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Introducing New Peptide Extracts from *Saccharomyces cerevisiae* and *Achatina achatina* Fluids with Strong Inhibitory Activities on Human *α*-Amylase

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Abstract

This study aimed at exploring for new natural peptides with strong inhibitory capabilities on *α*-amylase, the main metabolic enzyme that regulates mellitus diabetes, in order to contribute in controlling this global pandemic. It has consisted in heat shock (to 60˚C, 70˚C, 80˚C, 90˚C and 100˚C for 10, 20 and 30 minutes) of crude proteins extracted from biomass and extracellular parts of *Saccharomyces cerevisiae* under cultivation, and from the digestive fluid of the giant snail *Achatina achatina*, and *in-vitro* assays of the resulting solutions, as effectors, in human *α*-amylase catalyzing reactions. The results showed that whatever the temperature and time of treatment, an increase (from 2.65 to 3.98-fold) in proteins concentration was noticed. When blended up to 75 microliters in reaction mixtures, the three peptide extracts showed beyond 11% of inhibition of initial *α*-amylase activity. By reducing samples volume, only 5 microliters of the studied peptide extracts representing 4.70 µg of *S. cerevisiae* biomass peptides, 0.55 µg of *S. cerevisiae* extracellular peptides or 1.05 µg of peptides from the digestive fluid *A. achatina* were quite sufficient to induce complete (100%) inhibition of the human *α*-amylase activity. Compared to the inhibitory effect obtained from 2.50 µg of acarbose, a renowned antidiabetic, the studied peptide effectors showed more pronounced inhibitory activities. So, we can positively state that *S. cerevisiae* as well as *A. achatina* are both capable of synthesizing proteins made up of small inhibitory peptides which deserve purification and structural analysis for potential exploitation as healthy antidiabetic drugs.

Keywords

α-Amylase Inhibitors, Healthy Antidiabetics, Peptide Drugs, *Saccharomyces cerevisiae*, *Achatina achatina*

1. Introduction

Ranked first among the main non-communicable metabolic diseases as obesity and hypertension, type 2 diabetes which was long considered as a disease specific to rich countries is now approaching the twenty-first century epidemic proportions globally [1] [2]. In this context, it is important that urgent arrangements are made at the level of the world health organization (WHO) for the management of this pandemic-like disease in each member state. This necessarily involves the subsidy of anti-diabetic drugs in order to make them accessible to all.

Fighting diabetes required the use of several therapeutic approaches, mainly involving chemically synthesized molecules such as hypoglycemic sulfonamides, glinides and metformin's [3]. Also, the uses of porcine insulin therapy as well as *α*-glucosidase and *α*-amylase inhibitors such as acarbose have already been reported [4]. However, although these therapies showed significant efficacy, the long-term use of synthetic drugs exposes the patient to harmful effects that can lead to complications such as cardiovascular collapses, renal failure, neurological disorders and heart disease which are all, in the worst case, fatal [5] [6].

To alleviate this problem, the search for new bioactive molecules, with little or no side effects on health, would be a good alternative. Thus, for nearly a century, natural low molecular masses peptides, in particular di- and tri-peptides have been implemented as therapeutic agents in developing countries because of their advantage of being easily assimilated [7]. In addition of having lower risks of toxicity in that their degradation releases amino acids that are essential and beneficial molecules for human health [8], peptides have recently been suspected to have biological activity related to diabetes prevention [7].

Due to their easy access and rapid growth, microorganisms represent an inexhaustible source of peptides and are much more use for research work. This is the particular case of yeasts, which have already proved their worth in the medical field for the production of antibiotics, vaccines and proteins of therapeutic use such as insulin [9]. As an illustration, it has already been reported that the yeast *Saccharomyces cerevisiae* is an important industrial tool [10] for the production of proteins of commercial interest and also considered as a screening tool for new drugs [11]. Beside microorganisms, animals and plants are not to be excluded when it comes to exploring molecules of protein nature. Indeed, some of these biological resources, in addition to be very rich in proteins, are often locally available, inexpensive and easily accessible for research purposes. As an example, the digestive fluid of the African giant land snail *Achatina achatina* represents an abundant source of proteins [12] [13] that deserve further investi-

gation as is the case of this study.

This work is in line with the search for new and/or healthy bioactive molecules with therapeutic properties that can effectively contribute to the regulation of metabolic syndromes such as obesity, hypertension and diabetes. In the present study, available and easily accessible biological material, notably the baker's yeast *S. cerevisiae* and the giant snail *A. achatina*, were used. The experiment consisted in extracting proteins from these bioresources, hydrolyzing them by thermal heating, and testing the resulting peptide extracts, as effectors, in alpha amylase-catalyzing reactions with rice starch as substrate.

2. Materials and Methods

2.1. Biological Materials

The freeze-dried baker's yeast *Saccharomyces cerevisiae* was purchased from the super market. The African giant snail *Achatina achatina* was obtained from the wholesale market of Adjamé (Abidjan, Côte d'Ivoire).

2.2. Reagents and Chemicals

Several reagents and products of analytical grade used to perform this study were purchased from Sigma Aldrich Corporation. The main ones were: *α*-Amylase from human saliva (Type XIII-A, lyophilized powder, 300 - 1500 units per milligram of protein; 3,5 dinitro salicylic acid (DNS); copper sulphate; Folin-Ciocalteu's phenolic reagent; ammonium persulphate (APS); bis acrylamide; LPD (liquid potato dextrose) broth; YGC (Yeast extract Glucose Chloramphenicol) agar and rice starch.

2.3. Extraction of the Digestive Fluid of *Achatina achatina*

The digestive fluid was extracted by following [14] method. Snails were kept fasting for 3 days to allow digestion of all ingested organic matter. Then, their shells were gently broken by using a sledgehammer and the digestive fluids collected in an ice bath. The crude fluid solution was subsequently centrifuged at 10,000 rotation per minute (4˚C) for 30 minutes and the resulting supernatant stored at 4˚C for further assays.

2.4. Obtaining the Initial Yeast Suspension and Dilution

A sample of 0.1 gram of dried baker's yeast was dissolved in 10 ml of a sterile physiological water and homogenized with a Bioblock Scientific (USA) vortex to obtain a homogeneous suspension. A drop of this suspension was used for the subculture of the strain by streaking on a YGC agar. Then, the resulting medium was plated and incubated in a Venticell brand oven (Germany) at 30˚C for 24 hours. After incubation, biochemical identification of the strains was performed.

2.5. Biochemical Identification of Yeast Colonies

The identification of the yeast species was carried out by macroscopic naked eye

observation and microscopic fresh state analysis as described by [15].

2.6. Cultivation of *Saccharomyces cerevisiae*

Four (4.0) milliliters of the initial *S. cerevisiae* suspension were used to inoculate 36 ml of a previously prepared pH 6.5 liquid potato dextrose (LPD) culture medium. Then, the inoculum was incubated at 30˚C in a water bath for 18 and the growth culture broth absorbances measured twice (0 h and 18 h) at 600 nanometer using a Pioway (CHINA) UV-Visible spectrophotometer.

2.7. Obtaining Yeast Extracellular and Intracellular (Biomass) Protein Extracts

The cell biomass of *S. cerevisiae* was obtained following a classical centrifugation method. The yeast broth obtained after 18 hours of cultivation was centrifuged at 10,000 rpm for 5 minutes using a cold (4˚C) Sigma centrifuge (Germany). Supernatants which constituted the extracellular proteins extract were set aside and stored at 4˚C for further treatments. The pellet of each test tube representing impure biomasses were gathered together and rinsed several times by suspending in sterile distilled water before centrifugation as previously. After removal of the rinsing water, the pure yeast biomass was weighed and suspended in a 1:1 (w/v) ratio of physiological water before cell lysis.

The cell lysis operation has first consisted in freezing the biomass solution containing *S. cerevisiae* cells at −20˚C for 20 minutes followed by heating it for the same time (20 min) in a 100˚C boiling water. Then, the resulting lysate was centrifuged at 14,000 rpm (4˚C) for 5 minutes and the supernatants, representing the biomass protein extracts, were mixed and stored cold at 4˚C for further assays.

2.8. Salt Precipitation of Proteins by Using Ammonium Sulphate

The protein extracts from *S. cerevisiae* biomass and extracellular cultivation medium as well as the one of the digestive fluid of *A. achatina* were partially purified by salt precipitation. Extracts were transferred to a glass beaker containing a stir bar, and place into a cold magnetic stirrer chamber. While the sample is stirring, solid ammonium sulphate salt was added to bring the final concentration to 80% saturation and the geomagnetic disturbance beaker was moved to 4˚C overnight. The resulting solution was centrifuged at 10,000 rpm for 10 minutes and the pellet representing total precipitated proteins was re-suspended in twice volume of sterile distilled water and dialyzed in distilled water by using 40 kDa dialysis bags to remove residual salts. The final protein extracts were stored cold at 4˚C for further analysis.

2.9. Production and Quantification of Protein Hydrolysates

Biomass, extracellular and digestive fluid crude protein extracts were submitted to heat shock at different temperatures (60˚C, 70˚C, 80˚C, 90˚C and 100˚C) for

10, 20 and 30 minutes each. The different extracts obtained after these heat treatments were then introduced into D-Tube Dialyzer Maxi with molecular weight cut-off (MWCO) of 3.5 kDa for selective screening of lower (<3.5 kDa) molecular weight peptides. The resulting peptide solutions, which will subsequently be used as effector samples in human saliva α-amylase catalyzing reactions, are stored cold at 4˚C.

Native and heat shoch treated proteins concentrations were both determined according to a renowned procedure involving the phenolic Folin-Ciocalteu's reagent [16]. Purple staining absorbencies were spectrophotometrically determined at 660 nm and proteins concentrations related to a standard (1 mg/ml) bovine serum albumin (BSA) solution.

2.10. Human α-Amylase Catalyzing Reactions

The standard enzymatic catalyzing reaction was performed at 37˚C for 30 minutes. The total reaction mixtures were 200 µl made up of 100 µl of sodium acetate buffer (100 mM, pH 5.0), 75 µl of substrate [1% (w/v) starch from rice] and 25 µl of Human saliva α-amylase. After incubation, 250 µl of a DNS solution were added to both stop the reaction and quantify the released reducing sugars by heating the mixture to 100˚C for 5 minutes [17]. Absorbances of the obtained products were measured at 540 nanometer after cooling the test tubes in an ice bath and subsequent dilution by adding 2 ml of distilled water in each test tube. Assay values were related to a standard (1%, w/v) glucose solution.

For inhibition tests, two experimental batches were performed for each effector sample. The first batch was carried out with 50 µl of the peptides extract used as effector while the second batch uses 75 µl sample. Each volume (50 or 75 µl) of the effector solution was used by substituting the acetate buffer. The effector samples were first pre-incubated at 37˚C for 5 minutes in the presence of the amylolytic enzymes solution (25 µl) before starting reactions by adding the substrate. An acarbose solution (0.1 mg/ml) was used as the benchmark inhibitor of α-amylase activity.

2.11. Native-Polyacrylamide Gel Electrophoresis (PAGE)

To materialize the effect of the different heating treatments on the native crude protein extracts, electrophoretic tests were performed by using native and treated digestive fluid and the biomass extracts onto a 1.0 mm gel thickness plate [18]. The method consisted in the separation of proteins mixture onto two gels: a concentration gel made up of 4% polyacrylamide, and a separation gel (10% polyacrylamide). Samples heated to 80˚C for 10, 20 and 30 minutes were prepared by mixing 25 µl of each with 20 µl of the loading buffer [0.5 M Tris-HCl pH 6.8, 25% (v/v) glycerol, 0.5% (w/v) bromophenol blue]. A total volume of 25 µl of each sample was applied onto the gel wells and the migration was performed for 1 hour and 30 minutes in a constant power intensity and voltage. Tris/Glycine buffer pH 8.8 was used as the mobile phase and protein fragments were disclosed by staining with brilliant Coomassie blue.

2.12. Statistical Analysis

All tests were carried out in triplicate. So, values were statistically analyzed by using the IBM SPSS Statistics 20.0 software. The comparisons between variables were made by analysis of variance (ANOVA). The Duncan's multiple rank test at 95% confidence level was used to determine significant differences between means.

3. Results

3.1. Morphological Aspects of the Studied Baker's Yeast after Cultivation

After 24 h of incubation on YGC agar, a significant growth of the baker yeast's strain was observed. Naked eyes observations showed growth colonies characterized by rounded shapes, regular edges, and whitish and smooth appearances (Figure 1). The microscopic observation of fresh colonies disclosed individualized rounded cells with well-differentiated nuclei. Also, we can clearly identify cell walls and vacuoles, and noticed that the studied microbial cells growth occurs by budding (Figure 2).

3.2. Yeast Growth and Cell Biomass

After 18 hours of cultivation period, the yeast cells inoculum absorbances increased from 0.102 to 5.46 suggesting an exponential growth of the microorganism with estimated biomass weight of about 14.52 ± 0.53 g.

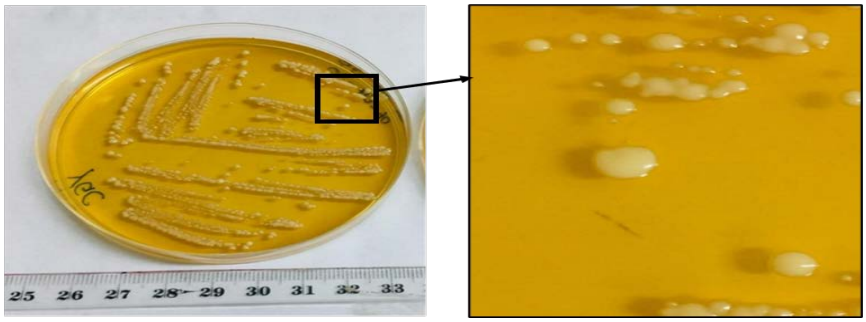

Figure 1. Macroscopic appearances of the studied *Saccharomyces cerevisiae* colonies on YGC agar.

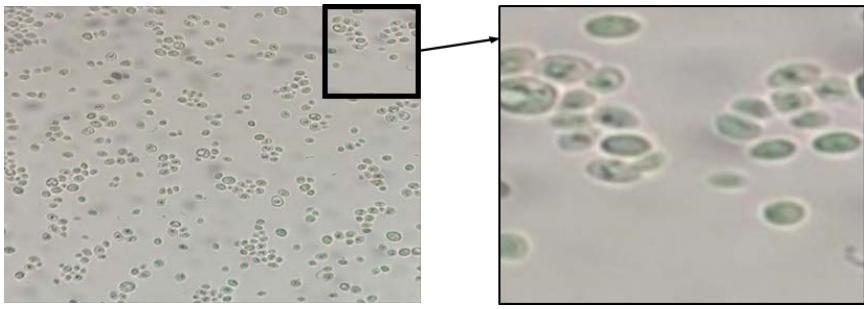

Figure 2. Microscopic observation (G × 40) of the fresh state of the studies *Saccharomyces cerevisiae* strain cells.

3.3. Proteins and Peptides Concentration of Extracts

As shown in Table 1, the initial concentration in proteins of the different extracts is generally improved regardless of the exposure temperatures and times. By and large, the most significant increase in concentration was observed for heated *S. cerevisiae* extracellular proteins extract, followed by that of the digestive fluid of *A. achatina*. Concentration values respectively increased from 0.11 to 0.50 mg/ml after 20 minutes of heating to 60˚C, and from 0.25 to 1.00 mg/ml during the same time of heating to 70˚C. Regarding proteins from *S. cerevisiae* biomass, their increase in concentration was lower after heat shock: it has varied from 0.94 to 2.59 mg/ml (Table 1).

3.4. Effects of the Effector Solutions on α-Amylase Activity

Figures 3-5 depict the variation in activity of the human saliva α-amylase in the presence of the three kind of low molecular mass peptide effectors. With effector samples obtained after treatment at 60˚C, α-amylase activity was by and large improved for up to 178% except when 50 µl of peptide samples from the digestive fluid of *A. achatina* were used (Figure 5(a)). Apart from this temperature, the peptide effectors obtained after exposure of crude protein extracts to 70˚C, 90˚C and 100˚C, exhibit an inhibitory effect on the α-amylase activity although incorporated in the reaction mixture at 50 µl or 75 µl (Figures 3-5). The overall inhibition rates vary from 11% to 95% (Figure 3 and Figure 4).

Table 1. Protein concentrations of unheated and heated extracts from biomass and extracellular cultivation medium of the yeast *Saccharomyces cerevisiae* and from the digestive fluid of the giant snail *Achatina achatina*.

Extract source and heating times	Proteins concentrations at different temperatures (mg/ml)				
	60˚C	70˚C	80˚C	90˚C	100˚C
Biomass of *S. cerevisiae*					
0 min[a]	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.94 ± 0.02
10 min	1.04 ± 0.07	2.02 ± 0.06	1.61 ± 0.03	1.70 ± 0.07	1.77 ± 0.07
20 min	0.75 ± 0.03	1.78 ± 0.05	1.49 ± 0.05	1.74 ± 0.10	2.13 ± 0.08
30 min	0.68 ± 0.05	1.62 ± 0.02	1.80 ± 0.04	2.59 ± 0.05	1.39 ± 0.03
Extracellular growth medium of *S. cerevisiae*					
0 min[a]	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
10 min	0.11 ± 0.01	0.20 ± 0.00	0.24 ± 0.01	0.26 ± 0.01	0.23 ± 0.02
20 min	0.50 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.28 ± 0.00
30 min	0.45 ± 0.01	0.18 ± 0.02	0.28 ± 0.01	0.23 ± 0.01	0.19 ± 0.01
Digestive fluid of *A. achatina*					
0 min[a]	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
10 min	0.64 ± 0.03	0.47 ± 0.04	0.66 ± 0.02	0.59 ± 0.02	0.57 ± 0.03
20 min	0.39 ± 0.11	1.00 ± 0.01	0.79 ± 0.02	0.57 ± 0.03	0.52 ± 0.03
30 min	0.42 ± 0.02	0.61 ± 0.02	0.71 ± 0.01	0.57 ± 0.02	0.42 ± 0.03

a. Unheated (native) extracts.

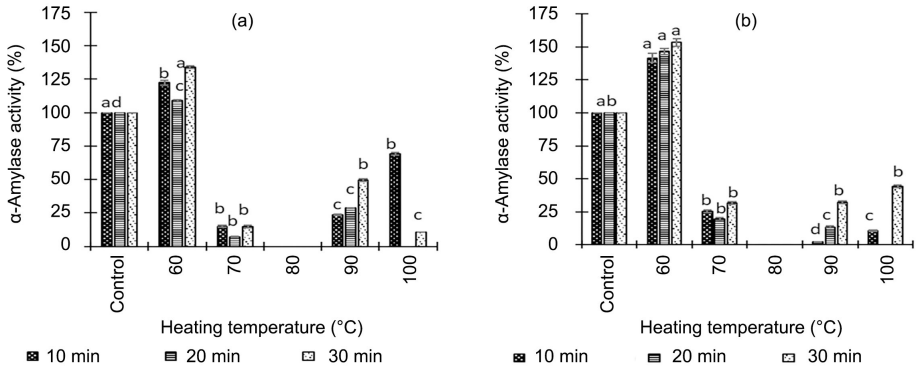

Figure 3. Activity of the Human saliva *α*-amylase in the presence of peptide extracts obtained from the cell biomass of *Saccharomyces cerevisiae* and used as effectors. Assays were performed with 50 µl (a) and 75 µl (b) of effector samples.

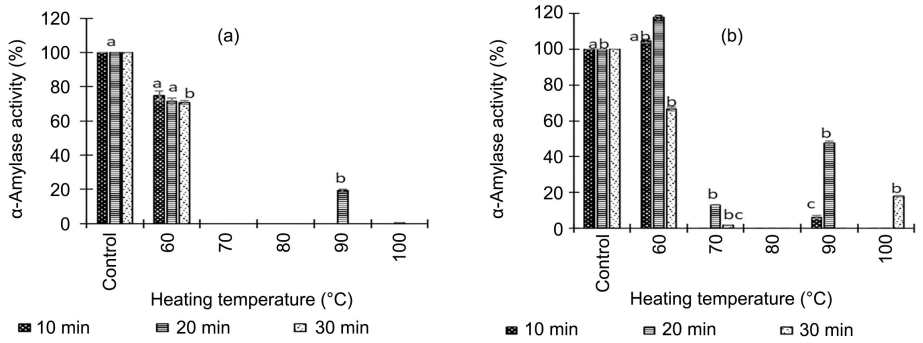

Figure 4. Activity of the Human saliva *α*-amylase in the presence of peptide extracts obtained from extracellular growth broth of *Saccharomyces cerevisiae* and used as effectors. Assays were performed with 50 µl (a) and 75 µl (b) of effector samples.

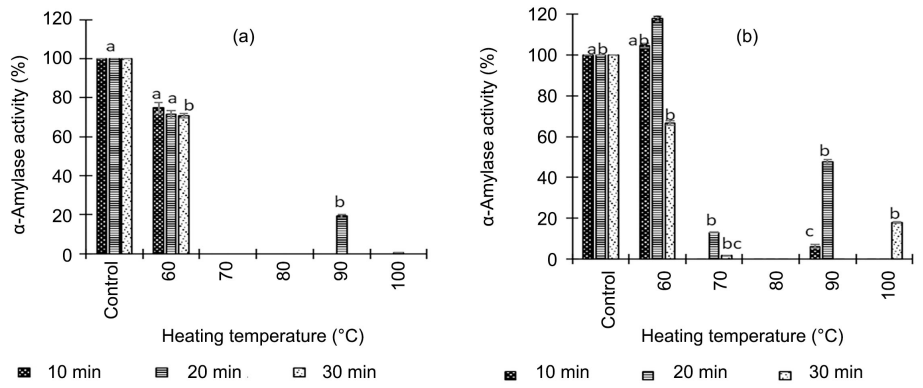

Figure 5. Activity of the Human saliva *α*-amylase in the presence of peptide extracts obtained from the digestive fluid of *Achatina achatina* and used as effectors. Assays were performed with 50 µl (a) and 75 µl (b) of effector samples.

The highest inhibition rates were observed with the peptide extracts (effectors) obtained by heating to 80˚C, regardless of the time of exposure to this temperature. As summarized in Table 2, only 5 µl of biomass, extracellular and digestive fluid effector solutions, produced by heating to 80˚C for 10 minutes, was sufficient to completely inhibit *α*-amylase activity.

Table 2. Protein Inhibition rates of Human saliva *α*-amylase in the presence of different amounts of the effector solutions obtained after heating to 80˚C for 10, 20 and 30 minutes.

Concentration of effectors and heating times		Inhibition rates (%)				
		5 µL	10 µL	15 µL	20 µL	25 µL
Acarbose[a] (0.10 µg/µl)	Standard	37.5	55.0	58.8	62.5	75.0
Biomass of *S. cerevisiae* (0.94 µg/µl)	10 min	100	80.0	98.0	100	100
	20 min	100	85.0	100	60	100
	30 min	100	100	100	82	100
Extracellular growth medium of *S. cerevisiae* (0.11 µg/µl)	10 min	100	61.0	100	64.0	100
	20 min	100	100	100	100	100
	30 min	100	100	100	100	100
Digestive fluid of *A. achatina* (0.25 µg/µl)	10 min	100	1000	100	100	78.0
	20 min	30.0	82.0	48.0	100	100
	30 min	93.0	100	100	95.0	100

a. Antidiabetic Drug.

3.5. Electrophoretic Profiles of Protein and Peptide Fragments

Both of electrophoretic profiles presented in Figure 6 clearly show that the protein extracts that have been exposed to heat treatments have more low molecular weight bands compared to their untreated counterparts loaded onto lane 1.

4. Discussion

The good growth of the biological material purchased at the supermarket on the culture media, namely yeast extract glucose chloramphenicol (YGC) and yeast potato dextrose (YPD), confirms on the one hand the purity of the selected strain, and on the other hand the identity of the studied yeast species. Indeed, these culture media are specific and suitable for the culture of *Saccharomyces cerevisiae* stains [19]. The analysis of the macroscopic morphological characteristics and the microscopic observation of the colonies in the fresh state confirm that it is surely the yeast *S. cerevisiae* that is use in the present study. Indeed, the colonies obtained on Petri dishes showed egg-shapes with a well-differentiated nuclei and large sizes. Also, it was noticed that they reproduce by budding. Such morphological characteristics have already been reported by a number of authors [20] [21], thus clearly confirming that the yeast strains used in the present work are probably those of *Saccharomyces cerevisiae*.

The proteins concentration of the effector solutions is different and variable regarding the origin. The relatively high protein content of the biomass reflects the fact that, by and large, yeast cells, and particularly *Saccharomyces cerevisiae* contain between 40 and 58% of proteins [22]. In contrast, the low protein levels observed in the extracellular culture medium could be the consequence of two major factors. On the one hand, the low amount of protein (1.9% of dry matter)

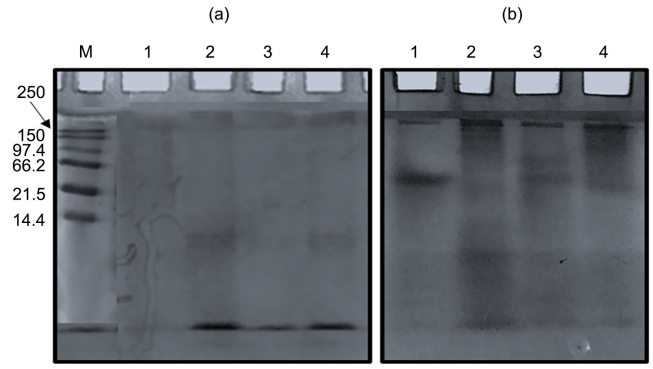

Figure 6. Native PAGE profiles of crude and heated (80˚C) protein extracts from (a) the biomass of *Saccharomyces cerevisiae* and (b) the digestive fluid of *Achatina achatina*. Lane 1: native samples; lane 2: samples heated for 10 minutes; lane 3: samples heated for 20 minutes; lane 4: samples heated for 30 minutes; M: Protein markers.

already reported in the potato, a richly starchy tuber, that served as the main raw material for the preparation of the LPD culture medium; and on the other hand, the consequence of the use of the proteins initially contained in the culture medium as a nitrogen source for yeasts growth [23].

The proteins concentration of the peptide effector solutions obtained in different heating conditions follow the same trends as that of their native untreated counterparts. However, the results of our study clearly show that the protein contents determined by using the Lowry and co-worker's method are higher for the same extract when it is previously heated. This increase in proteins concentrations could as well be the result of the linearization of amino acid chains which follow from the breakdown of low energy (hydrophobic, electrostatic, Van Der Waals…) and three-dimensional proteins structure bonds [24] [25]. Indeed, the structural disorganization of proteins participates in the exposure of aromatic amino acids and peptide double bonds which are specifically targeted by the Folin-Ciocalteu's reagent used in the Lowry and co-workers' method. So, our results suggest the heating of protein extracts from 70 to 100˚C before their quantification by using [16] method. As a result, the present study gives a significant contribution in improving this international renowned method for better quantification of soluble proteins.

Peptide extract samples obtained by heating to 60˚C have generally contributed to the improvement in alpha amylase activity when used as effectors in the reaction mixture. These results, although opposite to the general trend observed for treatments at higher temperatures (70˚C - 100˚C), could have a plausible interpretation. Indeed, 60˚C is bearable temperature for the maintenance of the three-dimensional structural integrity of mesophilic proteins, which is essential for the expression of their biological activities. Thus, the most plausible explanation that would follow from this contribution in improving the α-amylase activity is the combination of amylolytic enzymes activities originating both from 25

µl of the enzyme solution and from 50 or 75 µl of the non-denatured effector solutions.

Experimental temperatures above 60˚C, in particular 70˚C, 80˚C, 90˚C and 100˚C, are more favorable to the structural disorganizations of the majority of proteins exposed to them [26]. We can therefore affirm that a good number of proteins from the biomass and extracellular growth medium of the yeast *S. cerevisiae*, as well as those from the digestive fluid of the mollusk *A. achatina*, were affected. This could be supported by the fact that the use of the resulting effector solutions has showed opposite effects compared to those observed 60˚C treated samples. Indeed, they have significantly contributed to inhibition of the Human salivary α-amylase activity until the enzyme is completely inhibited. These results show that although thermal heating is not a specific method of protein hydrolysis like the commonly used proteases, it nevertheless contributed to the production of small peptide extracts with molecular weights lower than 3.5 kDa, and having in addition the ability to inhibit α-amylase, a key enzyme in diabetes metabolism. Therefore, the peptide extracts produced in the present study could be further investigated for the isolation and characterization of the peptide molecules involved in this inhibitory action, for potential biotechnological applications both in-vitro and *in-vivo*.

The presence of several protein bands of low molecular weight compared to those observed in the electrophoretic profile of their original crude extracts, confirms the fact that some of the proteins contained therein were indeed hydrolyzed under the effect of heat. Therefore, the possibility of attributing these variable inhibitory activities to peptide molecules is obvious since the starting solutions used are protein extracts. Our assertion is supported by the fact that peptides inhibitory effect has already been reported on enzymes activity in [27]. Due to the non-specificity of thermal hydrolysis, several peptides of different structures as well as various protein subunits as shown on native-PAGE profile could be found in the produced proteins hydrolysates. So, the probability of withdrawing the same peptide molecules in this heterogeneous environment, for each reaction tube during assays, is very uncertain. This could explain the inconsistency of some inhibition rates observed for a number of assays. Even though enzymatic hydrolysis of proteins is more specific, similar inconsistency in activity were also reported for rice bran protein hydrolysates [27].

The culture broth and biomass protein hydrolysates of *Saccharomyces cerevisiae* as well as protein hydrolysates from the digestive fluid of *Achatina achatina* have shown good inhibitory capabilities as compared to that of acarbose, a potent inhibitory drug, usually used in the treatment of type 2 diabetes [28]. When used in the reaction after thermal denaturation at 80˚C for 10 minutes, small amounts of the effector solutions (4.70 µg of biomass proteins, 0.55 µg of extracellular growth medium and 1.05 µg of proteins from the digestive fluid) were sufficient for complete inhibition of the Human saliva α-amylase activity. With comparison to acarbose, the inhibitor of reference used in this study, our peptide

inhibitors showed more efficiency because acarbose was not capable of completely inhibiting the *α*-amylase activity even though blended at 2.50 µg into the reaction mixture. In view of these interesting results, the studied proteins hydrolysates could be explored as promising and competitive sources of *α*-amylase and *α*-glucosidase inhibitors. Therefore, they could significantly contribute in slowing carbohydrates breakdown and thus reducing blood glucose concentration and subsequently regulate hyperglycemia in type 2 diabetes patients.

5. Conclusion

The present study aimed at producing cheap and easily accessible bioactive molecules of peptide kind from Ivorian agri-resources which could be explored as therapeutic agents for the regulation of type 2 diabetes. The results of this *in-vitro* study showed the possibility of *Saccharomyces cerevisiae* extracellular and biomass proteins hydrolysates as well as proteins hydrolysates from the digestive fluid of *Achatina achatina* to inhibit Human *α*-amylase activity. The best denaturation procedure was obtained by heating protein extracts to 80˚C for up to 30 minutes, because the resulting peptide solutions showed complete inhibition of *α*-amylase. The molecules suspected of inducing these inhibitory effects would be peptides whose presence was confirmed by native polyacrylamide gel electrophoresis profiles. Further characterization is necessary to the potential use of the suspected inhibitory molecules in both *in-vitro* and *in-vivo* mellitus diabetes regulation studies.

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Authors Contributions

Conceptualization: E.M. Bédikou and S. Niamké; Acquisition of data: M. Kadjo, C. Ehon, C. Assémian and E.M. Bédikou; Analysis of data: E.M. Bédikou, D. Koffi and A. Assamoi; Original draft preparation-Writing: E.M. Bédikou and S. Niamké.

Conflicts of Interest

No funders were involved in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors reported no conflict of interest.

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