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Enhancement of α-Glucosidase Production by *Saccharomyces cerevisiae* C8-5 and *Candida tropicalis* C0-7, Two Yeast Strains Isolated from *Tchapalo*, a Traditional Sorghum Beer of Côte d'Ivoire

Yapi Yapi Eric*, W.A.M. Alloue-Boraud, Coulibaly W. Hermann and Djè Koffi Marcellin

Biotechnology and Food Microbiology Laboratory, Department of Food Science and Technology, University of Nangui Abrogoua, Abidjan, 02 BP 801, Côte d'Ivoire

**Corresponding author*

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A B S T R A C T

The hydrolase enzymes using in industrial processes have always given particular interest because of their non-toxic and protective nature. In the present study, various physicochemical parameters for α-glucosidase production from strains of *Saccharomyces cerevisiae* C8-5 and *Candida tropicalis* C0-7 were optimized by using a starches of some agricultural products of Côte d'Ivoire. It was found that *S. cerevisiae* C8-5 and *C. tropicalis* C0-7 produced maximum α-glucosidase after 48 hours of fermentation at 37 °C, pH 7 and without agitation speed (0 rpm) using corn starch and millet starch (1 %). The more important α-glucosidase activity were obtained with *S. cerevisiae* C8-5 by using 1 % of corn starch (15.96 U/ml). It is conclued that corn and millet starches could be used in enzyme production, particulary the production of α-glucosidase.

Introduction

Fermentation remains one of the old technologies used for food production and preservation (Holzapfel, 2002). In Africa, several fermented foods make a significant contribution to people's nutrition. Traditional beverages in particular, sorghum beer (*Tchapalo*) are experiencing considerable growth among the various populations in Côte d'Ivoire. However this beer is the result of an uncontrolled fermentation process, which contributes of the variability of its organoleptic properties from one producer to another (Alloue-Boraud *et al.*, 2014). In order to contribute to the production of stable and healthy beers with constant organoleptic characteristics, several controlled fermentation trials have been carried out with selected starters of lactic bacteria and / or yeasts. Thus, the traditional *Dolo* beers (Burkina-Faso) and *Burukutu* (Nigeria) were produced from starters composed of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* (Glover *et al.*, 2009; Adewara *et al.*, 2013). In Côte d'Ivoire, the study of N'guessan *et al.*, (2011) selected a starter (*S. cerevisiae* + *C. tropicalis*) for the production of *Tchapalo*. During the fermentation process, starter strains produce several metabolites such as organic acids,

alcohols, volatile compounds and enzymes that improve the final quality of the product. The use of yeast for enzyme production has several advantages, such as moderate temperature for microbial growth, high metabolic diversity and rapid cell growth, and is more energy efficient. This results in shorter fermentation cycles and easy adaptation of microorganisms to different growing conditions (Oliveira *et al.,* 2015). Production of microbial enzymes is a necessary event in the industrial sectors, due to the high and superior performances of enzymes from different microbes, which work well under a wide range of varied physical and chemical conditions (Rajendra *et al.,* 2016).

Enzyme mediated processes are rapidly gaining interest because of reduced process time, intake of low energy input, cost effective, nontoxic and eco-friendly characteristics (Li *et al.,* 2012; Choi *et al.,* 2015). Among the enzymes produced, amylolytic enzymes are contributed to the degradation of starch. These enzymes account for 25% to 33% of international market of marketed enzymes and are used in many industrial processes that require partial or total hydrolysis of starch (Özdemir *et al.,* 2014).

Amylolytic enzymes are extensively used in biotechnology and have important applications in both the food and the pharmaceutical industries (Marin *et al.,* 2006). Among the amylolitic enzymes, α-glucosidase plays an important role in the process of metabolizing starch. Indeed α-glucosidase is the final enzyme involved in the metabolism of starch to glucose, and is used with α-amylase for the saccharification of starch (Gupta et Gautam, 1993). *α*-glucosidases (*α*-D-glucoside glucohydrolase; EC 3.2.1.20) hydrolyse the *α*-glycosidic linkages from the non-reducing end of oligosaccharides and polysaccharides with the release of *α*-glucose (Marin *et al.,* 2006). This enzyme is used in food industry for the production of high-quality sugar syrups and confectionary, and it is employed to improve the texture of baked products (Muhammad *et al.,* 2016).

Further, microbial enzymes are used in the treatment of health disorders associated with deficiency of human enzymes caused by genetic problems. α-glucosidase can hydrolyze polysaccharide into glucose at terminal non-reducing α-1-4-glycosidic linkage in small intestine, where the enzyme is located. The product from α-glucosidase is monosaccharide such as glucose, which is absorbed into intestine wall. Thus, the inhibition of α-glucosidase can, as well, control diabetes by delaying carbohydrate degradation and glucose absorption (Watcharachaisoponsiri *et al.,* 2016). However, the cost of enzyme production is a major limitation for it's commercialization and it's mainly depends on the physico-chemical fermentation conditions (Nascimento and Martins, 2004).

In order to ensure the expansion of the enzyme production industry, it's essential to produce enzymes at a lower cost. Culture media are essential in the process of producing enzymes, the use of a less expensive culture medium and stimulating the production of enzymes would significantly lower the cost of production. Indeed, according to Garcia-Martinez *et al.,* (2010), culture media represent 30 to 40% of the total cost of enzyme production. The choice of an appropriate production medium is therefore essential for microorganisms, both for their growth and obtaining enzymes (Bouatenin *et al.,* 2016). In order to reduce the cost of enzyme production, alternative culture media have been developed. Thus the use of agroindustrial residue losses for enzyme production can become economically important in the application of these biocatalysts on a large scale (Oliveira *et al.,* 2015). It's in this context that several carbon

sources such as banana peels, sorghum grains obtained during the production of *Ogi* in Nigeria and millet have been used in fermentation processes as substrates for enzyme production (Abu *et al.*, 2005, Maktouf, 2013, Oliveira *et al.*, 2015). Côte d'Ivoire has a variety of agricultural products potentially rich in starch among which are distinguished cereals (maize, millet, sorghum...), tubers (cassava, yam) and others. These products are for the most part used in human, animal or industrial food and their starches have never been valued in enzymes production. These starches could be used as a source of carbon for enzyme production and thus to constitute a substitute of synthetic starch whose accessibility remains difficult because of the relatively high price.

Exploration of α-glucosidase from yeast source and cost-effective production strategy are indispensable factors in order to meet the escalating demand at commercial level.

The current study was to investigate the enzymatic potentialities of *Saccharomyces cerevisiae* C8-5 and *Candida tropicalis* C0-7 particularly, α-glucosidase production for using corn starch and millet starch. Various physical and chemical parameters of growth medium were optimized to attain the maximum production yield of α-glucosidase under submerged fermentation technique.

Materials and Methods

Yeast strains and culture conditions

Yeast species of *C. tropicalis* and *S. cerevisiae* used as starters in this study were belonged to the culture collection of the Food Technology Department (University of Nangui Abrogoua). They were isolated from traditional sorghum beer from the district of Abidjan (Southern Côte d'Ivoire). They were identified by PCR-RFLP of the ITS region and sequencing of D1/D2 domains of the 26S rRNA gene (N'guessan *et al.*, 2011). Before their growth on solid state medium, yeasts were cultivated on 868 medium with chloramphenicol at 30 °C for 24 h. This medium contained (w/v): glucose monohydrate 2 %, yeast extract (Organotechnie, France) 1 %, peptone casein (Organotechnie, France) 1 % and agar (Merck, Germany) 1.5 %.

Inoculum preparation

A pure colony (24 hours) of each microorganism was inoculated in Erlenmeyer of 250 ml containing 50 ml of medium 863 (glucose 20 g/l, yeast extract 10 g/l, pepton 10 g/l and chloramphenicol 0.5 g/l). These medium were incubated during 12 hours at 28°C (Bataiche, 2014).

α-glucosidase production

The α-glucosidase production medium is composed per liter of 1% peptone, 1% corn starch or millet starch, 0.5% yeast extract, 0.3% K_2HPO_4 and 0%, 1% KH_2PO_4. In Erlenmeyer of 250 ml containing 54 ml of liquid fermentation medium constituted were inoculated with 6 ml of inoculum. For cocultures, inoculum volume was set out again according the ratios between the microorganisms.

For each starch source, four fermentation media were constituted as follows: (1) individual pure fermentation medium with *C. tropicalis* and *S. cerevisiae*; (2) mixed fermentation media of both yeast strains, respectively, in ratios of 2:1 and 1:1 (cell/cell). These media were incubated at 30°C in orbital shaker (shaking incubator) set in 150 rpm during 96 hours. At 0 h, 24 h, 48 h, 72 h and 96 h, samples of 8 ml were collected for α-glucosidaseactivity and pH assay. The samples were centrifuged at 5000 rpm at 4°C for 20 mn. The supernatants were collected and α-

glucosidase assay was carried out using p-Nitrophenol -α-D- glucopyranoside (p-NPG) method.

α-glucosidase assay

α-Glucosidase activity was assayed by measuring the release of p-nitrophenol from the substrate p-nitrophenyl- α -D-glucoside (p-NPG). An assay mixture (0.25 ml) consisting of a 0.1 M phosphate-potassium (pH 7.0), 15 mM of p-NPG and enzyme solution. After incubation for 20 min at 40 °C, the reaction was stopped by the addition of Na_2CO_3 at a concentration of 0.1 M, and absorbance of the reaction mixture was measured at 410 nm. One unit of the enzyme activity was defined as the amount of enzyme liberating 1 µmol of p-nitrophenol per minute.

Effect of physicochimicals parameters on α-Glucosidase production

Effect of incubation time

The effect of incubation time on enzyme production was investigated by checking the enzyme activity on 0 h, 24 h, 48 h, 72 h and 96 h of incubation of fermentation media (pH 7) in orbital shaker (Shaking incubator, Biobase) at 30 °C set in 150 rpm. α-glucosidase activity was assayed by measuring the release of p-nitrophenol from the substrate p-nitrophenyl- α -D-glucoside (p-NPG).

Effect of incubation temperature

The effect of incubation temperature on enzyme production was investigated by fermentation in different substrates and incubated at 28 °C, 30 °C, 37 °C and 40 °C at pH 7 in orbital shaker (shaking incubator, Biobase) set in 150 rpm for 48 hours. α-glucosidase activity was assayed by measuring the release of p-nitrophenol from the substrate p-nitrophenyl- α -D-glucoside (p-NPG).

Effect of medium pH

The effect of pH on enzyme production was investigated by adjusting the pH of differents fermentation media to 4, 5, 6, 7, 8, 9 and 10. The media were incubated at 30 °C in orbital shaker (shaking incubator, Biobase) set in 150 rpm for 48 hours. α-glucosidase activity was assayed by measuring the release of p-nitrophenol from the substrate p-nitrophenyl- α -D-glucoside (p-NPG).

Effect of agitation speed

The effect of agitation speed was investigated by incubating a differents media at 0 rpm, 50 rpm, 100 rpm, 150 rpm and 180 rpm in orbital shaker (shaking incubator, Biobase) at 30 °C for 48 hours.α-glucosidase activity was assayed by measuring the release of p-nitrophenol from the substrate p-nitrophenyl- α -D-glucoside (p-NPG).

Statistical assay

The results obtained during this study were the subject of a statistical processing with software R version 3.2.2. The averages obtained from three values were compared by variance analysis (ANOVA), then by Turkey test with level of significance 5%.

Results and Discussion

Effect of time incubation on α-glucosidase production

The enzymatic activities obtained during the fermentation carried out with 12 hours of preculture are recorded in the table 1. All enzymatic activities are increased to the beginning until the 48th hour of fermentation. The maximum activities were respectively to 5.592 ± 0.18 U/mL, 3.00 ± 0.146 U/mL, 9.147 ± 0.098 U/mL and 4.334 ± 0.22 U/mL for *S. cerevisiae* C8-5, *C. tropicalis* C0-7, the

cocultures (1:1) and (2:1) in medium formulated by 1 % of corn starch. In medium formulated by 1 % of millet starch, important activities were obtained by a pure culture of *S. cerevisiae* C8-5 after 48 hours of fermentation. The activity value was 6.37 ± 0.53 U/mL. The cocultures (1:1) and (2:1) are enregistred their important activities at 72 hours of fermentation with a respective values of 4.88 ± 0.34 U/mL and 4.75 ± 0.54 U/mL.After 48 hours, all activities are dicreased.Overall, pH values experienced slight acidification during the first 24 hours before becoming basic for the rest of fermentation.

The most important activities were obtained in a pH range between 6.75 and 7.2, whether its corn starch or millet starch that is used as a carbon source. This observation is similar than Muhammad *et al.,* (2014) and Muhammad *et al.,* (2016) studies. These authors obtained α-glucosidase activity from *Bacillus licheniformis* KIBGE-IB4 at pH 7 using medium with a sweet potato skin and a synthetic medium respectively. This slight acidification could be due to the organic acids synthesis during fermentation by the strains used. The most important α-glucosidase activities for cultures observed at 48 hours of fermentation has an interesting industrial aspect in terms of production time, especially for pure cultures of *S. cerevisiae* 8-5 and *C. tropicalis* C0-7, unlike other fungal strains that produced enzymes after 120 hours of fermentation (Gianesi *et al.,* 2006). However, it should be noted that the optimum time of fermentation varies from one microbial species to another for the production of enzymes. Thus the maximum production of xylanase by *Geobacillus stearothermophilus* KIBGE-IB29 was postponed to 24 hours of fermentation while *Aspergillus hortai* showed maximum productivity of endo-1,4-β-D-glucanase after 96 hours of fermentation (Bibi *et al.,* 2014; El-Hadi *et al.,* 2014).The study of Ackan *et al.,* (2011) also showed that the production of α-amylase by *Bacillus subtilis* RSKK96 required 72 hours of fermentation. In the case of our study, after 48 hours of incubation for all cultures, the α-glucosidic activity decreases. This decrease could be attributed to the alteration of the medium pH, the scarcity of nutrients or the synthesis of inhibitory metabolites (Pérvez *et al.,* 2015).

Effect of incubation temperature on α-glucosidase production

The influence of incubation temperature on α-glucosidase activity of the different cultures of the strains is presented in Figure 1. The different activities increase with the increase of incubation temperature. All cultures recorded their highest activity at 37 ° C and were respectively 5.67 U/mL, 3.75 U/mL, 3.85 U/mL and 3.14 U/mL for *S. cerevisiae* C8- 5, *C. tropicalis* C0-7, cocultures (1:1) and (2:1) in medium formulated with 1 % of millet starch (Fig. 1B). In production medium containing 1 % of corn starch, the most important activities were recorded with the pure cultures of *S. cerevisiae* C8-5 and *C. tropicalis* C0-7 with respective activity values of 10.924 U / mL and 8.148 U / mL (Fig. 1A) at 37 °C. Above this temperature (37 °C), all activities recorded a slight decrease in their values. Overall, the pure cultures of the strains yielded the largest activities compared to cocultures.

Incubation temperature is one of the most important environmental factors that directly affects the growth of microbial cells and the synthesis of different biomolecules by controlling the physiological activities and microorganisms metabolism (Irfan *et al.,* 2014; Garciaa *et al.,* 2015). The incubation temperature still plays a key role in the production and stability of the enzymes. Of the two environment types, the best activities were obtained with pure cultures at 37 °C.

Since the strains used for the enzyme production are a mesophilic nature, they are capable to produce α-glucosidase at 37 ° C. The optimum temperature for microbial growth usually varies from one microorganism to another and depends on mesophilic or thermophilic nature of microorganism (Kumar and Takagi, 1999).A similar result was observed with the use of *Bacillus licheniformis* KIBGE-IK4 for maximum production of maltase at 37 °C (Muhammad *et al.,* 2014). The decrease of α-glucosidase activity observed above 37 °C could be justified by the fact that high temperatures would improve the metabolic activity of microbial cells by increasing the kinetic energy of biomolecules. After reaching a certain level of speed, these biomolecules will begin to denature and cause slowing or stopping of microbial cell growth as well as the production of enzyme. Decreasing of α-glucosidase activity above 37 °C could also be justified simply because at high temperature, enzyme production could be negatively affected because of the thermal denaturation of the enzyme.

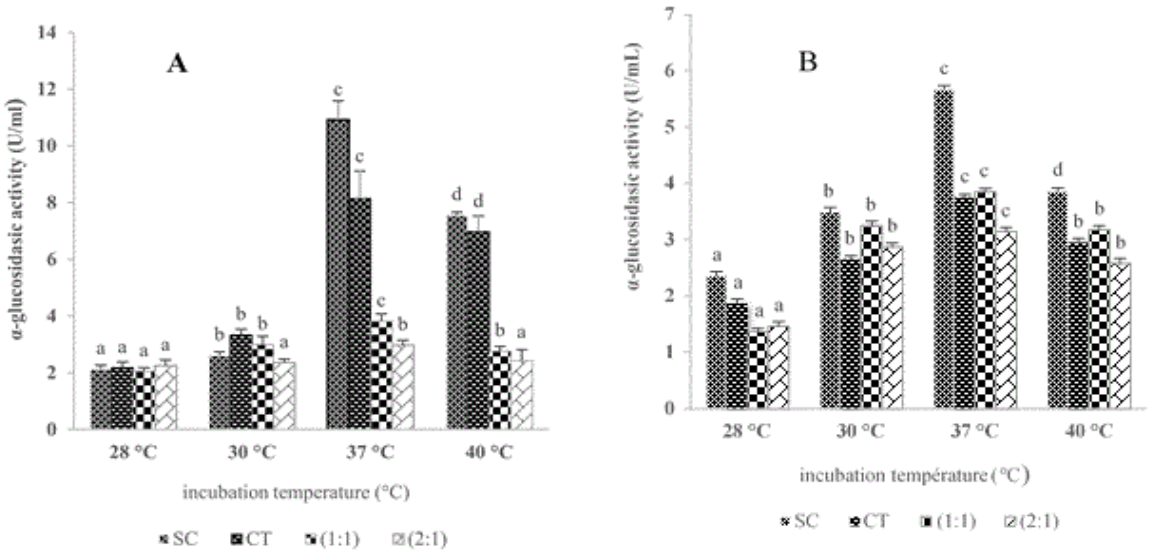
Increasing of temperature beyond the optimal temperature may also alter the structure of the cell membrane and thereby stimulate protein catabolism, ultimately causing cell death (Amin *et al.,* 2014). Thus, authors such as Pandey *et al.,* (2000) and Vidyalakshmi *et al.,* (2009) meant that high temperatures slowed the growth of bacteria and thus inhibited enzyme production. Our results are similar to those obtained by Karim *et al.,* (2015) who, after studying the production of endo-1,4-β-D-glucanase by *Bacillus licheniformis* KIBGE-IB2, found that activity above 37 °C had decreased. Indeed, higher temperatures (> 40 °C) and a large variation in physiological pH lead to denaturation of enzymes, which limits the use of these macromolecules in non-physiological conditions (Rajendra *et al.,* 2016. However, some authors have showed in their studies that α-glucosidase activity can be achieved at incubation temperatures above 37 °C. This is the case of Pant *et al.,* (2015) and Muhammad *et al.,* (2016) who obtained maximal protease and α-glucosidase activities from *Bacillus subtilis* and *Bacillus licheniformis* KIBGE-IK4 respectively at 45 °C and 40 °C. Our study also revealed that all crops recorded the lowest value of their activities at lowest incubation temperature (28 °C). Indeed, the temperature below the optimal microbial growth temperature would be a limiting factor of the facilitated transport of nutrients and the exchange of products between the intracellular medium and the extracellular medium. Low temperatures would significantly decrease both the permeability of the cell membrane and the rate of metabolic reactions (Rajoka *et al.,* 2006).

This result is different from that recorded in other studies that have mentioned optimal production temperatures of the enzyme close to 37 °C. This is the case of the production of α-amylase by *Bacillus subtilis* and *Bacillus* sp at 35 °C (Krishna and Chandrasekaran, 1996; Vidyalakshmi *et al.,* 2009).

Effect of medium pH on α-glucosidase production

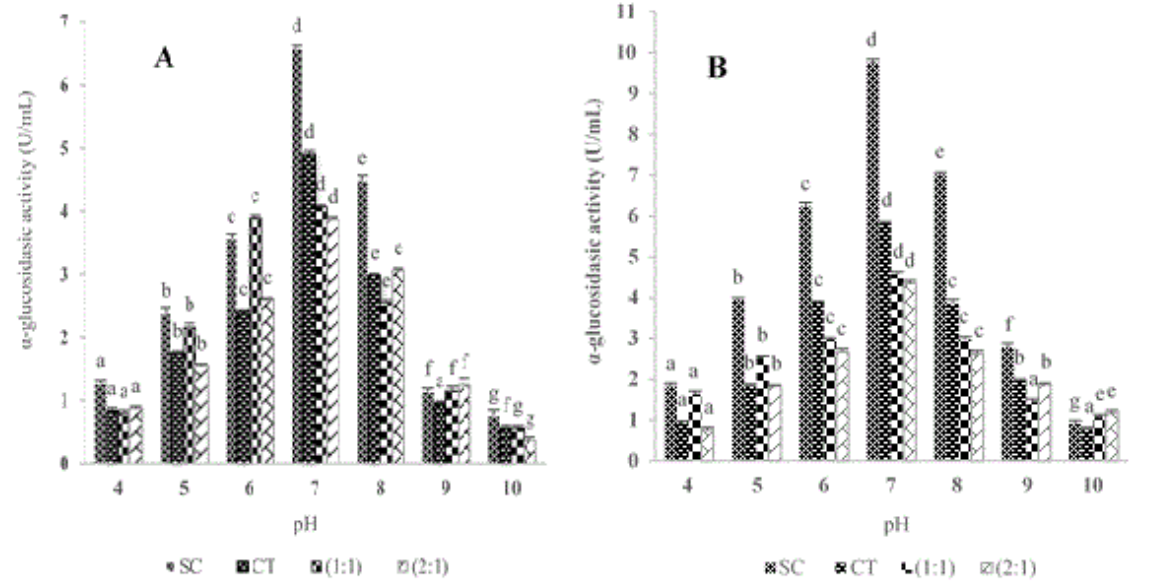
The effect of the pH of fermentation medium on α-glucosidase production is showed in Figure 2. Thus the activities of the strain cultures increase from pH 4 to pH 6 to reach their maximum at pH 7. These maximums are 6.587 U/mL, 4.903 U/mL, 4.075 U/mL and 3.875 U/mL respectively for *S. cerevisiae* C8-5, *C. tropicalis* C0-7, cocultures (1:1) and (2:1) in medium formulated with 1 % of millet starch (Fig. 2A). In medium containing 1 % of corn starch, the important activities were obtained at pH 7 and are respectively 9.764 U/mL, 5.835 U/mL, 4.578 U/mL and 4.352 U/mL for *S. cerevisiae* C8-5, *C. tropicalis* C0-7, cocultures (1:1) and (2:1) (Fig. 2B).

Fig.1 Effect of incubation temperature on α-glucosidase production (A: 1% of corn starch; B: 1% of millet starch)



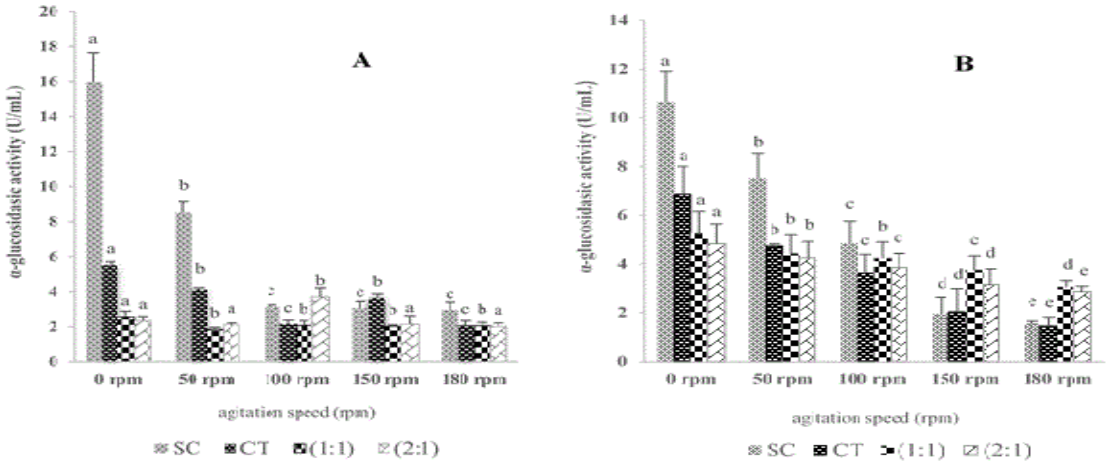
NB: The bonds of same colour carrying the same letters do not present a significant difference to the level of 5%

Fig.2 Effect of fermentation medium pH on α-glucosidase production (A: 1% of millet starch; B: 1% of corn starch)



NB: The bonds of same colour carrying the same letters do not present a significant difference to the level of 5%

Fig.3 Effect of agitation speed on α-glucosidase production (A: 1% of corn starch; B: 1% of millet starch)



NB: The bonds of same colour carrying the same letters do not present a significant difference to the level of 5%

Table.1 Effect of time incubation on α-glucosidase production (A: 1 % of millet starch; B: 1 % of corn starch 1 %)

A		Fermentation time (Hour)				
		0	**24**	**48**	**72**	**96**
SC	**Enzyme activity (U/mL)**	0 ± 0 ^{a}	3.64 ± 0.65 ^{b}	6.37 ± 0.53 ^{c}	4.56 ± 0.45 ^{d}	3.75 ± 0.087 ^{b}
	pH	7 ± 0	6.62 ± 1.05	6.94 ± 0.97	7.14 ± 1.02	7.47 ± 0.89
CT	**Enzyme activity (U/mL)**	0 ± 0 ^{a}	3.45 ± 0.76 ^{b}	4.84 ± 0.87 ^{c}	4.27 ± 0.57 ^{d}	3.55 ± 0.35 ^{b}
	pH	7 ± 0	6.53 ± 0.78	6.76 ± 0.89	6.98 ± 0.98	7.23 ± 0.75
(1:1)	**Enzyme activity (U/mL)**	0 ± 0 ^{a}	3.38 ± 0.336 ^{b}	4.12 ± 0.65 ^{c}	4.88 ± 0.34 ^{d}	3.78 ± 0.27 ^{e}
	pH	7 ± 0	6.48 ± 0.63	6.71 ± 0.75	7.05 ± 0.88	7.23 ± 1.12
(2:1)	**Enzyme activity (U/mL)**	0 ± 0 ^{a}	3.33 ± 0.47 ^{b}	3.89 ± 0.43 ^{c}	4.75 ± 0.54 ^{d}	3.67 ± 0.58 ^{c}
	pH	7 ± 0	6.56 ± 0.95	6.86 ± 0.87	7.12 ± 0.67	7.48 ± 0.88

		Fermentation time (Hour)				
		0	**24**	**48**	**72**	**96**
SC	**Enzyme activity (U/mL)**	0 ± 0 ^{a}	2.17 ± 0.21 ^{b}	5.59 ± 0.18 ^{c}	3.655 ± 0,58 ^{d}	3.417 ± 0.32 ^{d}
	pH	7 ± 0	6.7 ± 0.05	6.83 ± 0.02	7.45 ± 0,065	7.87 ± 0.035
CT	**Enzyme activity (U/mL)**	0± 0 ^{a}	2.27 ± 0.232 ^{b}	3 ± 0.146 ^{c}	2.057 ± 0.3 ^{b}	1.757 ± 0.18 ^{d}
	pH	7 ± 0	6.85 ± 0.015	6.95 ± 0.03	7.23 ± 0.025	7.64 ± 0.035
(1:1)	**Enzyme activity (U/mL)**	0 ± 0 ^{a}	2.436 ± 0.24 ^{b}	9.14 ± 0.098 ^{c}	2.71 ± 0.31^{d}	2.037 ± 0.07 ^{e}
	pH	7 ± 0	6.64 ± 0.0208	6.76 ± 0.015	7.03 ± 0.015	7.26 ± 0.015
(2:1)	**Enzyme activity (U/mL)**	0 ± 0 ^{a}	3.35 ± 0.75 ^{b}	4,33 ± 0.22 ^{c}	3.5 ± 0.16 ^{b}	2.137 ± 0.248 ^{d}
	pH	7 ± 0	6.55 ± 0.025	6.86 ± 0.032	7.12 ± 0.025	7.55 ± 0.025

NB: On the same line, the values carrying the same letters do not present a significant difference to the level of 5 %

A pure culture favorised more production of α-glucosidase compared to cocultures and the most important activities were obtained with *S. cerevisiae* C8-5. Beyond pH 7, strain cultures are experiencing a gradual decline in activity and this decrease is accentuated between pH 9 and 10 where all activity values are less than 1 U/mL.

Among the parameters influencing enzyme production, pH is also a very important chemical factor affecting microbial growth and metabolite production (Banargee and Bhattacharya, 1992). In fact, the growth of microorganisms as well as the progress of many enzymatic reactions, are strongly influenced by the pH which in turn influences the enzymatic structure and the ions transport, metabolites and enzymes across the cell membrane (Liang *et al.,* 2010). The results of our study showed that the production of α-glucosidase was better ensured when fermentation media were brought to pH 7, regardless of the carbon source used. Most bacterial and fungal strains can be grown at pH 6.0-7.0 for growth and enzyme production (Castro *et al.,* 1992; Gupta *et al.,* 2003). Optimum pH played an important role in the enzyme production because it can either lower the production of the enzyme by inhibiting the growth of the microorganism, or by creating an inadequate toxic environment that leads to denaturation or inactivation of the enzyme produced (Bajaj and Abbass, 2011). During the production of enzymes, microorganism were generally confronted at two different pH that are the pH of culture medium or external pH and the intracellular pH or internal pH. In the majority of microorganisms, the internal pH value is close to neutral pH. Thus the production of α-glucosidase recorded in our study would be facilitated by the dependence between the two types of pH. Indeed, the molecules are transported towards inside of cell under the influence of external pH. Once inside the cell, these molecules are used by the internal pH for protein production, enzymatic activities and excretion of metabolites during fermentation (Liu *et al.,* 2003). Thus extreme pH values could damage the cell membrane and thus cause cell death (Liu *et al.,* 2003). The production of enzymes with near-neutral pH has been mentioned in several studies. This is the case of carboxymethylcellulase (CMcase) and of the alkaline protease produced respectively by *Aspergillus hortai* and *Bacillus* sp in liquid fermentation medium at an optimum pH to 7 (El-Hadi *et al.,* 2014; Ibrahim *et al.,* 2015). However, any change in medium pH may influence the ionization state of various nutritionally important components and may also reduce their availability to microbial cells. This pH change could lead to the accumulation of toxic fermentation products, which would inhibit a multiplication of microbial cells responsible to enzymes secretion. Indeed enzymes reach their maximum activities at their different optimal conditions and therefore, would be inhibited by any fluctuation of these conditions (Bisswanger, 2014). At optimum pH, the catalytic site of enzyme is at ionization level. For this reason any change in the optimal incubation temperature could affect the integrity of secondary, tertiary and quaternary structure of the enzyme which, in turn would affect enzymatic activity (Meryandini *et al.,* 2006).

Effect of agitation speed on α-glucosidase production

The study of the effect of agitation speed on the production of α-glucosidase is shown in Figure 3. The α-glucosidase activity decreased with the increasing of agitation speed. Thus the most important activities were obtained when the fermentation media are not subjected to agitation (0 rpm). In medium containing 1 % of corn starch (Fig. 3

A), *S. cerevisiae* C8-5 recorded the highest activity at 15.96 U/mL followed by *C. tropicalis* C0-7 at 5.532 U/mL.

In medium formulated with 1 % of millet starch, the important activities are obtained from pure cultures in particular, that of *S. cerevisiae* C8-5 with an activity of 10.657 U/mL. As for cocultures, the best activities are obtained with coculture (1: 1) and are between 6.854 U/mL and 3.063 U/mL respectively at 0 rpm and 180 rpm (Fig. 3B). Overall, fermentations from pures cultures of *S. cerevisiae* C8-5 and *C. tropicalis* C0-7 resulted in significantly higher α-glucosidase activities than those obtained from media formulated with cocultures (1:1) and (2: 1).

To achieve a good fermentation in a liquid medium, agitation is one of the most important parameters that influenced the production of enzymes. According to Jang and Chang (2005), agitation affects oxygen mass transfer and would be a crucial factor for enzyme production during fermentation. The results of our study are revelated that α-glucosidase production was greater in the media subjected to an agitation speed of 0 rpm and with the pure cultures of the strains. In fact, the uniform distribution of nutrients and the oxygen demand satisfaction during the fermentation are under the control of the agitation speed (Andhyaru *et al.,* 2014). These results could be explained by the fact that high agitation rates would have a negative impact on cell growth and thus on enzyme production (Xia *et al.,* 2014). This remark explains the low activity values obtained in our work when were subjected to higher agitation speeds (> 100 rpm).

Indeed, the fermentation was carried out in Erlenmeyer flasks of 250 ml capacity with 50 ml in order to have a volume of oxygen corresponding to quarter of total volume of Erlenmeyer flask. Thus during fermentation, the agitation speed would favor a uniform distribution of the oxygen mass given the aerobic nature of the strains. This oxygen mass which, subjected to a high speed of agitation would be quickly exhausted and, consequently, would put the strains in a state of stress. The latter would produce other metabolites that either inhibit the enzyme production or would be inactive for its production. In contrast to media subjected to low agitation speeds, the oxygen quantity would be less quickly exhausted and facilitate the production of the desired enzyme. This observation was made by Asisipho *et al.,* (2017) during the cellulase and xylanase production by *Bacillus* species. These authors mentioned that beyond 100 rpm the production of xylanase was negatively affected.

The study allowed us to quantify the amylolytic and especially α-glucosidase activities of *S. cerevisiae* C8-5 and *C. tropicalis* C0-7 strains. The results obtained revealed that the use of a 12 hours preculture made it possible to obtain the most important enzymatic activities in this work. After 48 hours of fermentation, all strain cultures had reached their maximum activity. *S. cerevisiae* C8-5 was the most prolific strain for α-glucosidase production. The medium enriched with 1% corn starch favorized the production of this enzyme with a pure culture of *S. cerevisiae* C8-5. The optimization of the parametres of α-glucosidase production was concerned the variation of fermentation time, pH of fermentation medium, agitation speed and the incubation temperature. The study of these parameters has revelated that α-glucosidase production was maximum at 48 hours of fermentation, at pH 7, at temperature of 37 °C and without agitation of fermentation medium (0 rpm). Thus, our strains are produced amylolytic activity and particularly α-glucosidase activity which has been greater with a pure culture of *S. cerevisiae* C8-5. The

corn starch and millet starch could be used as carbon source for α-glucosidase production. That would reduce the synthetic starch dependence and could constituite a veritable substrate for industrial production of enzyme.

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