

BRIEF COMMUNICATION

Interference of *Pisum sativum* L. Root and Leaf Extracts with Nitrite Determination

J. SAHULKA and LUDMILA LISÁ

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

Abstract. Crude extracts of *Pisum sativum* L. roots interfere with nitrite determination only slightly, boiled root extracts considerably. Crude leaf extracts can interfere with nitrite determination in some cases even more than boiled root extracts. The precipitate of zinc phosphate helps to remove from crude leaf extracts some but not all the interfering material. Heating does not increase the interference in adult leaf extracts but it increases it in the extracts of very young *Pisum sativum* leaves.

When investigating anaerobic nitrite production by excised *Pisum sativum* L. roots we found that nitrite determination was made impossible by heating the assay medium with root segments at the end of the assay (SAHULKA 1977). To ascertain if this was caused by nitrite decomposition or if nitrite determination was hampered by some compounds formed and released from the roots during heating, we investigated the influence of heated and re-chilled extracts of *Pisum sativum* roots. This is of importance from the point of view of nitrate and nitrite reductase determinations. Boiled extracts in controls, or heating at the end of nitrate and nitrite reductase assays have been used or recommended by several authors (e.g. FERGUSON 1969, JONES and SHEARD 1973, FERRARI and VARNER 1971). ASLAM (1977) recently supposed that boiled maize root tip extracts inhibit nitrate reductase activity. Our results show that heated root extracts interfere with nitrite determination.

The roots of six-day-old seedlings of *Pisum sativum* L., cv. Raman, germinated under aseptic conditions, were homogenized in a mortar with a fourfold amount (w/v) of 0.05 M phosphate buffer pH 7.5, the homogenates were centrifuged at 15 000 g, 0 °C, 10 min, and the supernatants obtained were used either "unboiled" or were heated in boiling water in the first two series of experiments for 10 min (Fig. 1A, 1B) and in the third series for varying times (Fig. 1C). Heated and chilled extracts were again centri-

Received October 31, 1977; accepted December 15, 1977

* Address: Ke dvoru 16/15, 160 00 Praha 6, Czechoslovakia.

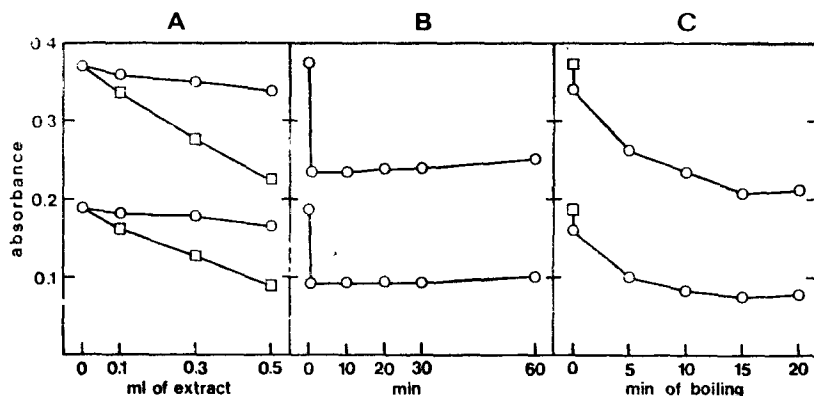


Fig. 1A. The effect of rising amounts of "unboiled" ○, and heated □, extracts of whole primary roots of *Pisum sativum* L. seedlings on nitrite determination (10 nmol and 20 nmol NaNO_2). The extracts were heated for 10 min. All the differences caused by heating are statistically significant, $P < 0.01$. Some differences caused by the addition of "unboiled" extracts, i.e. with 10 nmol NaNO_2 0.5 ml are also statistically significant, with 20 nmol NaNO_2 all differences. Fig. 1B. Interference in nitrite determination resulting from heated *Pisum sativum* L. root extracts after varying times of standing of heated extracts with nitrite solution. Time of heating 10 min. Always 0.5 ml of extract.

Fig. 1C. The influence of time of heating of the extracts on the extent of extract-derived interference in nitrite determination. □ Nitrite solution without extract. ○ Nitrite solution with 0.5 ml of extract.

fused for 10 min at $10\,000\times g$ and the supernatants obtained were used for the experiments. The influence of the extracts was investigated in 1 ml mixtures containing either 10 or 20 nmol NaNO_2 ; all the mixtures always contained the same amount of extraction buffer. Control mixtures did not contain nitrite. Nitrite was determined colorimetrically by adding 1 ml of 1% sulphanilamide in 1 N HCl and 0.5 ml of 0.02% N-(1-naphthyl)-ethylene-diamine dihydrochloride. These reagents are generally used for nitrite determination in enzyme assays.

"Unboiled" crude extracts of *Pisum sativum* roots interfere with nitrite determination only slightly, whereas heated extracts considerably (Fig. 1A). The effect on nitrite determination was approximately the same when the reagents were added to the mixture at any time between 0 and 60 min (Fig. 1B). The interference occurred even when the boiled extract was added to nitrite solution after sulphanilamide. The highest interference was observed after 15 min heating (Fig. 1C). We did not attempt to isolate and characterize the compounds interfering with the determination. Coagulated proteins were removed from the heated extracts during centrifugation.

Crude unheated extracts of *Pisum sativum* L. leaves prepared in the same way as crude root extracts interfered with nitrite determination in some cases even more than boiled root extracts (data not shown). The precipitate of Zn-phosphate formed after the addition of Zn-acetate to mixtures containing phosphate buffer, leaf extracts and nitrite helped to remove some material interfering with nitrite determination, but the interference was not removed completely. Heating crude extracts of mature leaves of *Pisum sativum* and *Brassica oleracea* did not further increase the interference, while heating crude extracts of young expanding leaves from one-week-old seedlings

of *Pisum sativum* increased it considerably (data not shown). All these facts should be taken into account when determining nitrate and nitrite reductase activities.

We intend to investigate in further experiments whether the interference with nitrite determination by leaf extracts can be overcome entirely.

References

- ASLAM, M.: Presence of heat-inducible nitrate reductase inhibitors in corn root tip. — *Plant Sci. Lett.* **9** : 89—92, 1977.
- FERGUSON, A. R.: The nitrogen metabolism of *Spirodela oligorrhiza*. II. Control of the enzymes of nitrate assimilation. — *Planta* **88** : 353—363, 1969.
- FERRARI, T. E., VARNER, J. E.: Intact tissue assay for nitrite reductase in barley aleurone layers. — *Plant Physiol.* **47** : 790—794, 1971.
- JONES, R. W., SHEARD, R. W.: Application of automated dialysis and colorimetry to the assay of nitrate and nitrate reductase in plant extracts. — *Can. J. Plant Sci.* **53** : 207—213, 1973.
- SAHULKA, J.: The influence of pretreatment with different cations on anaerobic nitrite production by excised *Pisum sativum* L. roots. — *Biol. Plant.* **19** : 338—345, 1977.