Effects of High Hydrostatic Pressure on the Inactivation of Bacterial Metaloproteases Enzymes Associated to Milk Spoilage

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To prevent deterioration of dairy products, the inactivation of extracellular proteases produced by psychrotrophic microorganisms able to degrade milk proteins is very relevant. Proteolytic digestion of the milk can lead to clotting and gelation of milk casein. High hydrostatic pressure (HPP) is the most-widely adopted novel non-thermal technology for the commercial pasteurization of foods. HPP produces minimal changes in flavor, color, or taste of food. Pressurization also modifies the conformation of enzymes altering their active sitesand consequently, their biological activities. To investigate, the pressure-induced inactivation of a crude extracellular protease produced by a *Stenotrophomonas maltophilia* strain isolated from a dairy plant was evaluated as a model. The enzyme was extracted after 72 h of microorganism growth at 30 ° C by using a complex medium (CM), containing peptone (5g / L), beef extract (3 g / L) supplemented with 1% skimmed milk powder. Four micrograms of the crude extract were placed in sample plastic bags (2cm x 3cm) , heat sealed free from air bubbles, treated at 300 MPa and 30 °C or 600 MPa and 35°C both for 15 minutes followed by storage at 4°C until protease activity determination. A slight decrease in protease activity of *Stenotrophomonas maltophilia* was observed when the pressure was increased at pH 8,3: inactivation of 14,3% at 300 MPa and of 17,3% at 600 MPa. The reduced inactivation effect seems to be a general pattern since no proteolytic inactivation was observed when the crude extract form the reference strain, *Pseudomonas fluorescens* (ATCC 13525), was tested at the same conditions. The partial inactivation of the proteolytic enzymes of *Stenotrophomonas maltophilia* strain depended on the pH, processing temperature and processing time. Further investigation is required in order to evaluate the possibility of increasing milk spoiling bacterial metaloproteases.

Keywords: metalloproteases, high pressure, milk spoilage.

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