TITLE: EVALUATION OF SUBSTRATES FOR THE PRODUCTION OF A BACTERIAL PROTEASE AND ANTIOXIDANT HYDROLYSATES

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ABSTRACT:
Proteases are valuable biocatalysts employed in the detergent, leather and food industries, and *Bacillus* spp. are major sources of these enzymes. Substrates used in culture media affect protease production; thus, evaluation of substrates is a relevant part of upstream bioprocessing. Microbial processes are also studied to obtain protein hydrolysates, since released peptides may possess antioxidant potentials. In such processes, microorganisms secrete proteases which then hydrolyze the proteins. Thus, the co-production of proteases and antioxidant protein hydrolysates was evaluated during cultivations with *Bacillus* sp. CL18 using different substrates. Cultivations were performed in mineral medium with 10 g/L of either feathers, casein, feather meal (FM), fish scales (FS), soybean meal (SM), soy protein (SPI), whey protein (WPI), or lyophilized whey (LW). During incubations (30°C, 125 rpm, 0-7 days (d)), culture supernatants were evaluated for proteolytic activity (azocasein; U/mL), soluble protein (Folin-phenol) and scavenging of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS; %). WPI and LW induced low protease production (<221 U/mL). Highest production in other media were (U/mL; d): feathers (323; 5), FM (426; 4), SM (590; 1), casein (700; 2), FS (730; 1), and SPI (1260; 2). FS (10 g/L) was selected due to higher productivity (U/mL/day). FS+glycerol (10 g/L) resulted in higher protease production (1,420 U/mL; d 6), but lower productivity. In FS+NH_4Cl (1 g/L) medium, increased production (1,815 U/mL; d 2) and productivity were observed. Initial soluble protein contents were increased during cultivations with feathers, FM, WPI, casein, SPI, and FS+glycerol, whereas no prominent increases occurred with LW, SM, FS, and FS+NH_4Cl. Radical scavenging was not affected in LW medium (~15%, 0-7 d). In other media, scavenging was increased as compared to initial (d 0) values. Specifically, from 1 to 59% (d 5) with feathers; from 38 to 67% (d 4) with SM; from 60 to 82% (d 4) with casein; from 24 to 67% (d 5) with WPI; from 14 to 68% (d 4) with FM; and from 20 to 72% (d 2) with SPI. Initial scavenging (~8%), was incremented to 24, 35, and 60% after 2 d of cultivation in FS, FS+NH_4Cl, and FS+glycerol, respectively. SPI and FS-containing media were particularly suitable to obtain, simultaneously, proteases and bioactive hydrolysates. From a biorefinery perspective of residual biomass transformation, FS appears as a promising substrate.

Keywords: bioconversion, enzyme, protein hydrolysate, bioactivity