Obtaining concentrated extract of lipases from orange waste

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Abstract

The crude extracts were obtained from orange wastes processed for juice. These crude extracts were concentrated by precipitation with ammonium sulfate. The lipases contained in the concentrated extracts were identified by zymography with the MUF-butyrate as substrate.

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Materials

100ml CHAPS, 5% filtered solution DG098 by G-Biosciences

1 Gallon TBS [10X] (Tris buffered saline) (100mM Tris.HCl, 1.5M NaCl, pH 7.5) R030 by G-Biosciences

1kg Ammonium Sulfate RC-019 by G-Biosciences

4-Methylumbelliferyl butyrate 17695-46-4 by Sigma Aldrich

Protocol

Obtaining crude extracts

Step 1.

The oranges used for the study were from the species *Citrus sinensis* L. Osbeck Pear variety. The orange samples were processed using in line extraction of the J. B. T. Food Tech company. During this process were generated three wastes fractions, called peel, core and frit. About 10 pounds of each fraction was collected manually from the output location of the equipment, individually packaged and identified.

Each waste fraction was analysed separately. After processing, the samples were packaged, stored for 2 days at 5ºC, milled mechanically to a particle volume of approximately 0.5 cm3, frozen and lyophilised (Liotop® L101) at -60°C for 24 hours. After lyophilisation, the matrices were again crushed and stored at approximately 7ºC until the time of analysis (until 6 months after the sampling).

Obtaining concentrated extracts

Step 2.

The crude extracts were fractionated through salting out using 60% ammonium sulfate (Ecibra ®) and through dialysis using dialysis tubing cellulose membrane 43mm (Sigma-Aldrich®). The extracts were lyophilized (Liotop® L101) at -60°C for 24 hours after fractionation and frozen until analysis.

Lipase zymography (MUF-butirate)

Step 3.

The lipase activity was identified through zymography by using MUF-butyrate substrate (Sigma Aldrich®).

The concentrated extracts were resuspension in Tris buffer (0.05 M, pH 7.4) containing CHAPS (4%) and protease inhibitor (Sigma-Aldrich 1mM); then, they were homogenized in probe and centrifuged.

Different polyacrylamide concentrations, ranging from 5 to 15%, were tested in order to prepare the 10% native polyacrylamide gel. The gel was prepared with 4 mL distilled water, 2.5 ml HCl Tris buffer (0.5 M, pH 8.8), 3.35 mL acrylamide/bis-acrylamide (30/0.8%), 5 µL TEMED, and 50 µL ammonium persulfate. Each gel well was added with 20 µL of each sample and the run was performed at 150 volts.

After the electrophoresis, the gel was placed in a vessel containing 100 mL Tris buffer (50 mM, pH 8.0), 2% triton X-100 and 10 µM methylumbelliferyl (MUF) -butyrate for 15 min, at 37°C. The bands were analyzed under ultraviolet light (UV) to allow finding lipase in the extracts.

The results were expressed as mean ± standard deviation (SD) and analyzed through Tukey test; all pairs of groups were compared through One-Way ANOVA (non-parametric); $p < 0.05$ was considered statistically significant, whereas $p < 0.0001$ was considered highly significant. The bands were quantified in the Image J 1.15 software. The statistical analysis was performed in the GraphPad Prism 6 software.