A Research Note

Effect of Selected Antioxidants on the Activity of a Mixture of Crude Pseudomonas Lipases

P. L. HARRIS and S. L. CUPPETT*

Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583-0919

(Received for publication June 4, 1990)

ABSTRACT

Commercially acceptable concentrations (0.02%) of butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), Tenox 4, and Tenox 8 were evaluated for their effect on the lipolytic activity of mixed Pseudomonas crude lipases (MCLs). MCLs were prepared from lipases produced by raw milk isolates of Pseudomonas fluorescens, P. cepacia, and P. putida. GRAS antioxidants, L-ascorbyl 6-palmitate (AP) and d-α-tocopherol (AT), at 0.5, 1.0, and 2.0% concentrations, were also evaluated for their effect on lipase activity. Antioxidants and enzyme preparations were incubated for 30 min at 25 or 40°C. At 25°C, all antioxidants tested inhibited lipase activity to some extent. At 2% concentration, AP completely (100%) inhibited lipolytic activity, and AT inhibited lipolytic activity by 89%. At 40°C, the effectiveness of AP and AT as lipolytic inhibitors decreased, and the low concentrations of BHT, BHA, TBHQ, Tenox 4, and Tenox 8 stimulated lipolytic activity.

Antioxidants, as defined by the United States Food and Drug Administration, are substances used to preserve food by retarding deterioration, rancidity, or discoloration as a result of oxidation (3). Commercially available antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), d-α-tocopherol (AT), and L-ascorbyl 6-palmitate (AP) (8,13,20), are used extensively in the food industry (7,12,13).

Microbial lipases have been responsible for the reduction in storage stability of a variety of products (16,19,22). Although the heat treatments used in milk processing effectively destroy most heat-labile microorganisms, heat is only partially effective in eliminating the heat-resistant extracellular lipases which have been secreted into the raw milk by the microorganisms (5,9,10,18,21,23).

Heat-resistant extracellular lipases are inhibited in broth by metal ions, including Zn²⁺, Hg²⁺, Cu²⁺, Ni²⁺, Cd²⁺, Fe³⁺, Fe²⁺, and Co²⁺ (1,4,14,15) and partially inhibited by NaCl (4), ethylene diaminetetraacetic acid (EDTA) (6), and the amino acid, histidine (2).

The potential use of antioxidants as lipolytic inhibitors has not been investigated. Therefore, this study was designed to investigate the effect of selected antioxidants on the lipolytic activity contained in mixed crude lipase (MCL) derived from the individual isolates of Pseudomonas fluorescens, P. cepacia, and P. putida.

MATERIALS AND METHODS

Isolation and identification of lipolytic psychrotrophic bacteria

Lipolytic psychrotrophic bacteria were isolated from raw milk by the single-layer method (11). Identification was facilitated by the use of Oxiferm tubes (Roche, Nutley, NJ), API 20E strips (Analytab Products, Plainsview, NY), and additional biochemical tests (17). Isolates were obtained at 5°C, and, thereafter, maintained at 5°C on standard plate count agar slants.

Lipase production and mixed crude lipase (MCL) preparation

Ten 250-ml Erlenmeyer flasks, each containing 100 ml of reconstituted nonfat dry milk (NFDM) (10%), were sterilized at 121°C. After cooling to 21°C, the milk samples were inoculated with a culture of P. fluorescens, P. cepacia, or P. putida, and shaken using a Burrell Wrist Action Shaker (Burrell, Pittsburgh, PA) at 21°C for 24 h at a setting of 3. The cultures were then incubated at 21°C under stationary conditions for an additional 4 d. Cultures containing the same bacterial strain were pooled, and the microbial cells were separated from the supernatant by centrifugation at 11,500 g at 4°C for 30 min. The Pseudomonas lipases were precipitated from the supernatant by the addition of ammonium sulfate to 40% saturation. This fraction (40%) was collected and suspended in 50 ml of 5 mM N,N'-bis [2-hydroxyethyl]-2 aminoethane sulphonic acid (BES) buffer (pH 7.2) and dialyzed against 4 L of distilled water at 5°C using a 1.6 cm diameter Spectrapor (Spectrum Medical Industries, Inc., Los Angeles, CA) membrane tubing with a molecular weight cutoff of 6,000 - 8,000 daltons. After 24 h of dialysis, with three water changes, the MCLs were frozen, freeze dried, and stored at -20°C until needed. The Pseudomonas MCL preparation that was tested contained an equal weight (1:1:1) ratio of the lipases from P. fluorescens, P. cepacia, and P. putida diluted 1:200 (w/v) with reconstituted NFDM (10%).

Published as Paper No. 8948. Journal Series Nebraska Agricultural Experiment Station, Lincoln, NE 68583-0919.
Antioxidants

BHT, BHA, TBHQ, Tenox 4 (20% BHA, 20% BHT, and 60% corn oil), and Tenox 8 (20% BHT and 80% corn oil) were obtained from Eastman Kodak Co. (Kingsport, TN) and prepared at 0.02% concentrations (w/v) in dimethyl sulfoxide (DMSO). L-Ascorbyl 6-palmitate (AP) and d-α-tocopherol (AT), both with GRAS status, were obtained from Sigma (St. Louis, MO) and were used at levels of 0.5, 1.0, and 2.0% (w/v) in 95% ethanol.

Lipase assay

Lipase activity was determined by using the method of Versaw et al. (23), which is based upon the release of β-naphthyl (BN) from β-naphthyl caprylate (BNC) and is lipase specific. In this procedure, 0.05 ml of the MCL was added to an incubation mixture containing 0.2 ml 200 mM sodium taurocholate and 1.8 ml 50 mM BES buffer (pH 7.2) and then equilibrated at either 25 or 40°C. Then, 0.02 ml of 200 mM BNC and 0.05 ml of an antioxidant was added to each assay tube. Control tubes had 0.05 ml of either DMSO or 95% ethanol added, depending on the solvent used to solubilize the antioxidant. The tubes were incubated at 30 min. After incubation, 0.02 ml of Fast Blue BB salt (100 mM in DMSO) was added and the tubes incubated for an additional 5 min. The reaction was stopped by the addition of 0.2 ml of 0.72 N trichloroacetic acid (TCA). Then 2.71 ml of a 1:1 ethanol (95%)/ethyl acetate (v/v) mixture was added and the absorbance at 540 nm was determined. Lipase activity was reported in μmoles of β-naphthol released/ml/h. Treatment effects are reported in values relative (percentage increase or decrease) to the control.

A completely randomized experimental design was used with six replicate treatments per variable. ANOVA (LSD) (20) statistical tests were performed.

RESULTS AND DISCUSSION

At 25°C, BHT, BHA, or TBHQ alone significantly (P<0.05) reduced lipolytic activity by the MCL (Table 1). BHT was a significantly (P<0.05) better lipolytic inhibitor than BHA and TBHQ. At 40°C, BHT, BHA, or TBHQ stimulated lipolytic activity, although not significantly (P>0.05). At both 25 and 40°C, the corn oil preparations of BHT and BHA (Tenox 4) and BHT (Tenox 8) behaved in a manner similar (Table 1) to BHT or BHA alone, but each response was decreased in magnitude, possibly due to the reduced levels of the antioxidants contained in these preparations.

At a 0.5% concentration and 25°C, AP significantly decreased (P<0.05) lipolytic activity by the MCL, but at 40°C this concentration of AP stimulated lipolysis, although this effect was not significant (P>0.05) (Table 1). AP significantly (P<0.05) inhibited lipolytic activity of the MCL at a 1.0% concentration and 25°C, but this effect was diminished but not significantly (P>0.05) at 40°C. AP significantly inhibited (P<0.05) lipolytic activity at a level of 2.0% at both temperatures (Table 1). The inhibitory effectiveness of AP was directly related to concentration and indirectly related to incubation temperature.

At all concentrations and temperatures evaluated, AT significantly (P<0.05) inhibited lipase activity of the MCL. The effectiveness of AT as a lipolytic inhibitor, at 25°C, was not proportional to the concentration added (Table 1). At 40°C, the effectiveness of AT as a lipolytic inhibitor decreased significantly (P<0.05) at both the 1.0 and 2.0% concentrations, in that, at this temperature (40°C), the reduction in lipase activity was less (59-62%) than the reduction (73-80%) observed at 25°C.

REFERENCES