CARTAS AL EDITOR

A RAPID AND SENSITIVE METHOD FOR THE DETECTION OF PROTEASE INHIBITORS

Dear Sir:

Most edible legumes and several other foods contain protease inhibitors which may have antinutritional effects if they are not destroyed by appropriate heating. Therefore, various procedures have been described for their detection (1-3).

We have succesfully developed a simple method which can be performed with very small amounts of sample, and which has resulted most useful in testing protease inhibitors in legume seeds.

Finely ground seeds are extracted with 0.85o/o NaCl solution (seed to solvent ratio 1:10) by continuous stirring at room temperature for 1 hr, and clarified by centrifugation and filtration.

A casein-agarose solution is then prepared as follows: 16.6 ml of 0.8o/o agarose solution, 1.8 ml of 0.1M $CaCl_2$ and 3.6 ml of 0.2o/o casein, all dissolved in Michaelis buffer (barbital sodium-sodium acetate - HCl, pH 8.6, ionic strength 0.05). In the blank solution casein was replaced by buffer.

Standard microscopic slides are covered with one or the other of the previously melted agarose solutions to form 2-mm thick layers using gel electrophoresis holders. After solidification, 6-8 holes of 3 mm diameter are punched in each slide and the agarose removed with the help of a hypodermic needle.

Extract samples of 5 µl are filled in one of the holes of a slide

with and without casein. To allow diffusion of the inhibitor the slides are kept in a humid chamber at 37°C for one hour. Then the casein-containing slides are covered with a piece of filter paper Whatman No. 1 (76x26 mm) previously soaked with a trypsin (Sigma, from bovine pancreas type III) solution of 25 μg/ml in Michaelis buffer, and incubated again for one hour at 37°C in the humid chamber. After removing the filter paper strips both slides are stained for 30 min with a solution of 0.2% amido black in 7% acetic acid and destained with 7% acetic acid until the agarose is colorless.

When the slides with the casein-free agarose were treated with the protease, the same blue spots as in the blank slides were observed.

Where inhibitor is present, a blue circle appears around the hole in the casein-agarose gel plates, indicating that the casein has not been digested. This slide is compared with the casein-free one. Usually the proteins of the sample diffuse little into the agarose and are not digested by trypsin under the experimental conditions described. The samples marked *a*, *b*, *c*, and *d* in Fig. 1 showed the typical pattern of undigested casein. In sample *e* no protein was detected in the casein-free slide nor inhibition circle in the casein-containing one. Sample *f* had no detectable trypsin inhibitor.

By employing this procedure many samples can be analyzed in a short period of time without using any expensive laboratory equipment. Parts of single seeds, chromatographic fractions or heat-treated food products can easily be tested by this method due to its high sensitivy, since 5 ng of Kunitz trypsin inhibitor (Sigma type 1-S) can be detected. Proteases other than trypsin can be used for testing the presence of their respective inhibitors.

A. Callejas
A. Palozzo
W. G. Jaffé
Universidad Central de Venezuela
Facultad de Ciencias
Escuela de Biología, Apartado Postal 47097 Los Chaguaramos
Caracas 1041 A, Venezuela

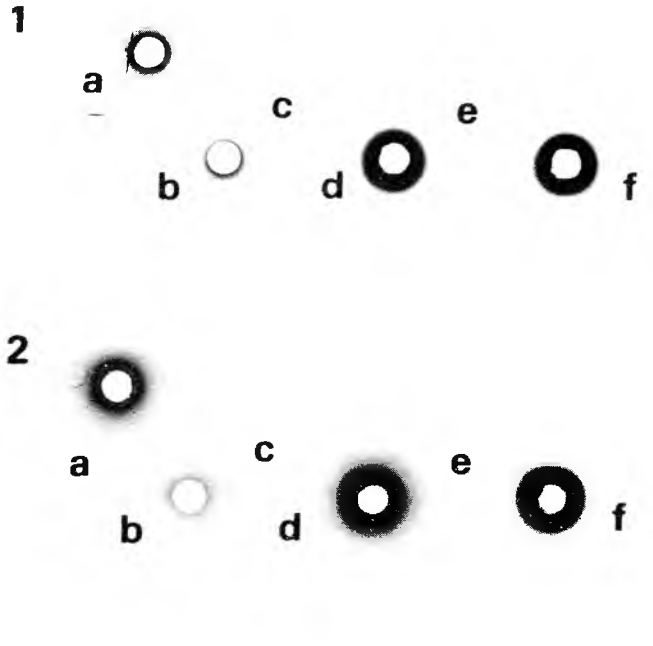

FIGURE 1

Inhibitory activity of several legume seed extracts

Plate 1: Casein-free agarose gel.
Plate 2: Agarose-casein gel;

a *Phaseolus vulgaris* var. cubagua
b *Abrus precatorius*
c *Cassia muschiata*
d *Glycine max.*
e *Lonchocarpus* s.p.
f *Clitoria generallis*

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CONTAMINATION OF GUATEMALAN COTTONSEED OIL WITH MOLD METABOLITES

Dear Sir:

Cottonseed oil, produced locally, is the main edible oil consumed in Guatemala. Quality control analyses carried out in our laboratory[1] for nine years have revealed invariably the presence of an extraneous substance in the oil. This material is neither a natural component nor an additive used to lengthen the shelf-life of this commodity.

The extraneous component can be detected in the unsaponifiable fraction by thin layer chromatography (TLC) assays. With this technique, an intensely fluorescent, aquamarine colored band can be observed. The location of the fluorescence is immediately above the band corresponding to the sterol fraction.

Contamination with aflatoxin was discarded after TLC assays showed that the fluorescent band of the oil has a different R_f than the one related to the mycotoxin.

In order to investigate if the compound is produced by mold infestation of the cotton plants, ground seeds were placed in a favorable medium for fungal growth. After a few days, several kinds of molds were present in the Petri dishes. Every one of them was isolated in order to obtain pure colonies.

One of these cultures produced pigments extractable in diethyl ether. A solution of these pigments, obtained after washing the Petri dishes with the solvent, was assayed by means of TLC. The presence of several resolved yellow pigments and a fluorescent band similar to the one in the oil could be observed in the plates.

This mold was identified as *Aspergillus chevalieri*. Pure cultures of this mold were obtained from the Northern Research

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1 Laboratorio Unificado de Control de Alimentos y Medicamentos (LUCAM).

Laboratory collections. This culture was compared with the local strains by TLC. In both cases the yellow pigments and the aquamarine fluorescence were present.

It has not been possible to establish unequivocally that the fungal metabolite from *A. chevalieri* is the same material found in the oil. It must be mentioned here that fluorescent bands have been observed in the unsaponifiable fraction of palm and rubber seed oils produced in Central America. It is possible that these fluorescent compounds present in vegetable oils produced in the tropics have their origin in mold infestation of the crops.

In order to verify this hypothesis further work is necessary in connection with the identification of the molecular structure of the fluorescent compound. Studies to find out the effects of this substance in the diet of the population should be carried out also.

A final observation is that this compound survives the hydrogenation process. The fluorescence can be detected in margarines and shortenings derived from cottonseed oil.

Jorge Zúñiga Rojas, Ph. D.

Senior Chemist, Laboratorio Unificado de Control de Alimentos y Medicamentos (LUCAM), OPS / OMS / INCAP / Gobierno de Guatemala.
INCAP
Apartado Postal 11-88
Guatemala, Guatemala.

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