Contribution to the study of alliinase, the active principle of garlic

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Context and objectives [1]

Alliinases are a class of enzymes presents in the *Allium* genus (garlic, onion, etc.) that catalyzes the cleavage of the C – S bond of a cysteine sulfoxide in a sulfenic acid (figure 1). The latter converts into volatile compounds that confer the flavor, odor and tear-inducing properties of the Alliums.

Pungent compounds are only released when the plant is damaged what enables the contact between the enzyme and the substrate. Indeed, these are usually separated and cannot react in an entire cell.

The compounds released once the enzymatic reaction occurred are significant: they confer flavor and odor to food preparations, and their bioactivities are regularly investigated.

A better understanding of this crucial enzymatic reaction goes through a study of the enzyme, with special attention to its extraction and characterization.

The present work aims to compare several extraction and purification methods, and to characterize the enzyme alliinase.

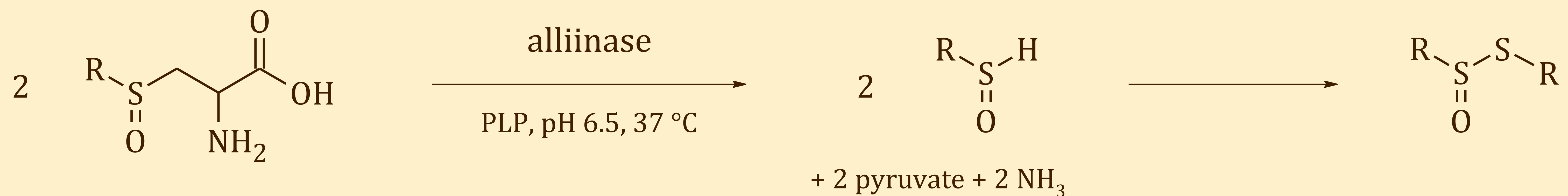

Figure 1: Enzymatic reaction of alliinase with a cysteine sulfoxide produces a sulfenic acid. Two molecules of the latter condense to form a thiosulfinate

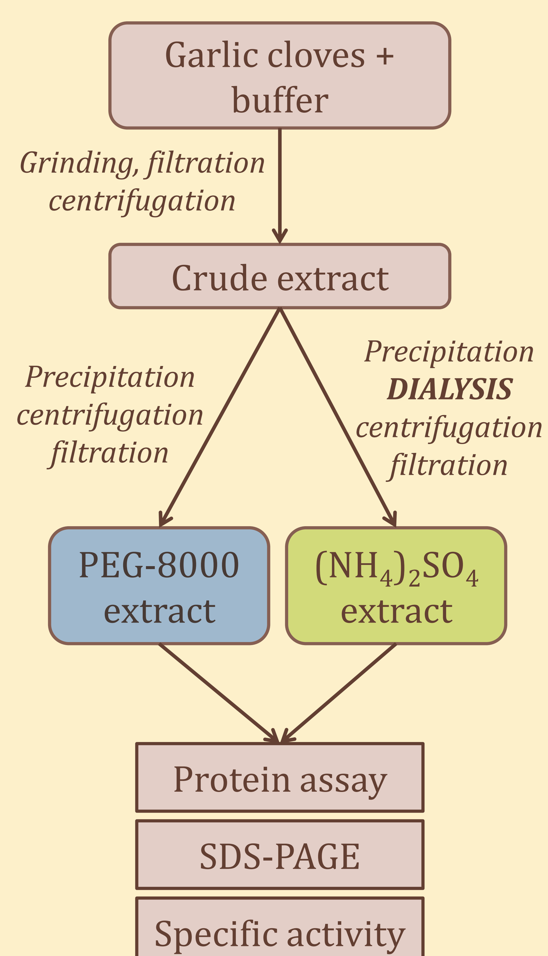

Figure 2: Extraction procedure.

Extraction of alliinase

The procedure is summarized in figure 2. The PEG-8000 extraction has been performed according to Rabinkov et al.[2], and the ammonium sulfate extraction has been adapted from the latter, with an additional dialysis step to remove the salt.

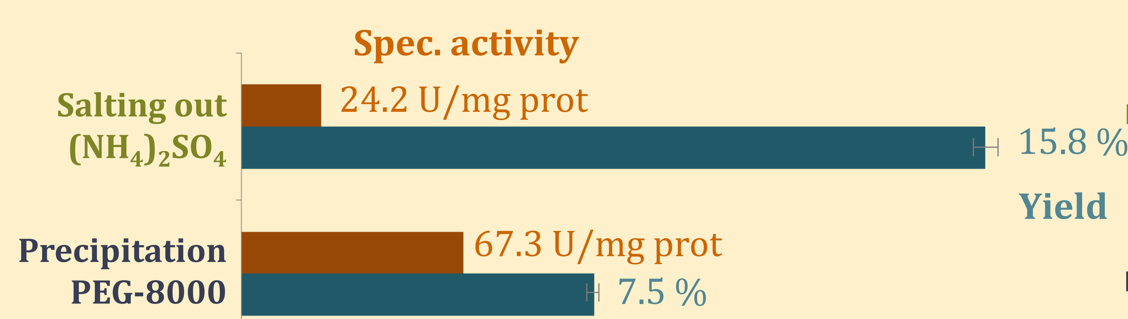

Figure 3: Specific activity is higher but extraction yield is lower with the PEG-8000 than with the salting out extraction method. SD: n=3.

The amount of recovered alliinase is higher in the PEG-8000 precipitation (figure 3). Furthermore, this procedure does not need a dialysis step.

Purification of alliinase

We have purified the extracts by affinity (ConA) and size-exclusion (Sephadex 200) chromatography. The purest extract has been produced by **salting out** and **ConA chromatography** with high yield (figure 4).

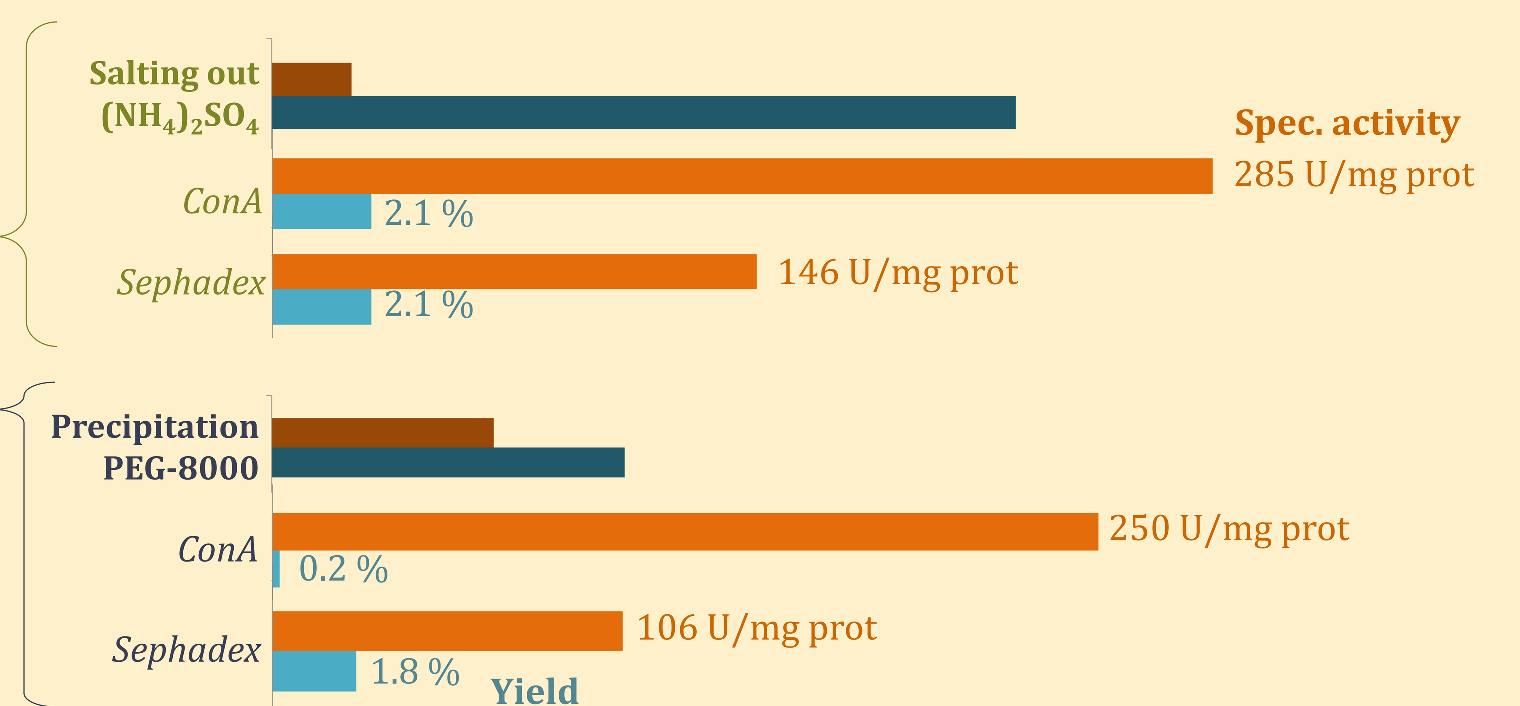

Figure 4: The salting out extraction followed by affinity chromatography (ConA) provides the highest specific activity and yield.

Study of the protein extracts

1. *Protein assay*

We have determined the protein content according to the Lowry procedure[3] after a precipitation of the free amino-acids in the extract by trichloroacetic acid. Calibration has been performed with BSA, and absorbance measured at 660 nm.

2. *SDS-PAGE*

Electrophoresis has been applied to assess the purity of the protein extracts. This method has been not used for quantification. An example of the resulting gel is given at figure 5.

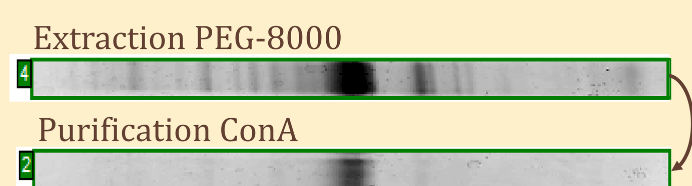

Figure 5: SDS-PAGE assesses the efficiency of the purification

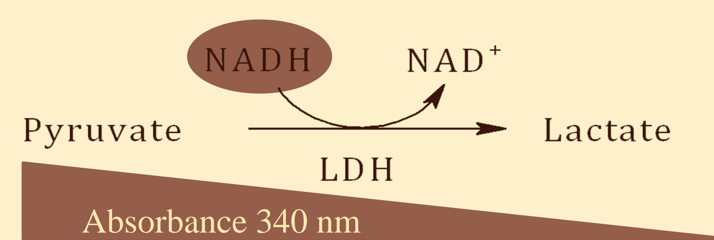

Figure 6: Indirect measure of the alliinase activity through NADH disappearance (340 nm)

3. *Specific activity*

Measurement of the activity has been done at 37 °C and pH 6.5 in the presence of PLP by an indirect spectrophotometric method (figure 6): the enzymatic reaction releases equimolar amounts of pyruvate and ammonia (figure 1). Pyruvate is turned into lactic acid by lactate dehydrogenase (LDH) in the presence of NADH. The disappearance of NADH has been measured at 340 nm.
The highest specific activity measured in our extracts is **285 U/mg of protein**, which is consistent with the specific activities from the literature (228 to 660 U/mg of protein, depending on the garlic origin [4]).

Characterization of the alliinase

1. *Determination of K_M and V_{max}*

V_i has been measured for 9 concentrations of substrate (between 0.1 and 25 mM) by assessment of the pyruvate production (see "Specific activity"). K_M and V_{max} have then been determined by the Lineweaver-Burke plot [5].

Our PEG-8000 extract purified on ConA shows a **V_{max} of 1.14 x 10^{-2} mM.min^{-1}** and a **K_M of 0.87 mM**, which is lower than the values reported in the literature: Miron et al.[6] measured a V_{max} of 1.55 x 10^{-2} mM.min^{-1} and a K_M of 1.93 mM. Kuettner et al.[4] suggested that those parameters vary with the garlic origin (in our case Spanish garlic).

2. *Stability*

We have evaluated the stability of PEG-8000 and purified (ConA) extracts with and without glycerol at four temperatures (RT, 4, -20 and -80 °C) by measuring the activity over time.

Glycerol does not seem to play a key role: the activities are similar regardless of the glycerol content. Purified extract does not show any activity after 4 days at room temperature or 9 days at 4 °C, while the unpurified extract shows a half-life of 9 days both at RT and 4 °C. Both the extract and the pure enzyme are still fully active after 6 weeks if stored at – 20 °C and – 80 °C.

Conclusions

- We recommend the salting out extraction (ammonium sulfate) followed by affinity chromatography on ConA to obtain pure alliinase with good yields. The specific activity and V_{max} for this enzyme are lower than in previous reports, making Spanish garlic a poor candidate for alliinase extraction.
- Alliinase can be stored at -20 °C for over 6 weeks without loss of activity.

Literature cited

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[6] Miron, Shin, Feigenblat, Weiner, Mirelman, Wilchek, Rabinkov (2002) *Anal Biochem*, **307**, 76–83

Further information

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