Determination of amylase activity in crude extracts from sesame (*Sesamun indicum* L.) seeds

Determinação da atividade amilásica em extratos brutos de sementes de gergelim (*Sesamun indicum* L.)

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ABSTRACT

Amylases are enzymes belonging to the hydrolase class. They can be produced and extracted from microorganisms, animals and plants, and have multiple industrial and biotechnological applications. This study, for the first time, reports the amylase activity of unhulled (after germination) and hulled sesame (*Sesamum indicum* L.) seeds. The results obtained using the Bernfeld test indicate that the crude extract with a 0.5 mol L^{-1} NaCl solution after 60 min of heating the shelled sesame seeds presents higher amylase activity. The ideal pH and temperature were determined as 6 and 50 °C, respectively, differing from the amylase values found in other seeds but the close to values found in bacteria. Due to the wide and diverse applicability of amylases, the search for new natural sources of these enzymes is important. Sesame seeds can now be considered a potential source of amylases and the extraction of these enzymes is described in this article.

RESUMO

As amilases são enzimas que pertencem à classe das hidrolases, podem ser encontradas e extraídas a partir de microorganismos, animais e vegetais, apresentam múltiplas aplicações industriais e biotecnológicas. Neste trabalho, pela primeira vez, é relatada a atividade amilásica em sementes de gergelim (*Sesamum indicum* L.) sem casca (depois da germinação) e com casca. Os resultados obtidos por meio do teste de Bernfeld indicam que o extrato bruto com a solução de NaCl 0,5 mol L^{-1} após 60 min de aquecimento das sementes de gergelim sem cascas apresenta maior atividade amilásica. O pH e a temperatura ideais determinados foram de 6 e 50 °C, respectivamente, sendo diferentes dos valores de amilases encontradas em outras sementes mas próximos dos valores encontrados em bactérias. Devido à ampla e diversificada aplicabilidade das amilases, é importante a busca por novas fontes naturais destas enzimas. As sementes de gergelim podem então ser consideradas uma potencial fonte de amilases e a extração destas enzimas estão descritas neste artigo.

INTRODUCTION

The amylases α (1,4-α-D-glucan-glucanohydrolase, E.C 3.2.1.1) and β (4-α-D-glucan maltohydrolase, E.C 3.2.1.2) are enzymes that belong to the hydrolase class, capable of breaking the non-terminal α-1-4-glycosidic bonds of the starch into glycogen, maltose, dextrin and glucose (Souza & Oliveira, 2010). The optimal pH described in the literature for these enzymes are between 4.5 and 6.5, the optimal temperatures are between 55 and 75 °C and molecular masses are found between 12 kDa and 67.4 kDa, depending on their origin (Greenwood; MacGregor, 1965; Biazus et al. 2009; Divakaran et al. 2011). Comparative studies about genes that produce amylases from cereals and microorganisms reveal that the differences among structures and enzymatic characterizations may come from ancestors, that are different for cereals and microorganisms (Ju et al. 2019). For example, in maize, the α-amylase has a molecular mass of 67.4 kDa (Biazus et al. 2009), while the one in *Lactobacillus manihotivorans* has 13.5 kDa (Aguilar et al. 2000).

Amylases may be of animal or vegetable origin. For practical applications, they are extracted mainly from cereal seeds, such as wheat (Ilram-ul-Haq et al. 2002; Kayastha, 2014) and barley (Greenwood & MacGregor, 1965), but they can also be found in fruits like mango (Mehrnoush & Yazid, 2013) and jambolana (John, 2017). These enzymes present several applications in industrial processes such as in the production of textiles, pharmaceuticals, beer, detergents and biodiesel as a biocatalyst (MacGregor, 1977). Thus, it is important to search for new amylase sources. In cereals like barley (Greenwood & MacGregor, 1965), wheat (Lunn et al. 2001) and rice (Damaris et al. 2019), amylase and other enzymes are usually found in high amounts during the germination process because of their important role during the plant initial development, until photosynthesis can be performed. This process involves biochemical and physiological reactions and, as a consequence of the activities of these enzymes, the starch content in the seeds decreases, since it is converted into glycose that is used in respiration, energy production and plant formation (Greenwood & MacGregor, 1965; Lunn, 2001; Damaris et al. 2019).

Among the various grains that are abundantly consumed by the population, sesame presents various health benefits due to the high levels of vitamins (especially of the B complex), calcium, iron, phosphorus, potassium, magnesium and proteins, and also high oil content, especially unsaturated fatty acids like omega 9 and 6. Some studies show that sesame

oil is a viable alternative for the production of biodiesel (Saydut et al. 2008). In this work, the identification, extraction and characterization of optimal pH and temperature of amylase activity in unhulled (after germination) and hulled sesame (*Sesamum indicum* L.) seeds were carried out.

MATERIAL AND METHODOS

1. Preparation of crude extract

Unhulled sesame seeds, 2 days after germination, and hulled sesame seeds were ground to provide 100 g of samples (named USS and HSS, respectively) for initial amylase extraction. In order to remove the oil fraction (Khorrami et al. 2018), 150 ml of acetone was added, followed by centrifugation for 10 min at 8000 × *g* and 4 °C, what was repeated five times with both samples. Crude extracts were obtained by mixing 10 g of ground seeds to 100 mL of each different extractor liquid: distilled water (H_2O), ethanol 70% (*V*/*V*), sodium chloride solution (NaCl) 0.5 mol L^{-1} and sodium phosphate buffer solution 0.5 mol L^{-1} pH 6.9, for 1 h under continuous stirring and then centrifugation for 10 min at 8000 × *g* and 4 °C, after which the supernatant was recovered.

After obtaining the crude extracts from each extracting solution, three samples were prepared: one unheated sample and two heated to 100 °C, one for 30 min and the other for 60 min. Heating was performed to determine the thermal stability of the amylases. Heated samples were then centrifuged for 10 min at 8000 × *g* to separate precipitated material.

2. Determination of amylase activity

The amylase activity was determined as described by Bernfeld (Bernfeld, 1955). Absorbance readings were performed on a SmartSpec Plus spectrophotometer (Bio-Rad). Assays were performed in triplicates.

3. Determination of the optimal pH and temperature and protein quantification

pH values assayed were 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 provided by buffers Citrate (pH 4.0 and 5.0), MES-NaOH (pH 6.0), Tris-HCl (pH 7.0) and Glycine-NaOH (pH 8.0, 9.0 and 10.0). Temperatures assayed were 30, 40, 50, 60 and 70 °C in a water bath (Quimis, Diadema, São Paulo). Determination of total protein content was performed according to Bradford (Bradford, 1976).

RESULTS AND DISCUSSION

1. Determination of amylase activity

Figures 1a and 1b show the final absorbance values (proportional to amylase activity in the Bernfeld assay when different liquids were used for amylase extraction and after different times of heating treatment, for samples

USS and SSH, respectively. Table 1 shows total protein concentration estimated by the Bradford assay for both samples.

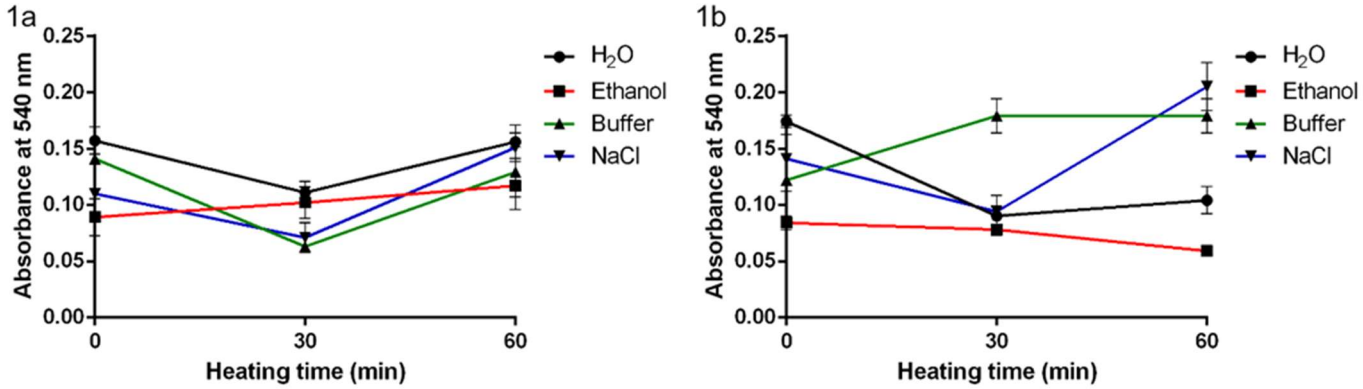

Figure 1. Amylase extraction test with distilled water (H_2O), ethanol 70% (*V*/*V*), sodium chloride solution 0.5 mol L^{-1} and sodium phosphate buffer solution 0.5 mol L^{-1} pH 6.9; absorbance values from the Bernfeld assay. **1a)** USS sample. **1b)** HSS sample. The points are average of triplicates and the bars correspond to the standard deviations (+1 and -1 σ).

Table 1. Total protein concentration estimated by the Bradford assay for USS and HSS samples.

Extractor liquid (100 mL)	Total protein concentration / mg mL^{-1}	
	USS	HSS
Distilled H_2O	0.21	1.80
Ethanol 70% (*V*/*V*)	0.34	0.11
Sodium Phosphate Buffer 0.5 mol L^{-1} pH 6.9	0.52	0.61
NaCl 0.5 mol L^{-1}	0.12	1.20

Figure 1a evinces amylase activity (absorbance at 540 nm) for all samples, from ethanol, buffer, water and NaCl solution, even after submission to heating up to 60 min, what is indicative of the presence of amylase. The heating step is used in many protein purification protocols because several contaminants do not resist high temperatures and precipitate, there remaining only thermostable proteins (John et al. 2001; Khorrami et al. 2018). It seems that sesame amylase is at least partially thermostable. This enzyme is essential for the growth and development of the seeds, so it is produced in the

aleurone layers during the germination process (Gubler et al. 1995).

Figures 1a and 1b show that the crude extract for samples USS and HSS diluted with NaCl solution after heating at 60 °C show absorbances of 0.16 and 0.20, respectively, whereas table 1 shows that this solvent extracted total proteins to give final concentrations of 0.12 and 1.20 mg mL^{-1} for sample USS and HSS, respectively. These results indicate that the NaCl and water solutions demonstrated greater amylase activity in the USS samples, while the NaCl solution demonstrated the highest amylase activity in the HSS samples. The sodium phosphate buffer solution, despite extracting more proteins in the USS samples, did not demonstrate greater amylase activity when compared in the tests performed. The amylases extracted from rice and barley were extracted in sodium phosphate buffer (Zhang et al. 2025 and Hossain et al. 2025). From these results, the NaCl solution demonstrated the best amylase activity, differentiating it from that of rice and barley.

4. Effects of pH and temperature on amylase activity

Figures 2a, 2b, 3a and 3b show that the pH and temperature optima were 6 and 50 °C, respectively, for both samples USS and HSS.

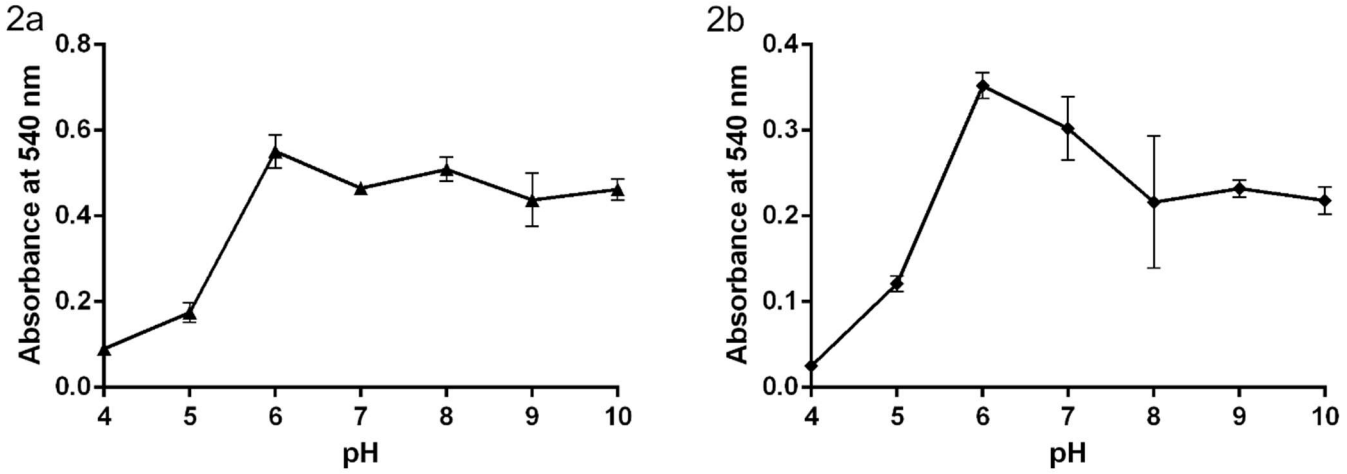

Figure 2. Determination of the optimal pH, plots of absorbance (Bernfeld assay) × pH. **2a)** USS sample. **2b)** HSS sample. The points are average of triplicates and the bars correspond to the standard deviations (+1 and -1 σ)

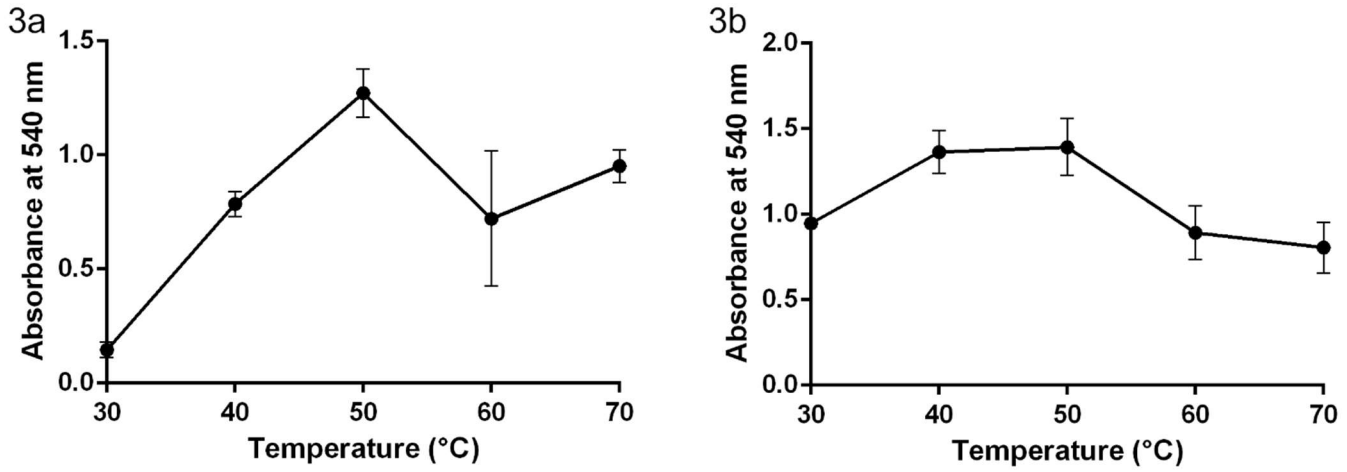

Figure 3. Determination of the optimal temperature, plots of the absorbance (Bernfeld assay) × temperature. **3a)** USS sample. **3b)** HSS sample. The points are average of triplicates and the bars correspond to the standard deviations (+1 and -1 σ)

The results obtained for both sesame samples are different from those obtained for wheat (Lunn et al. 2001), that presents optimal pH and temperature of 5 and 70 °C, barley (Greenwood & MacGregor, 1965), 5.5 and 45-50 °C and mango (Mehrnoush & Yazid, 2013), 7.0 and 50 °C. It is possible to observe that the sesame amylase presents peculiar characteristics in relation to the optimal pH, although the optimal temperature is the same as that of barley and mango.

At comparing to amylases obtained from microbial systems, such as α-Amylase from *Bacillus cereus* GL2, with pH and optimum temperature of 6 and 50 °C (ALHAZMI and ALSHEHRI, 2025), and an α- Amylase isolated from soil samples, with activity up to pH 8.5 (Abo-Kamer et al. 2023) (which showed antibiofilm activity against *Pseudomonas aeruginosa* and later showed to be also from *B. cereus*), one observes that the sesame amylase has an optimum pH similar to those, what makes it even more attractive for future studies.

Due to the wide range of industrial applicability of amylases, especially in the food industry, the characterization of new amylases extracted from low cost sources is important. In this way, this paper has shown that hulled sesame seeds present a significant amount of proteins and that the NaCl solution was the liquid that extracted most of the amylase activity. This extract characterization showed that the optimal pH and temperature are 6 and 50 °C, respectively, revealing an amylase with peculiarities in the optimal pH value when compared to amylases present in other seeds.

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