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THE INFLUENCE OF THREE DIFFERENT DRYING PROCEDURES ON SOME ENZYMATIC ACTIVITIES OF THREE VIPERIDAE SNAKE *VENOMS*

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ABSTRACT: Some enzymatic activities of lyophilized, sun-dried and vacuumdried venoms of copperhead *(Agkistrodon contortrix)*, saw-scaled viper *(Echis carinatus sochureki)* and lancehead *(Bothrops atrox)* snakes were determined and compared to the respective fresh venoms. It has been shown that none of the drying procedures has a general advantage over the others. It is concluded that the drying procedure used may influence the enzymatic activities of snake venoms to a different extent. It may be worthwhile to take the drying procedure into consideration, if snake venoms are used as enzyme source, since it may influence the yield of the enzyme to be purified.

KEYWORDS: Snake venoms; drying procedures; enzyme activities.

INTRODUCTION

It is known that dried snake venoms retain many of their biological activities over long periods of time[6, 8, 9, 10, 12]. Although this has never been demonstrated it is often said that lyophilized venoms are more stable than desiccator-dried venoms[11]. Low temperature processing and drying, however, are more complex scientifically and technologically than other drying procedures and the physicochemical changes which can take place during freeze-concentration and subsequent drying may be prominent[3, 4]. Thus, in this study the influence of lyophilization, vacuum-desiccation and sun-dryng on some enzymatic activities from venoms of the broad-banded copperhead *(Agkistrodon contortrix laticinctus)*, the

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saw-scaled viper *(Echis carinatus sochureki)* and the lancehead *(Bothrops atrox)* were investigated. Lyophilization and vacuum-desiccation are widely used laboratory procedures, where as sun-drying is a method used under field conditions.

MATERIAL AND METHODS

Snake venoms and drying procedures

Manual VENOM EXTRACTIONS were performed with *Agkistrodon contortrix laticinctus, Echis carinatus sochureki* and *Bothrops atrox* snakes. All animals were kept in our own snakefarm in Aesch, Switzerland. The venom pools obtained from some ten specimens of each snake species were divided into four aliquots and used in the experiments as fresh venom, lyophilized, vacuum-desiccated and sun-dried respectively. VACUUM-DESICCATION was performed in a vacuum-desiccator (Haereus, Zurich, Switzerland) during 4 hours under 10^1 Torr vacuum at a temperature of 40°C and the water being removed via the pump. LYOPHILIZATION was performed in a lyophilizator (FC-600, LSL Secfroid, Lausanne, Switzerland). Before venom application, the apparatus was cooled down to -30°C. After deep freezing of the venoms to -45°C within 3 hours 20 minutes, the lyophilization processus with 10^{-2} Torr was finished 66 hours later with a final product temperature of +30°C. As a sun-drying procedure venom aliquots were dried for 5 hours outside under the sun on a sunny mid-July day in Aesch near Basel, Switzerland.

Experimental procedures

The dry weights were determined by weighing venom aliquots before and after the drying procedures on an analytical balance (Mettler, Greifensee, Switzerland). The dry weight of the fresh venom samples was estimated by calculating the mean of the dry weights obtained with the different drying procedures. The possible error of $\pm$ 5 % in this dry weight estimation was taken into consideration for the evaluation of the results. SDS POLYACRYLAMIDE GEL-ELECTROPHORESIS (SDS-PAGE) was carried out in a PhastSystem electrophoresis unit (Pharmacia, Uppsala, Sweden). The venoms (1mg venom per ml bidistilled water) were diluted 1+1 with sample buffer (10mM Tris/HC1, 1mM EDTA, 2.5% SDS, 0.01 bromphenolblue, pH 8.0). The sample volume applied to the gels (PhastGel Gradient 8-25%, Pharmacia, Sweden) was 1 μl. The running conditions were 250 V, 10mA, 3.0W, 15°C and 65Vh at the beginning and 50V, 0.1mA, 0.5V, 15°C and 0 Vh at the end of the experiment. L-AMINOACID OXIDASE ACTIVITY was manometrically determined in a differential respiro-meter (IG-14, Gilson, Middleton, USA) using 50 mM L-Phenyl-alanine (Sigma, St. Louis, USA) in 1/15 M Sorensen-phosphate buffer as the substrate. The vessels in which the enzymic reaction took place were shaked over a 60 minutes time period at 37°C and the oxygen consumption recorded at 10 minutes intervals. Venoms were used in a concentration of 1mg per ml bidistilled water. DETERMINATION OF ENZYME ACTIVITIES USING CHROMOGENIC SUBSTRATES were performed according to the method described by MEIER et al.[9]. For this purpose, 1.79ml of Tris-imidazole buffer (pH 8.4, ionic strength 0.15) and 0.01ml snake venom solutions were preincubated at 37°C in a disposable polystyrene cuvette. Then, 0.2ml of a 2 μM substrate solution were added and the change in absorbance at 405nm was photometrically recorded. The enzymatic release of p-nitroaniline per 3 minutes was measured (A/3 min) and converted into enzyme milliunits (mU)

by multiplication of this value by 6'667[9]. One international enzyme unit (U) is the amount of enzyme, which catalyses 1 μmole of substrate per minute under standardized conditions. Substrates with high specificity for thrombin (Chromozym^R TH;Nα-Tosyl-Glycyl-L-Prolyl-L-Arginyne-p-nitroanilide-hydroacetate), plasma kallikrein (chromozym PK;Nα-Benzoyl-L-Prolyl-*L*-Phenylalanyl-*L*-Arginyne-*p*-nitroanilide-hydroacetate), glandular kallikrein (Chromozym GK; 2Hydroacetate-H-*D*-Valyl-L-Cyclohexylalanyl-*L*-Arginyne-*p*-nitroanilide), urokinase (Spectrozyme UK; Nα-Carbobenzoxy-*L*-Glutamyl (α-tertiary butoxy)-Glycyl-*L*-Arginyne-*p*-nitroanilide-hydroacetate) and plasmin (2Hydroacetate-H-*D*-Valyl-Phenylalanyl-*L*-Lysyne-*p*-nitroanilide *respectively were used*. All substrates are manufactured by Pentapharm Ltd. and distributed by Boehringer-Mannheim, FRG (Chromozym series) and American Diagnostica, USA (Spectrozyme substrate) respectively. The standard deviation of the test method has been evaluated with ± 8.5%. Thus, differences of more than ± 10% were judged being significant.

RESULTS AND DISCUSSION

The drying residues obtained with the different procedures vary within a comparatively small range only (Fig. 1). The high dry residues found support the hypothesis, that snake venoms are probably the highest concentrated secretory fluids produced by vertebrate organisms[2, 13]. The SDS-PAGE also did not reveal visible differences in molecular weight, indicating that no significant proteolytic degradation took place during drying (results not shown). This is, however, not surprising, since the electrophoretic pattern of all venoms investigated is very complex and it has been recently shown that e.g. the venom of *Agkistrodon contortrix* consists of some 80 different protein components[1]. When compared to fresh venom, every drying procedure led to a decrease in *L*-amino acid oxydase activity (figs. 2A, 3A, 4A). This confirms the labile character of this enzyme[5]. For two reasons the results of the experiments performed using chromogenic substrates have to be interpreted with some caution. Firstly, the different enzymes present in the venoms may interact synergistically. Thus, in contrast to the determination of *L*-amino acid oxidase activity, the activity measured with the chromogenic substrate may rather reflect the contribution of different venom components than the activity of on single enzyme. Consequently, the amidolytic activities measured may be attributed to known as well as to so far unrecognized venom proteinases. Secondly, it is not possible to correlate the amidolytic activities of venoms with the susceptibility of the substrates to mammalian proteinases[7]. However, although great caution was taken in interpreting these results, some interesting conclusions may be drawn. In the *Agkistrodon contortrix* venom, no significant differences were observed with the different drying procedures (fig. 2, B-F). A significant decrease in amidolytic activities was observed with sun-dried *Bothrops atrox* venom on the substrates for thrombin (fig. 3B) and urokinase (fig. 3D) respectively, whereas with this venom, no significant differences could be detected between vacuum-drying and lyophilization. The most striking differences were obtained with *Echis carinatus sochureki* venom (fig. 4). Obviously, there must a so far unrecognized inhibitor(s) be present in this venom, which will be destroyed during the drying procedures, thus leading to much higher enzymatic activities when compared to the fresh venom (fig. 4B, D, E, F). Surprisingly, sun — as well as vacuum-drying led to a significantly higher activity of the venom to the substrate for glandular kallikrein, whereas the activity of the lyophilized venom was in the same range as the fresh venom.

CONCLUSIONS

The drying precedure used may influence the enzymatic activities of snake venoms to a different extent. Thus, it may be worthwhile to take the drying procedure into consideration, if snake venoms are used as enzyme sources, since different enzymes present in the venom may be influenced in a different way according to the drying procedure used. Consequently, the drying procedure to which the snake venom is submitted may influence the yield of the enzyme to be purified.

RESUMO: Algumas atividades enzimáticas de venenos liofilizados, secos ao sol e secos a vácuo das serpentes *Agkistrodon contortrix, Echis carinatus sochureki* e *Bothrops atrox* foram determinadas e comparadas com os respectivos venenos frescos. Demonstrou-se que nenhum dos processos de secagem tem vantagem sobre os outros. Conclui-se que o processo de secagem usado pode influenciar as atividades enzimáticas dos venenos das serpentes até uma diferente extensão. Recomenda-se escolher o método mais adequado se os venenos de serpentes forem usados como fonte de enzimas.

UNITERMOS: Serpentes-venenos, processos de secagem, atividades enzimáticas.

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LEGENDS TO FIGURES

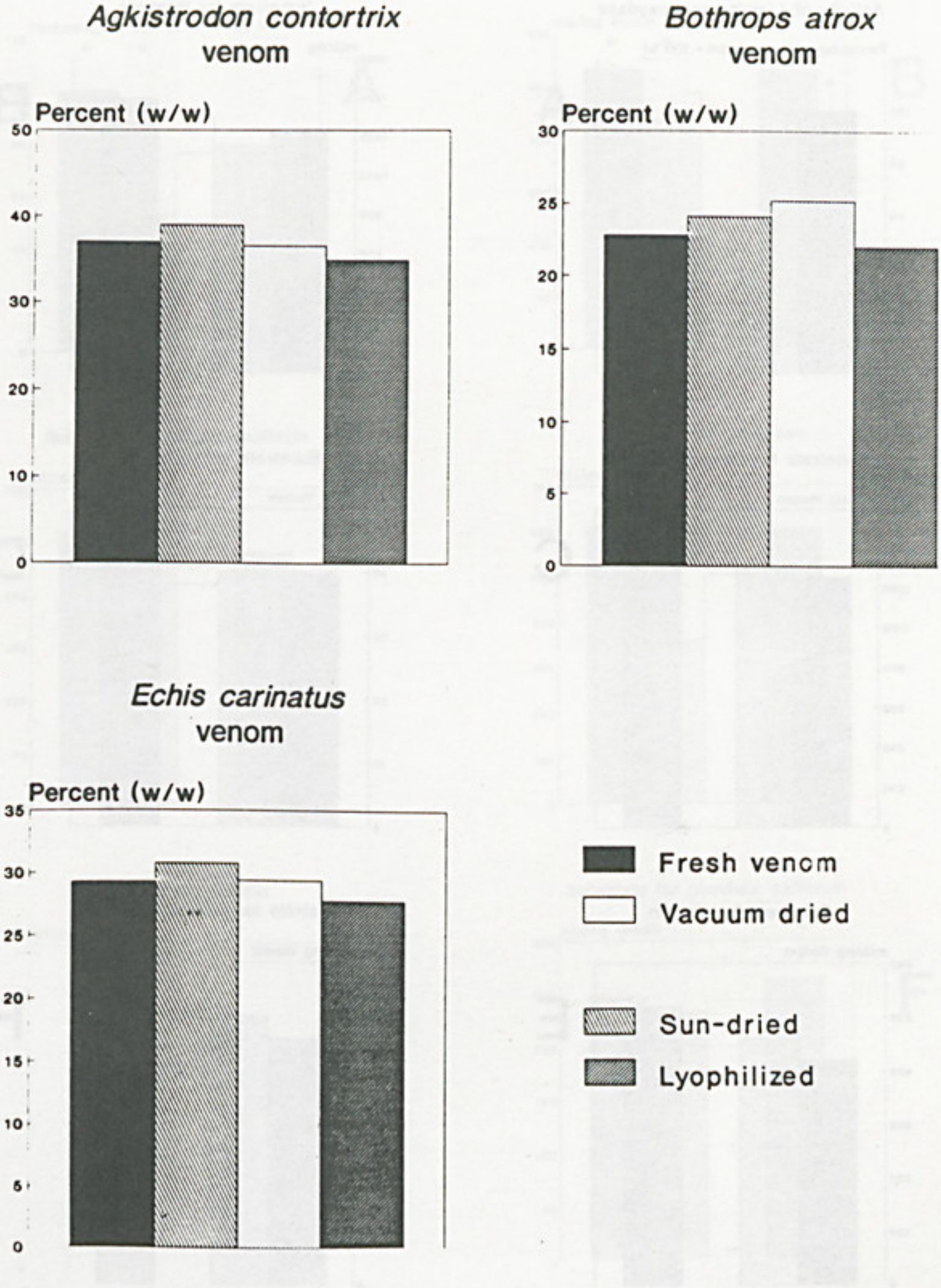

Fig. 1. Dry residues of the venoms. The differences between the values obtained were not significant.

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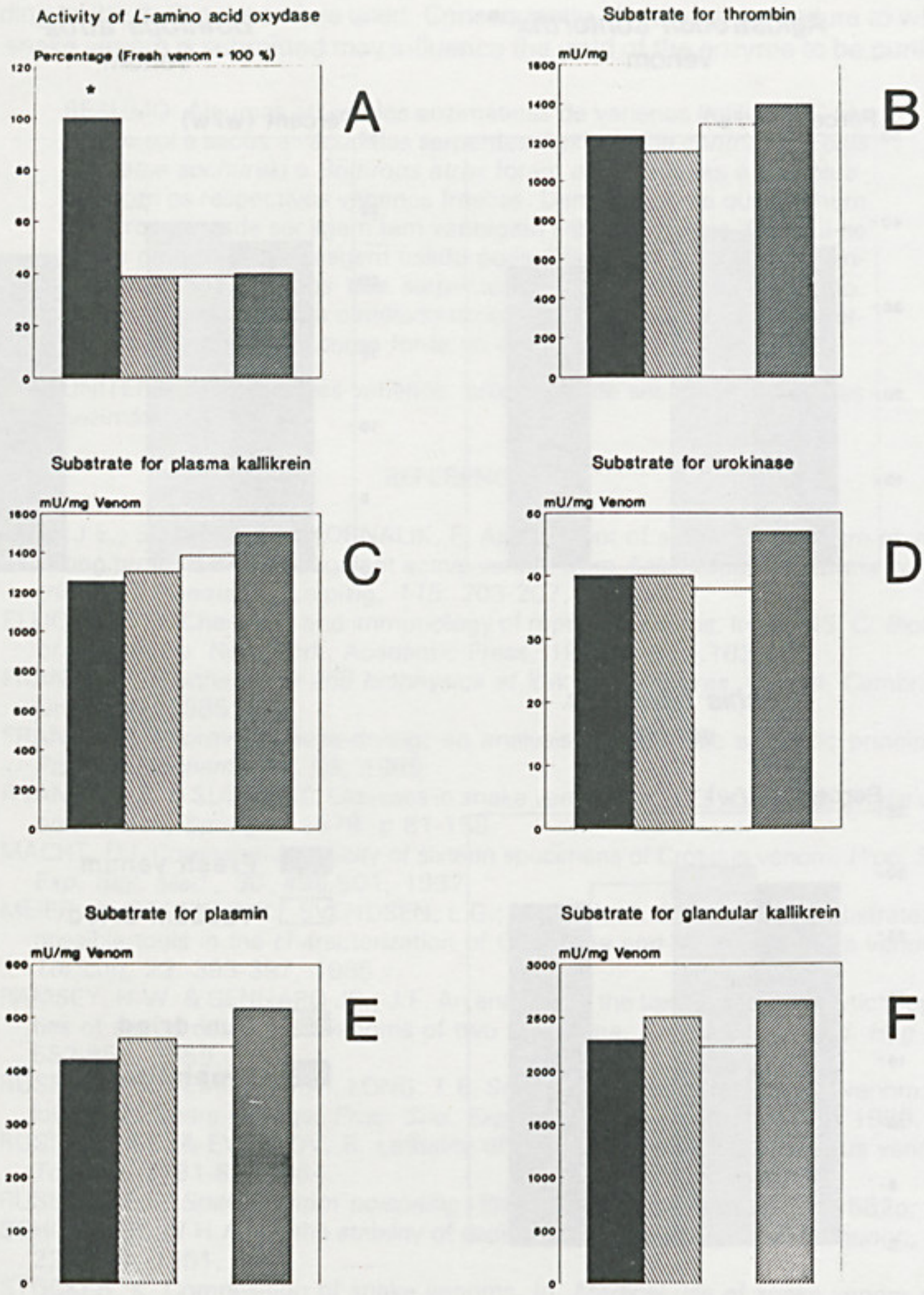

Fig. 2. Enzyme activities of *Agkistrodon contortrix* venom. With the exception of the L-amino acid oxidase determination, where every drying procedure leads to a significant activity decrease, all other differences are not significant with this venom.

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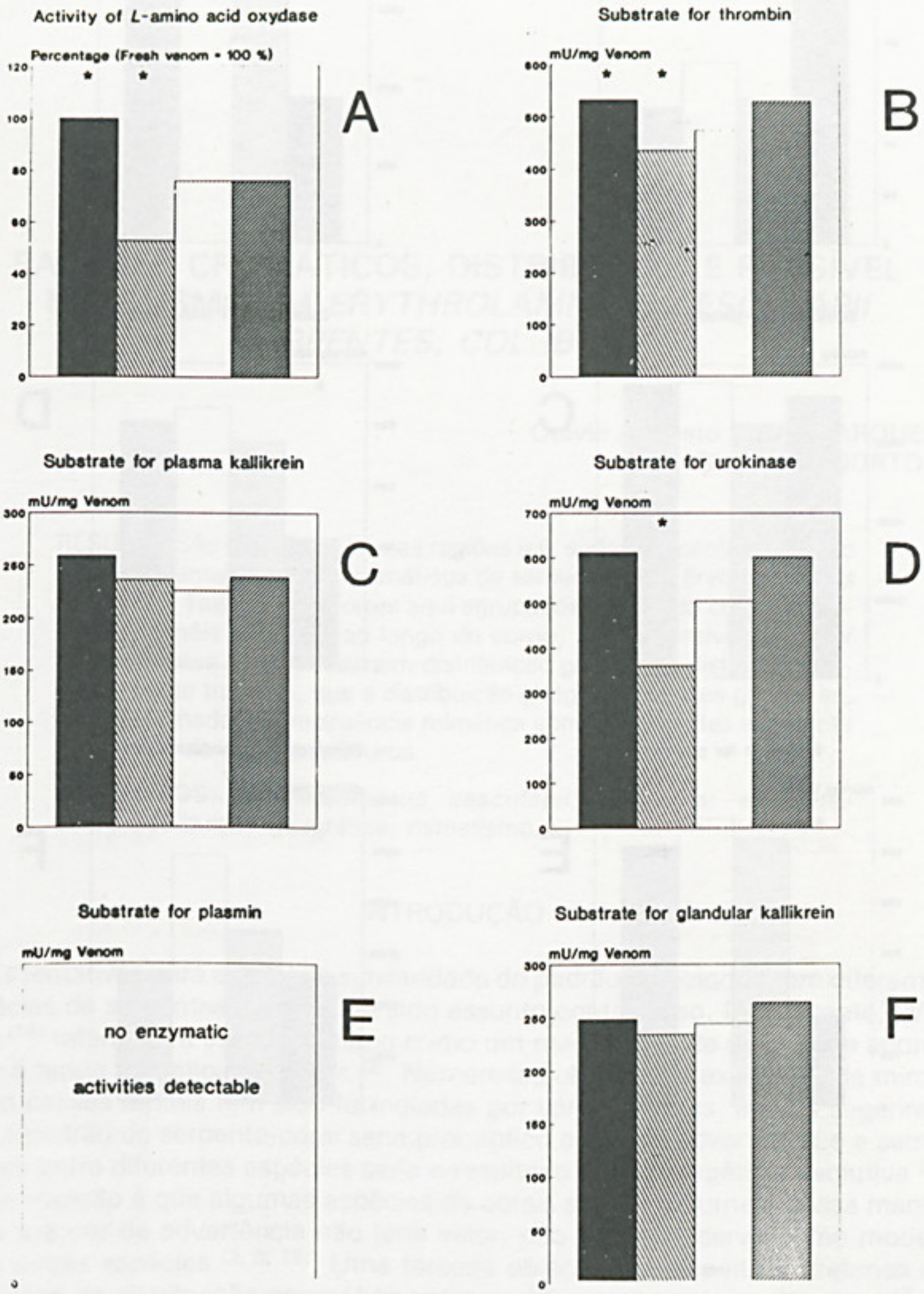

Fig. 3. Enzyme activities of *Bothrops atrox* venom. In comparison to fresh venom, sun-drying leads to a significant activity decrease of L-amino acid oxidase (A) and on the thrombin (B) and urokinase (D) substrates, respectively.

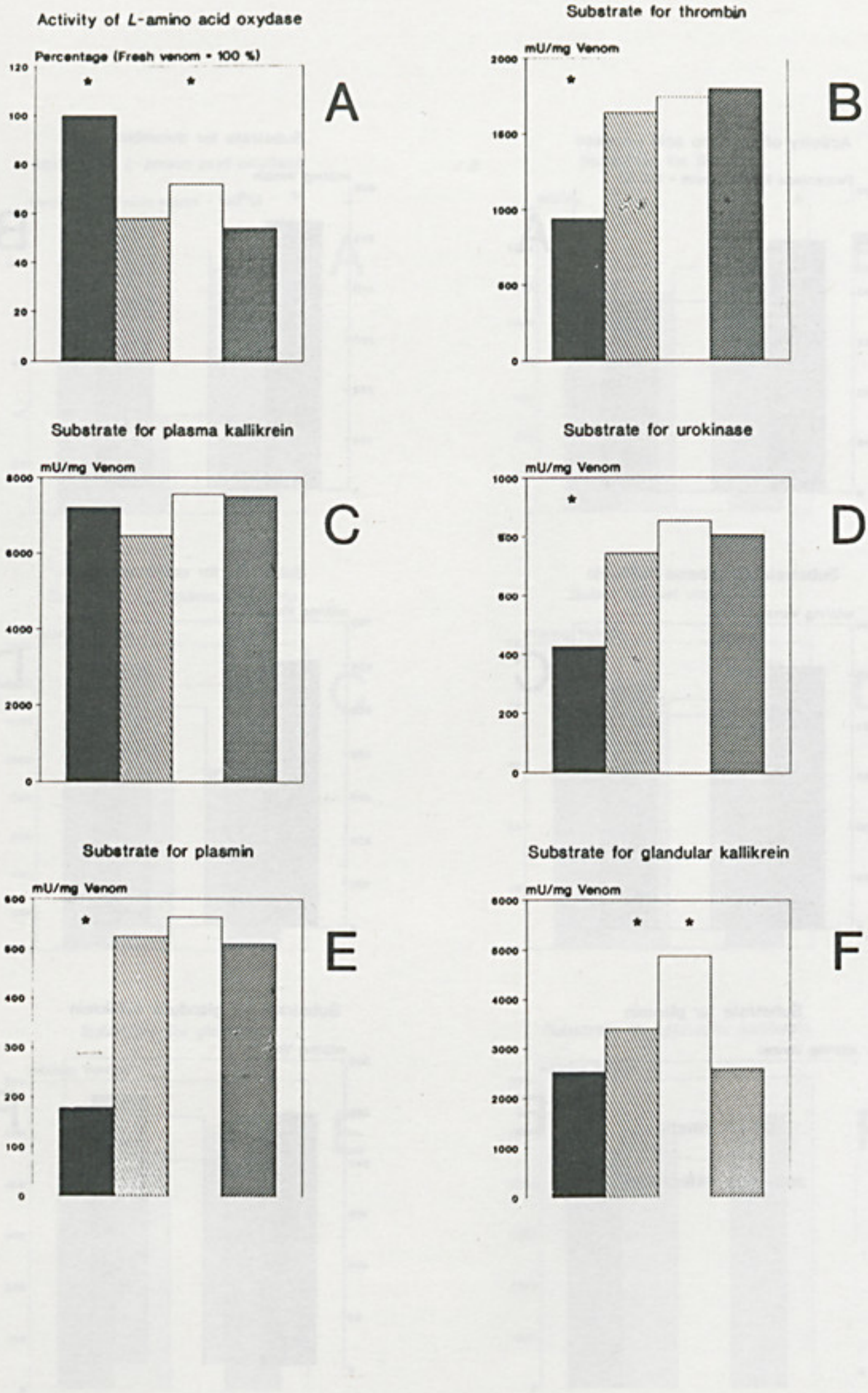

Fig. 4. Enzyme activities of *Echis carinatus* venom. The significant increase in activity observed with the thrombin (B), urokinase (D) and plasmin (E) substrates, respectively after drying, suggests the presence of one or more very labile inhibitors. Vacuum-drying seems to be the best drying procedure, in respect of enzyme activity as measured with the substrate for glandular kallikrein.