells/mL of must); E2: with 0.5 g/liter (33.5 × 10^6cells/mL); Daily consumption of soluble extract calculated into linear zone (between 0 and 4 days) of the progress of the process.
Source: Authors

Table 2. Evolution of the pH during the progress of the process of ethylic fermentation of the must of cacao in function of the rate of seeding of *S. cerevisiae* yeast employed.

Period (day)	Essay 1 (E1)	Essay 2 (E2)
	pH	pH
0	3.34	3.34
1	3.41	3.44
2	3.5	3.51
3	3.53	3.57
4	3.55	3.61
5	3.56	3.65
6	3.57	3.67

E1: Seeding with 0.2 g of yeast/L of must (13.4 × 10^6cells/mL of must); E2: with 0.5 g/L (33.5 × 10^6cells/mL).
Source: Authors

first days, until at lower degree of consumption of soluble extract estimated at 7.6% Brix compared to essay E_2 (with 0.5 g of yeast/liter) which went down less rapidly (3.175% Brix/day) until at 8.3% Brix. During the 2 last days of incubation, the consumption of the soluble extract was very slow, discrete until at day 6, respectively where we have 7% Brix for E1 and 7.5% Brix for E2 till the end of the incubation.

Evolution of pH

The evolution of the ethylic fermentation process of the juice of cacao by *S. cerevisiae* was noted by a progressive augmentation of pH (Table 2).

Starting from the initial pH value of 3.34 of the juice during the fermentation process, the profile of the pH ascended in the two cases tested.

For E_1 (with the smallest level of inoculation), the pH profile was low and stopped at the value of 3.57 compared to that of essay E_2 (with the more high level of inoculation) which stopped at pH –3.67 at the end of the incubation period of 6 days.

Evaluation of the technological parameters

The technological parameters, notably the speed of the fermentation process, the quantity of soluble extract transformed, the quantity of residual soluble extract, and the quantity of ethanol formed for–essay E1 tested are rationally valued (Table 3).

The quantity of residual soluble extract at the end of the fermentation process was estimated at 1.70 g/100 g of cacao juice for essay E1 which was effected by seeding the mucilage juice with 0.2 g of yeast/liter (13.4 ×

Table 3. Evaluation of the technological parameters of the process of ethyl fermentation of the must for each essay tested; E1 (essay with 0.2 g of yeast/L) and E2 (essay with 0.5 g of yeast/L).

Essay tested	Evaluation of the technological parameters of the process of ethylic fermentation						
	Initial quantity of soluble extract (g/100 g of juice)	Quantity of residual soluble extract (g/100 g of juice)	Quantity of transformed soluble extract (g/100 g of juice)	Efficacy of use of yeast (g of transformed soluble extract/100 g of juice/g of yeast used)	Rdt (%)	Finale quantity of formed ethanol (g/100 g of juice)	$Y_{Et/S}$ (g of formed ethanol/g of transformed soluble extract) % weight
E1	18.09	1.70	16.39	81.95	90.60	4.86	0.29
E2	18.09	2.82	15.27	30.54	84.41	ND	ND

Rdt: Yield of relative transformation to conversion of soluble extract; $Y_{Et/S:}$ coefficient of relative yield at the conversion of soluble extract into ethanol; E1: seeding with 0.2 g of yeast/L of must (13.4×10^6cells/mL of must); E2: with 0.5 g/L (33.5×10^6cells/mL). ND: no determined.
Source: Authors

10^6cells/mL of must); essay E2 was obtained by seeding 0.5 g of yeast/liter *(*33.5×10^6cellules/mL*)* with 2.82 g/100 g of cacao juice.

Appreciation of the performance in the ethylic fermentation process of the essays tested

To know the performance of the essays tested with the initial soluble extract of the mucilage juice of cacao, the efficacy of the yeast and the yield of the transformed soluble extract are presented in Table 3.

For the two essays tested (E1 and E2), the initial quantity of the soluble extract was estimated at 18.09 g/100 g of the mucilage juice. Yield of the transformed soluble extract was evaluated at 90.60% for essay E1 (with 0.2 g of yeast/liter of mucilage juice) and 84.41% for essay E2 (with 0.5 gram of yeast/liter of mucilage juice). The amount of the transformed soluble extract during the process was evaluated at 16.39 g/100 g of juice for E1 and 15.27 g/100 g of juice for E2. In the same sense, the efficacy of the yeast tested was evaluated at 81.95 g of the transformed soluble extract/100 g of juice/gram of yeast used for E1, and 30.54 g of the transformed soluble extract/100 g of juice/gram of yeast used for E2.

Concerning essay E1 (done with 0.2 g of yeast/liter of the mucilage juice), the final quantity of the ethanol formed was estimated at 4.86 g/100 g of the mucilage juice, which corresponded coefficient of converting the soluble extract into 0.29 g ethanol formed.

The mucilage juice of cacao fruit, the by-product of cacao fruit treatment containing organic and inorganic substances can inflict damages on the floral and microbial eco-systems in the environment where the activity takes place (Spevacek and Ritchson, 2020). Improving the ethylic fermentation process of mucilage juice of cacao could produce wine of commercial quality. It brought an added value to the industrial treatment of cacao fruit in preservation environment as revealed by Ngampika et al. (2022) on valorization of cacao capsules and Vu et al. (2018) on valorization of banana peels.

The process of ethylic fermentation could enhance the mucilage juice of cacao fruit to produce ethanol (Stewart and Speers, 2019). This process being gas production favours auto-agitation of liquid medium as the fermentation progresses by employing an appropriate fermenter, which is at the center of the installation of fermentation (Smith, 2021).

The interest to use *S. cerevisiae* in the ethylic fermentation process of the mucilage juice of cacao was proved after deducing that *Saccharomyces* is implicated in the traditional process of elaborating many African fermented foods (Flibert et al., 2016; Diakabana et al., 2019).

As the ethylic fermentation process progressed, the soluble extract diminished rapidly from 21% Brix from 0 to 4 days, revealing the degradation of the fermentable soluble extract by *S. cerevisiae* yeast (Diakabana et al., 2007). This value of the soluble extract content was stabilized at about 7 to 7.5% Brix from day two to the last fourth day of the fermentation. This indicates the soluble extract was not degraded by *S. cerevisiae* and constituted the fraction of the residual soluble extract (Diakabana et al., 2013).

The fermentation of the mucilage juice started as soon as the initial biomass of the yeast was realized with concentration of 13.4×10^6 cells/mL of cacao juice (0.2 g of dry yeast/liter) for essay E1 and 33.5×10^6 cells/mL (0.5 g of dry yeast/L) for essay E2 (Diakabana et al., 2019). In relation to that, this directed process was carried out for 6

days (incubation of the two cases tested). The average consumption speed of the soluble extract for essay E1 which was effected by seeding cacao juice with 0.2 g of yeast/liter (13.4×10^6 cells/mL of juice) was higher (3.35% Brix/day) compared to essay E2 (3.175% Brix/day) which was realized by seeding 0.5 g of yeast/liter (33.5×10^6 cellules/mL). That signifies high yeast rate (33.5×10^6 cellules/mL). Saturated concentration did not favour well the transformation of the fermentable soluble extract of the mucilage juice of cacao as obtained by Diakabana et al. (2019) in the fermentation of ginger juice.

The fermentation of the soluble extract for essay E2 was precociously stopped by using a seeding rate relatively high (33.5×10^6 cells/mL). This was done because the yeast culture had accomplished its metabolic role, which was notably negative effect of high dose of ethanol on yeast (Maskell, 2016; Castro and Bryant, 2021; Cisilotto et al., 2021). It was associated with the flocculation of the population of yeast (Speers, 2016) in the saturated concentration of inoculum (33.5×10^6 cells/mL) in relation to essay E1 which was effected with a feeble yeast (13.4×10^6 cells/mL of must). This precocious stop of the fermentation explains how higher quantity of residual soluble extract was obtained for essay E2 compared to essay E1.

The increased profile of the pH of the two essays tested and developed in the zone of acidic pH guaranteed the character of the food (Nout et al., 2003; Bousmaha et al., 2009; Tchibozo et al., 2012).

The augmentation of the pH was relative to the production of ethanol progressively, which became the exhausted source of assimilable nitrogen in the mucilage juice during the ethylic fermentation by *S. cerevisiae* (Akin, 2008). That is made clear by the feeble content of ammoniacal nitrogen estimated at 0.03% relative to composition of the dried beans of cacao despite the importance of the nitrogen protein estimated at 10.0%.

The feeble coefficient of yield relative to the conversion of the soluble extract into ethanol for essay E1 (yeasting with 0.2 g of yeast/liter) is probably caused by the Crabtree effect, notably the respiration of ethanol by *S. cerevisiae* and the high content of soluble extract during the long hours of the fermentative process (Engasser, 1988).

Conclusion

This work allows us to understand that the optimal value of the rate of yeast of the mucilage juice is 0.2 g of yeast/liter. The notable presence of residual soluble extract in the two cases indicates the soluble extract is not fermented by *S. cerevisiae* in the mucilage juice of cacao.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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