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Production, Partial Purification and Characterization of Two α-Amylase Isoforms from *Saccharomyces cerevisiae* strain YOP 1/2-2 Isolated from *Tchapalo* (Côte d'Ivoire)

Ouattara Lacinan[1], Koné Fankroma Martial Thierry[2*], Djoudy Alix Evrard[2], N'guessan Kouadio Florent[1] and Dabonné Soumaïla[2]

[1]Laboratory of Biotechnology and Food Microbiology, Department of Food Science and Technology, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.
[2]Laboratory of Biocatalysis and Bioprocessing, Department of Food Science and Technology, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Authors OL and KFMT designed the study, wrote the first draft of the manuscript and managed literature searches. Author DAE wrote the protocol and managed the analyses. Author NKF did the sample collection. Author DS supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Amylases play an important role in biotechnology and find applications in several industrial fields such as pharmaceutical, food, paper, cosmetics and detergents. Thus, it appears necessary to identify new sources of amylase, especially from microbial origin, due to the low energy consumption, cost-effective, high metabolic diversity, rapid cell growth, non-toxic and eco-friendly characteristics. In the present report, we carried out the production and partial purification of α-amylase by *Saccharomyces cerevisiae* strains isolated from *Tchapalo*, a traditional alcoholic beverage of Côte d'Ivoire. Five fungal isolates were screened initially for α-amylase production by using method of wells on Yeast Extract Peptone Dextrose Agar medium, a complete medium for yeast growth. One step DEAE-Sepharose Fast Flow was achieved for partial purification of α-

**Corresponding author: E-mail: fankrom@yahoo.fr;*

amylase obtained. Among yeasts, isolate *S. cerevisiae* YOP 1/2-2 was able to provoke starch hydrolysis halo of 15.067±0.12 mm on starch agar plate after 48 h of incubation at 30°C. The partial purification of resulting enzyme showed two protein peaks, designated α-amylase 1 (AMY1) and α-amylase 2 (AMY2) with amylolytic activity and specific activities of 1.57-1.58 U/mg protein. Both isoforms (AMY1 and AMY2) were thermostable with optimal activity at 50 and 55°C, respectively, and at pH ranged from 4.5 to 5.3 in 0.1 M sodium acetate buffer. EDTA and Cd^{2+} strongly inhibited α-amylase activity by 72-75% and 64-65%, respectively, whereas cations Ca^{2+} and Mn^{2+} showed 85-99% and 71% increased amylolytic activity, respectively. All these properties show the potential uses of α-amylases from *S. cerevisiae* in the industrial transformation of starch.

Keywords: Saccharomyces cerevisiae YOP 1/2-2; α-amylase; purification; characterization; Tchapalo.

1. INTRODUCTION

α-Amylase [α-(1,4)-D-glucan glucanohydrolase (E.C.3.2.1.1)] is an endoenzyme that belongs to family 13 of glycoside hydrolases (GH13) [1,2]. It randomly hydrolyzes osidic bonds of amylose, amylopectin, starch, glycogen and other polysaccharides containing three or more α-(1,4)-D-glucose bonds [3]. Amylases can be found in several sources (plants, animals and microorganisms) and are among the most important enzyme classes having approximately 25-30% of the world enzyme market [2,4]. However, those of microbial origin (bacterial and fungal) have found their application in biotechnology and into a wide range of industrial processes: food industry, pharmaceutical, textile, distilling, fermentation, paper and detergent [2,5]. With the increasing interest of fungal amylases, several investigations were carried out on extracellular amylolytic enzymes secreted by species such as *Aspergillus* [1,2], *Rhizopus* [3], *Talaromyces pinophilus* [6], *Lipomyces, Saccharomycopsis* and *Schwanniomyces* [7].

In recent years, researches have been done on amylase production by thermophilic microorganisms [4,8]. Amylases with thermostable and acidophilic properties play an important role in starch industry, during saccharification and liquefaction processes [9,10]. Also, the use of fungal α-amylases is increasingly in demand because of the ease of their culture, the cost effectiveness and the absence of pathogenic risks (GRAS: Generally Recognized as Safe) [11,12]. Indeed, microorganisms grow much faster as compared to both plants and animals, which help to speed up enzyme production [2]. The α-amylase properties such as thermostability, pH profile, pH stability, and Ca^{2+}-independency are critical in the development of fermentation process [13].

During the production of *Tchapalo*, a traditional sorghum beer from Côte d'Ivoire, lactic acid bacteria and yeasts have been identified as fermentative microorganisms [14]. These are involved in the spontaneous fermentation step of *Tchapalo* process, thus giving a product with widely varying quality [14,15]. Moreover, *Saccharomyces cerevisiae* (an edible yeast) was found to be the predominant species isolated from the fermentative yeast flora found in *Tchapalo* [16]. Previous studies have also revealed the enzymatic potentialities of *S. cerevisiae* to produce various enzymes including α-glucosidase and α-amylase [17].

Indeed, these enzymes are involved in the biodegradation of starch, the predominant substrate of *Tchapalo*. α-Glucosidase is the last enzyme involved in the catabolism of starch to glucose; it therefore acts after α-amylase hydrolysis. However, previous work has focused only on the production of α-glucosidase from strains of *S. cerevisiae* C8-5 [17,18]. Furthermore, few studies have described the production, purification and biochemical characterization of α-amylase produced by the yeast *S. cerevisiae*.

This study is the first report on the production capacity of α-amylase under submerged fermentation, by a local fungal isolate *S. cerevisiae*. The partial purification and characterization of thermostable extracellular α-amylase from *S. cerevisiae* isolated from *Tchapalo* were also studied.

2. MATERIALS AND METHODS

2.1 Microorganism

Five strains of *Saccharomyces cerevisiae* (*S. cerevisiae* TPA, *S. cerevisiae* TLM1-6, *S. cerevisiae* YOP 1/2-2, *S. cerevisiae* TLM1-8, *S. cerevisiae* BINGER TA1-1) used in this work were belonged to the culture collection of the Food Science and Technology Department (University NANGUI ABROGOUA, Côte d'Ivoire).

These five strains were randomly selected between all strains of *S. cerevisiae* previously isolated from samples of traditional sorghum beer (*Tchapalo*) from the district of Abidjan (Côte d'Ivoire) [19]. Theirs identifications were performed by PCR-RFLP of ITS region and sequencing of D1/D2 domains of 26S rRNA gene [19].

2.2 Media and Culture Conditions

Isolation of yeast was performed on Yeast Extract Peptone Dextrose (YPD) medium, containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose and 15 g/L agar, supplemented with 0,5 g/L chloramphenicol, allowing growth inhibition of Gram positive and negative bacteria. Colonies were formed and sub-cultured on YPD agar plates by streak plate technique and re-incubated at 30°C for 24 h to form pure colonies [20]. Cell morphology and homogeneity were studied after coloration of obtained colonies with methylene blue, and their observation with an optical microscope at 40X magnification [21]. Colonies with distinct morphological differences such as color, shape and size were selected.

2.3 Amylase Screening

Among the five strains of selected yeasts, it was necessary to select the strain with best performance for amylase production. The amylolytic power of microbial strain was determined by inoculating 10 µL of each isolate in 3 mm deep micro-wells on the surface of starch agar plate (10 g/L soluble starch, 10 g/L yeast extract, 10 g/L peptone and 0.5 g/L chloramphenicol) according to the method of Fossi et al. [11].

The plates were incubated at 30°C during 48 h, before measuring the diameter of each hydrolysis zone. The appearance of a clear hydrolysis zone after addition of Lugol's iodine solution revealed the amylase activity of strain. The amylolytic power was defined as the average diameter (mm) of hydrolysis halo provoked by a strain. The most active strains were selected as potential microorganisms for amylase production and all plates were stored at 4°C for further experiments.

2.4 Inoculum Preparation

Inoculum preparation was carried out according to the method described by Bataiche [22]. The yeast isolate stored in refrigerator (4°C) was sub-cultured and incubated at 30°C for 24 h on YPD agar medium. A pure colony (24 h) of microorganism was inoculated in 250 mL Erlenmeyer containing 50 mL of YPD medium supplemented with chloramphenicol 0.5 g/L. The inoculated medium was then incubated at 30°C during 12 h under shaking conditions (120 rpm).

2.5 Amylase Production

Extracellular amylase was produced in submerged fermentation. After cultivating at 48°C for 2 days, microorganisms with a clear halo around the spot were inoculated into the liquid cultivation medium. Thus, the production was carried out in 250 mL Erlenmeyer flask containing 6 mL of inoculum and 54 mL of liquid medium (1% yeast extract, 1% peptone, 1% soluble starch, 0.05% chloramphenicol) for enzyme production. The cultures were incubated at 30°C in orbital shaker (shaking incubator) at 150 rpm for 72 h. During starch hydrolysis, samples of 8 mL were collected each 12 h and centrifuged at 7500 rpm at 4°C for 20 min. The obtained supernatant was used for α-amylase activity, total protein content and pH assay. After fermentation, the samples were centrifuged at 7500 rpm and 4°C for 20 min and the supernatant constituted the crude amylase extract.

2.6 Enzyme Assay

Enzyme activity was determined by Bernfeld method [23], using 3,5-dinitrosalicylic acid (DNS) reagent to measure reducing sugars released during amylolysis. The reaction mixture containing 100 µL of enzyme solution with 200 µL of 1% (w/v) soluble starch in 0.1 M sodium acetate buffer (pH 5.6) was incubated for 30 min at 40°C [24]. After incubation, the reaction was stopped by adding 300 µL of DNS reagent. The reaction mixture was heated for 5 min in boiling water bath, then cooled on ice for 5 min before reading the absorbance at 540 nm using a spectrophotometer (INESA, Chine).Under the defined reaction conditions, one unit of enzyme activity was defined as 1 µmol of glucose released per min. Specific activity was expressed as the units of enzyme activity per mg of protein.

2.7 Protein Estimation

The protein content of the purified enzyme was determined according to the spectrophotometric assay of Bradford using a Coomassie brilliant

blue [25]. Bovine serum albumin was used as standard.

2.8 Partial Purification Procedure

The partial purification procedure was performed in cold room with 20 mM sodium acetate buffer (pH 5.6). The crude enzyme extract was loaded onto a DEAE-Sepharose Fast Flow column (2.5 x 4.0) equilibrated with the same acetate buffer. After washing the column with two bed volumes of acetate buffer, elution with a 50 mL increasing discontinue gradient (0 M; 0,2 M; 0,4 M; 0,6 M; 0,8 M; 1 M) of NaCl dissolved in 20 mM sodium acetate buffer (pH 5.6) was applied to the column. Proteins were eluted with a flow rate of 2 mL/min and fractions of 2 mL were collected. Protein content and α-amylase activity were determined in each individual purified fraction. The active fractions containing the majority of α-amylase activity were pooled and stored at 4°C for subsequent analyses.

2.9 Physicochemical Characterization of Partial Purified Enzyme

2.9.1 Effect of pH on activity and stability

The effect of pH on α-amylase activity was performed at 40°C under standard test conditions by measuring the hydrolysis of 1% soluble starch solution in a series of buffers (0.1 M) at various pH values (3.0 to 7.0). Buffers used were citrate phosphate buffer (pH 3.0 to 7.0) and sodium acetate buffer (pH 3.6 to 5.6).

For the pH-stability study, the enzyme solutions were preincubated during 15 min at 40°C in 0.1 M sodium acetate buffer at various pH values between 3.6 and 5.6. Then, the residual activity was measured under the standard assay conditions after adding substrate solution.

The results were expressed as percentage activity of zero-time control of untreated enzyme.

2.9.2 Effect of temperature on activity and stability

The temperature optimum of partial purified α-amylase was determined in 0.1 M sodium acetate buffer (pH 5.6) at temperatures ranging from 35 to 65°C under standard test conditions.

The study of thermal denaturation consisted in preincubation of partial purified enzyme at different temperatures ranging from 35 to 65°C for 15 min. Then the enzyme was incubated in 0.1 M acetate buffer (pH 5.6) under standard test conditions.

The thermal stability of the enzyme was determined in 0.1 M acetate buffer (pH 5.6) at 37 and 50°C after exposure to each temperature during 90 min. Aliquots were withdrawn at 15 min intervals and immediately cooled.

The residual enzymatic activity was determined in each case at 40°C under the standard test conditions and expressed as percentage activity of zero-time control of untreated enzyme.

2.9.3 Effect of some chemical agents

The effect of chemical agents (activators or inhibitors) on α-amylase activity was investigated at concentrations of 1 and 5 mM in 0.1 M sodium acetate buffer (pH 5.6). The different solutions of metal ion salts (KCl, NaCl, $CaCl_2$, $CdCl_2$, $MnCl_2$, $MgCl_2$, $CuSO_4$, $ZnSO_4$ and $FeCl_3$) and other chemical compound, ethylene diamine tetraacetic acid (EDTA) were individually mixed with the enzyme and incubated at 40°C for 30 min. Then, the α-amylase activity was measured under standard test conditions. The residual activity was expressed as a percentage of the control without the chemical agent.

2.10 Statistical Analysis

All analyses reported in this study were carried out in triplicate and reported as mean ± standard deviation. The data were analyzed by one-way ANOVA models followed by STUDENT test, with the help of the software STATISCA 7.1 (Statistica, 99th Edition, France).

3. RESULTS AND DISCUSSION

3.1 Amylase Profile of *Saccharomyces cerevisiae* strains

All tested *Saccharomyces cerevisiae* strains were able to grow on YPD agar medium at 30°C for 48 h. Colonies of yeast were usually white, creamy, round, smooth/glistening or dry, convex.

The YPD medium of 1% starch (selective medium) was used for amylase production by yeast isolates. A summary of results from amylase production of yeast isolates is given in Table 1. The result revealed that all strains showed amylase activity (lysis zone greater than 0.5 cm) with variable hydrolysis zone diameters.

Yeast strains showed significant amylase production activity, with diameters of the starch hydrolysis zone between 11 and 15.06 mm. These values were higher to those (5-11 mm) obtained by Shruti et al. [26] after using *Aspergillus flavus* in culture medium containing 1% starch. Among the five strains of yeast tested, the highest amylolytic power was found in the strain *S. cerevisiae* YOP 1/2-2. This isolate provoked a starch hydrolysis halo of 15.06±0.12 mm after its inoculation in 3 mm micro-well on starch agar medium followed by the incubation at 30°C during 48 h. Thus, the strain *S. cerevisiae* YOP 1/2-2 showing the highest amylolytic halo was selected for amylase production in submerged system and further analysis.

3.2 Biochemical Parameters of α-Amylase Production by *S. cerevisiae* YOP 1/2-2

Isolate YOP1/2-2 having the largest clear hydrolysis zone was selected for amylase production in submerged system. Kinetic parameters such pH, protein concentration and amylase activity of isolate during fermentation in liquid medium were screened.

3.2.1 pH evaluation of medium during α-amylase production

The *S. cereviseae* YOP 1/ 2-2 strain, cultivated on YPD medium with 1% starch, caused a relatively large decrease in pH at the beginning of experiment. Indeed, the initial pH = 6.9 decreased to 6.28 after 24 h incubation (Fig. 1). The decreasing pH observed was probably due to the fermentation of carbon sources available in the medium, following the formation of organic acids (succinic, acetic, formic, propionic…) [27], which increase the acidity of the environment [28].

In addition, Saito et al. [29] recorded a drop in pH from 5 to 3.8 after fermentation with *Rhizopus oryzae* ATCC 34612, leading to an accumulation of fumaric and lactic acids synthesized in the medium. Then the pH increased from 24 h to 72 h to reach a value of 6.67. This increase would be due to the degradation of proteins and the catabolism of amino acids releasing ammonia [29]. The increase in pH could also be explained by the metabolism of carbohydrates which releases CO_2 [27].

Table 1. Starch hydrolysis abilities by yeasts using micro-wells assay method

Strain no.	Codes	Diameter of hydrolysis zone (mm)
1	*S. cerevisiae* TPA	11.00 ± 0.01
2	*S. cerevisiae* TLM1-6	11.16 ± 0.29
3	*S. cerevisiae* YOP 1/2-2	15.06 ± 0.12
4	*S. cerevisiae*TLM1-8	12.00 ± 0.01
5	*S. cerevisiae* BINGER TA1-1	11.00 ± 0.02

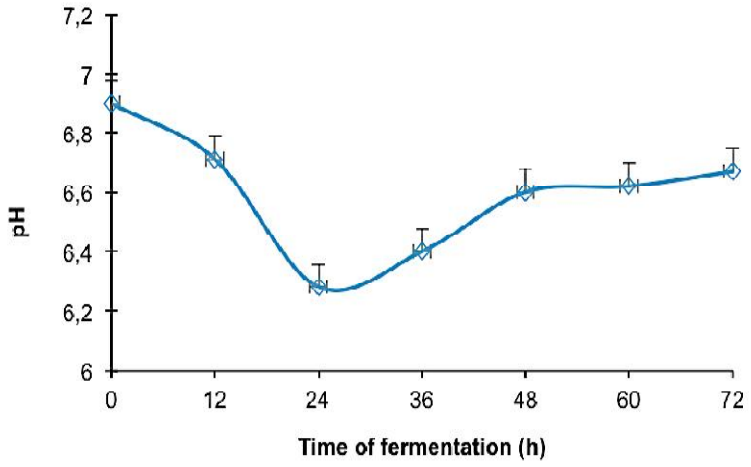

Fig. 1. Evaluation of medium pH during α-amylase production by *S. cerevisiae* YOP 1/2-2

3.2.2 Evaluation of α-amylase activity and protein levels during fermentation

Regarding the amylase activity and protein levels during fermentation, a similarity was observed between these two parameters. Indeed, the profile of amylase activity in the starch-based culture medium and that of protein content are similar. Amylase activity of *S. cerevisiae* YOP 1/2-2 was determined by measuring the reducing sugars released by substrate hydrolysis each 12 h. The amylase activity was expressed by *S. cerevisiae* after 12 h of incubation. It gradually increased to a peak at 24 h, then started to decline until 38 h before stabilizing (<20%) until 72 h (Fig. 2A). These results showed a high increase in amylase production (98%) from 12 to 24 h of hydrolysis in the fermentation medium. High yield of amylase production was reported after 48 h of incubation with some *Streptomyces* species [30,31]. α-Amylase production increased with cell growth. This type of profile has been reported for ascomycete yeasts cultivated on media based on wheat [11] and potato [32].

From 12 h incubation time in the fermentation medium, an increase of protein production was observed until a peak (98%) at 24 h, followed by a gradual decrease until 38 h and then a stabilization (<20%) until 72 h (Fig. 2B). This increasing is probably due to synthesis of enzymes, particularly α-amylase [33]. In addition, it appeared to be compatible with the kinetics of growth and amylase activity. During the decline phase, protein concentration drops below 20% following the proteolytic action of proteases released during cell lysis [34].

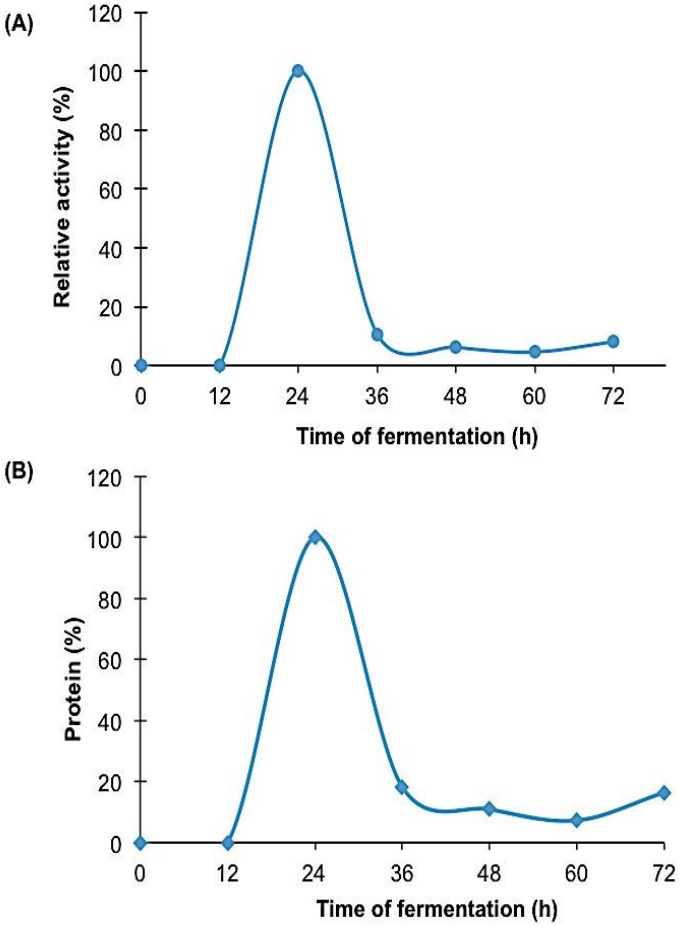

Fig. 2. Evaluation of α-amylase activity (A) and protein level (B) expressed by *S. cerevisiae* YOP 1/2-2 during fermentation

3.3 Partial Purification of α-Amylase

One step DEAE-Sepharose Fast Flow was achieved for partial purification of α-amylase present in the culture supernatant of *S. cerevisiae* YOP 1/2-2. The results of partial purification procedure are summarized in Table 2. Two protein peaks, designated α-amylase 1 (AMY1) and α-amylase 2 (AMY2), showing amylolytic activity were obtained, when washing the column with 20 mM sodium acetate buffer (pH 5.6) and at 0.2 M NaCl concentration (Fig. 3). At this stage, α-amylase AMY1 was 1.08-fold purified at a yield of 55.70% and with a specific activity of 1.57 U/mg protein, whereas AMY2 was 1.09-fold purified at a yield of 54.91% and with a specific activity of 1.58 U/mg protein.

Data recorded in this study are lower than those previously reported [2,35,36]. Indeed, α-amylase from *Bacillus methylotrophicus* strain P11-2 was purified (13.1-fold, 7.0% yield, 330.7 U/mg protein) by ammonium sulfate precipitation, dialysis, anion-exchange chromatography and gel chromatography [36]. The purification of α-amylase from *Aspergillus flavus* NSH9 (3-fold, 11.73% yield, 48.10 U/mg protein) was performed using ammonium sulfate precipitation, dialysis and anion-exchange chromatography [2]. In order to improve the specific activity and the purity level, the crude amylolytic extract from *S. cerevisiae* should be subjected to a series of treatments such as ammonium sulfate precipitation followed by dialysis before loading onto an anion-exchange chromatography column.

3.4 Optimum pH and Stability of α-Amylase

Both isoforms (AMY1 and AMY2) of partial purified α-amylase produced by *S. cerevisiae* have optimal activity at pH ranged from 4.5 to 5.3 in 0.1 M sodium acetate buffer (Fig. 4). Similar results were obtained with a variety of fungal species such as *A. niger* [37], *A. oryzae* [35], *Talaromyces pinophilus* 1-95 [6] and *A. flavus* NSH9 [2], which exhibited optimal amylolytic activity at pH 5.0-6.0. Therefore, AMY1 and AMY2 are acidic amylases like most fungal amylases [38].

Table 2. Purification steps of α-amylase from *Saccharomyces cerevisiae*

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Culture extract	25.31	17.37	1.45	1	100
DEAE-Sepharose Fast Flow					
AMY1	14.10	8.95	1.57	1.08	55.70
AMY2	13.90	8.80	1.58	1.09	54.91

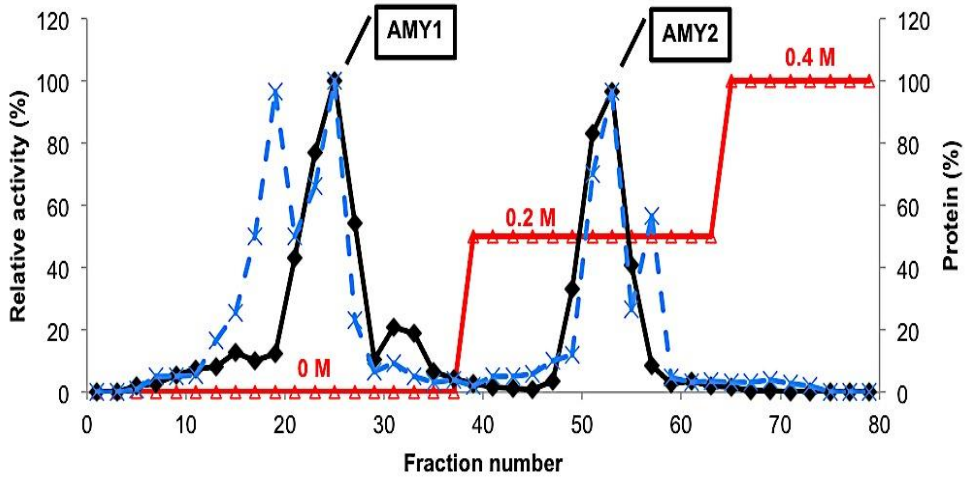

Fig. 3. Anion-exchange chromatography profile on DEAE-Sepharose Fast-Flow of α-amylase from *S. cerevisiae* YOP 1/2-2, showing enzyme activity (♦), chloride sodium gradient (Δ) and protein contents (x)

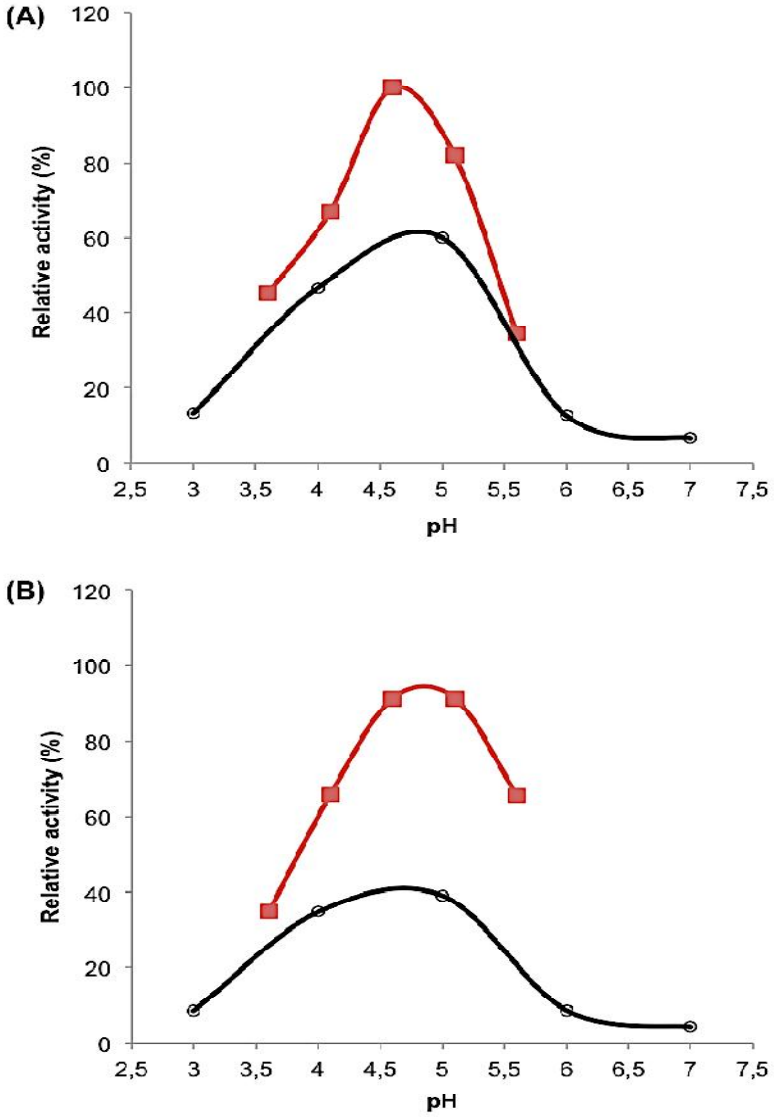

Fig. 4. Determination of optimum pH of partial purified α-amylases AMY1 (A) and AMY2 (B) from *S. cerevisiae* YOP 1/2-2

The buffers used were 0.1 M citrate phosphate buffer (○) from pH 3.0 to 7.0 and 0.1 M sodium acetate buffer (■) from pH 3.6 to 5.6.

The pH stability in 0.1 M sodium acetate buffer showed a maximal stability at pH values ranging from 4.6 to 5.6 for these two *S. cerevisiae* isoenzymes (Fig. 5). Thus, a pH above 5 would be a good compromise between the activity and the stability of the enzyme to achieve the specific hydrolysis of starch over a long time-period. This stability zone of *S. cerevisiae* amylases shows an advantage for wine-producing industries, which have a high tolerance to acidic pH [39]. Results suggest that the partial purified enzymes (AMY1 and AMY2) can be exploited in processes that require acidic pH.

3.5 Optimum Temperature and Thermo-stability of α-Amylase

The effect of temperature on partial purified amylases (AMY1 and AMY2) was determined within a broad pH range from 35 to 65°C (Fig. 6). Amylase AMY1 has a maximum activity at 50°C in 0.1 M sodium acetate buffer (pH 5.6), whereas

that from AMY2 is 55°C. The further increase in temperature resulted in a decrease in both amylolytic activities. Our results were similar to those of fungal thermostable α-amylases from *A. flavus* NSH9 (50°C) [2], *Talaromyces pinophilus* 1-95 (55°C) [6] and *Trichoderma pseudokoningii* (50°C) [40]. However, our values are lower than those of α-amylases from some fungal species (65-90°C) [12] and from the majority of bacterial species (70-100°C) [10,36].

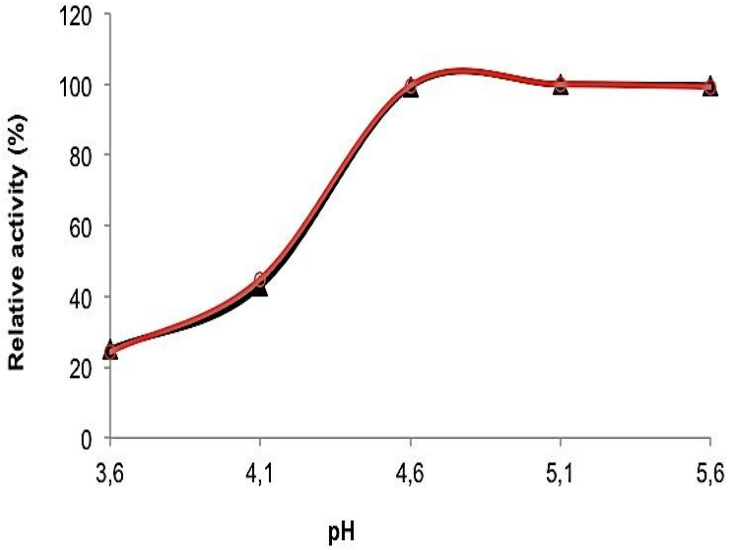

Fig. 5. pH stability of partial purified α-amylases AMY1 (▲) and AMY2 (○) from *S. cerevisiae* YOP 1/2-2

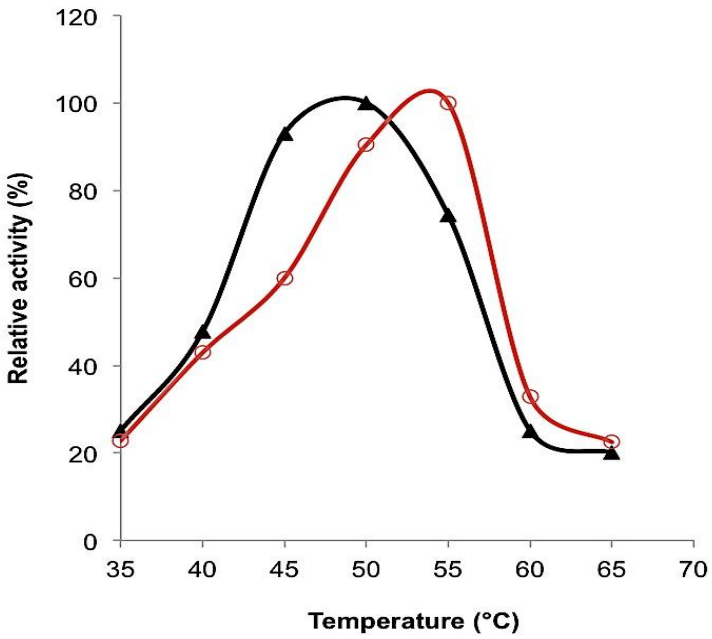

Fig. 6. Determination of optimum temperature of partial purified α-amylases AMY1 (▲) and AMY2 (○) from *S. cerevisiae* YOP 1/2-2

Thermostability is considered an important and useful parameter criterion for industrial application of amylases from microorganisms. As shown in Fig. 7, AMY1 and AMY2 from *S. cerevisiae* were fairly stable at temperature up to 50 and 55°C, respectively. Beyond these respective temperatures, the amylolytic activity decreased as the temperature increased. Results of thermal stability of partial purified α-amylases (AMY1 and AMY2) indicated that amylolytic activities from *S. cerevisiae* remained fully stable during 120 min at 37°C in 0.1 M sodium acetate buffer (pH 5.6) (Fig. 8). At their different optimum temperatures (50°C for AMY1 and 55°C for AMY2), the activities were less stable, showing 97% activity after 15 min of incubation. However, the half-life (50% residual activity) of these isoenzymes was found after 60 and 70 min for AMY1 and AMY2, respectively. It should be noted that thermostability of both α-amylases expressed from *S. cerevisiae* was comparable to that reported for some fungal α-amylases [2].

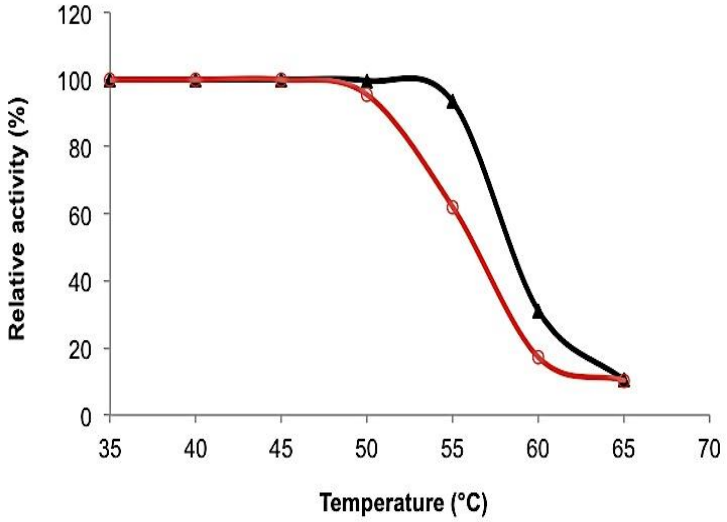

Fig. 7. Thermal denaturation of partial purified α-amylases AMY1 (▲) and AMY2 (○) from *S. cerevisiae* YOP 1/2-2

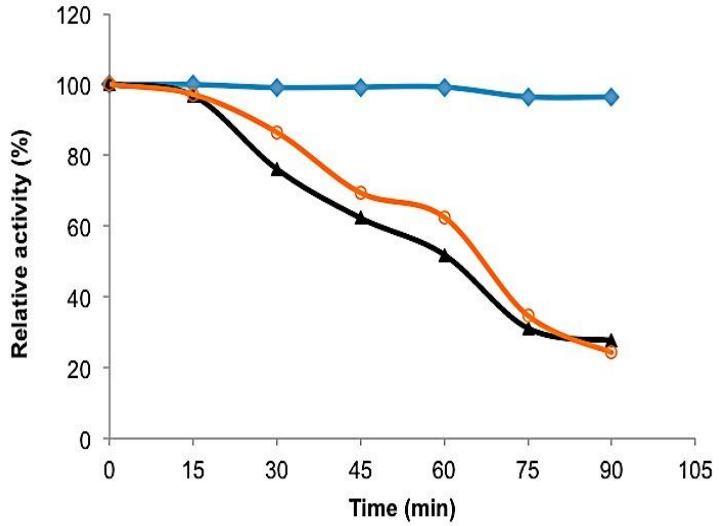

Fig. 8. Thermal stability of partial purified α-amylases from *S. cerevisiae* YOP 1/2-2 at 37°C (♦) and at 50°C (▲ for AMY1; ○ for AMY2)

3.6 Effect of Chemical Agents on α-Amylase Activity

The effect of selective inhibitors or activators on amylolytic activity expressed from *S. cerevisiae* was studied (Table 3). Ions Ca^{2+} and Mn^{2+} act as activators of both α-amylase isoforms (AMY1 and AMY2), whereas Cd^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+} and EDTA act as inhibitors. In addition, K^{+} and Na^{+} seem to not affect the enzyme activities.

Data also showed that the effect of all studied chemical agents on α-amylase activity is much more significant at 5 mM concentration. Indeed, at this concentration Ca^{2+} and Mn^{2+}showed 85-99% and 71% increased amylolytic activity, respectively. These cations may act as cofactors by enhancing the α-amylase activity. In previous work, the enhancement effect of 5 mM Ca^{2+} has already been revealed on α-amylases from *A. flavus* NSH9 (114%) [2] and from *Tepidimonas fonticaldi* strain HB23 (155%) [4]. However, Ca^{2+} was also found to inhibit the activity of some α-amylases, characterized by Ca^{2+}-independent [6,41].

In this study, EDTA and Cd^{2+} strongly inhibited α-amylase activity by 72-75% and 64-65%, respectively, when added at 5 mM to the reaction mixture. Inhibitory effect of EDTA (metal chelator) and Cd^{2+} has been reported in previous studies [36,40]. Agüloglu et al. [41] reported 79% inhibition (with 1 mM EDTA) and 87% inhibition (with 10 mM EDTA) on α-amylase from *Anoxybacillus flavithermus*, while Acer et al. [42] reported 63% inhibition with 1 mM EDTA on α-amylase from *Anoxybacillus sp*. AH1. The inhibition effect of EDTA on activity suggests that α-amylases from *S. cerevisiae* are metalloenzymes, as demonstrated by Lim *et al*. [38]. These enzymes require divalent metal cations such as Ca^{2+} in their active site to be fully active, like most of amylases which are Ca^{2+}-dependent. This would be an advantageous feature because most of the amylases used in industrial processes are reported to be metal ions dependent enzymes [43].

Table 3. Effect of some chemical agents on α-amylase activity from *S. cerevisiae* YOP 1/2-2

Chemical agent	Concentration (mM)	Relative activity (%)	
		AMY1	AMY2
None	0	100	100
KCl	1	90	89.34 ± 0.01
	5	95	96.21 ± 0.09
NaCl	1	92	85.91 ± 0.01
	5	97	99.65 ± 0.02
$CaCl_2$	1	139	140.89 ± 0.01
	5	185	199.31 ± 0.03
$CdCl_2$	1	40	44.00 ± 0.01
	5	36	34.36 ± 0.01
$CuSO_4$	1	58	58.41 ± 0.02
	5	41	41.23 ± 0.03
$MgCl_2$	1	65	65.29 ± 0.01
	5	48	48.10 ± 0.01
$MnCl_2$	1	131	121 ± 0.01
	5	171	171 ± 0.03
$ZnSO_4$	1	65	65.29 ± 0.02
	5	49	44.67 ± 0.02
$FeCl_3$	1	62	61.85 ± 0.01
	5	47	44.67 ± 0.02
EDTA	1	38	37.80 ± 0.01
	5	25	27.49 ± 0.01

4. CONCLUSION

In the present study, the fungus *Saccharomyces cerevisiae* YOP 1/2-2 isolated from *Tchapalo* can be used as a potential source for amylase production under submerged fermentation. Two extracellular α-amylase isoforms (AMY1 and AMY2) were partially purified by one-step anion-exchange chromatography on DEAE-Sepharose Fast Flow and biochemically characterized. Both isoforms exhibited good activity at acidic pH (4.5 to 5.3) and were fairly thermostable at temperatures up to 50 and 55°C. These enzymes would be potential candidates for starch liquefaction processes, which take place in the pH range from 5.00 to 6.50. α-Amylases from *S. cerevisiae* have been also identified as metalloenzymes and appear to be Ca^{2+}-dependent, as they are inhibited by EDTA and activated in the presence of Ca^{2+}. Thus, α-amylase from *S. cerevisiae* exhibits some properties that could be used in profitable industrial fermentation processes and in starch industry. Nevertheless, studies on optimization of enzyme production and purification procedures are needed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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