

**TITLE:** MICROBIAL PROCESSING FOR THE VALORIZATION OF AGRO-INDUSTRIAL BY-PRODUCTS

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**ABSTRACT:**

Brazil ranks 3<sup>rd</sup>, 6<sup>th</sup>, and 4<sup>th</sup> in the global production of poultry meat, cheese, and cassava, respectively. High amounts of feathers, whey, and cassava bagasse (from starch production) are generated as residues. Bioprocessing is an interesting approach for the conversion residual biomasses into value-added products. Therefore, these by-products were used, as single or combined substrates, to obtain proteases and antioxidant hydrolysates during cultivations with *Bacillus* sp. CL33A. Cultivations were initially carried out in mineral medium with feather meal (FM; 10 g/L), liquid whey (LW), lyophilized whey (WL; 5, 10, 15 g/L), or cassava bagasse (CB; 5, 10, 15 g/L plus 1 g/L ammonium sulfate). Thereafter, FM was evaluated at 10, 15, 20, 30, 40 g/L. FM concentration resulting in higher protease production was selected, and then WL, CB (1, 5, 10 g/L), and LW were evaluated as co-substrates. During incubations (30 °C, 125 rpm, 0-7 days (d)), the proteolytic activity (azocasein; U/mL), soluble protein (Folin-phenol), scavenging of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS; %), and Fe<sup>2+</sup>-chelating ability (ferrozine method; IC; %) were evaluated in culture supernatants. As a single substrate, FM resulted in higher protease production (488 U/mL; d 3), as well as higher soluble protein contents (5.7 mg/mL; d 4) and antioxidant activities (81% ABTS, 98% IC; d 4), in comparison to LW, WL or CB (66-204 U/mL protease; 1.4-2.9 mg/mL protein; 25-45% ABTS; 5-45% IC). Higher FM concentration up to 30 g/L increased protease production (3,045 U/mL; d 4), which was accompanied by increments in soluble protein (10.6 mg/mL) and antioxidant potentials (93% ABTS, 99% IC; d 3). Supplementing WL (5 g/L) to FM medium (30 g/L) further enhanced protease production (5,275 U/mL; d 4). The use of CB (particularly at 5 g/L) as co-substrate with FM (30 g/L) also incremented protease yields (7,025 U/mL; d 4). Effects of CB and WL were not significant towards antioxidant activities. Addition of LW to FM (30 g/L) drastically diminished the production of protease, which retarded the release of soluble proteins, delaying the enhancement of measured bioactivities. FM is a suitable substrate to obtain proteases and antioxidant hydrolysates. WL and CB, as abundant residues, are promising co-substrates to enhance protease production. Further research is guaranteed regarding protease applications, and the search for bioactive peptides within protein hydrolysates.

**Keywords:** bioprocess, protease, protein hydrolysate, antioxidant activity

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