



Abstract Obtaining Protein Hydrolysates from Hemp Seeds ⁺

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Hemp (Cannabis sativa ssp. sativa) is a widespread textile plant whose seeds, obtained after the commercial utilization of fiber, contain 20–25% protein, which is rich in all the amino acids necessary to maintain good health. Hemp seeds' protein has a very good bioavailability and digestibility, contains hypoallergenic peptides whose absorption is improved by the fiber complex, and has a subtle aromatic profile, being a balanced food source for human nutrition. The current trend is to relaunch this underexploited plant to obtain seeds protein with benefits in various diseases that have been medically proven. The aim of this paper was to perform the enzymatic hydrolysis of hemp seeds protein to obtain bioactive peptides. Hemp seeds' protein was used as a substrate for testing the following enzymes: Papain, Alcalase 2.4 L, Pepsin, and Pancreatin. The hydrolysis efficiency was evaluated for each enzyme by modifying the following parameters: enzyme/substrate ratio, pH, temperature, and reaction time. Enzymatic hydrolysis of hemp seed proteins was performed according to the methods described by [1,2], with minor modifications. Hemp seed protein (5% w/v protein weight) was dispersed in distilled water at room temperature and homogenized at 10,000 rpm for 2 min. The temperature and pH of suspensions were adjusted to the values of each enzyme to ensure an optimum activity. The suspensions were gently agitated on a stirrer. The substrate/enzyme ratio was calculated from the amount of protein in the sample. During digestion, the pH values were kept constant by the addition of 2M NaOH or 1M HCl. After inactivation of the enzymes (90 °C for 15 min), the protein hydrolysates were neutralized and centrifuged at $5400 \times g$, 20 min, at 4 °C, to remove undigested proteins. The supernatant containing a mixture of amino acids, oligopeptides, and polypeptides was recovered, dried, and stored at -20 °C for further analysis. The specific degree of hydrolysis for each enzyme in descending order was: papain > pepsin > alcalase > pancreatin. The enzymes that achieved a higher degree of hydrolysis were papain, after 1 h (2.25%), and pepsin after 2 h (1.87%). Hydrolysis with alcalase reached the degree of hydrolysis of 1.12% after 2 h, being faster than that with pancreatin, which recorded 1% after 3 h. After the initial phase of increasing hydrolysis, the rate of hydrolysis decreases as time increases, entering a stationary phase. Currently, interest for protein hydrolysates containing bioactive peptides is growing due to their potential to be used in the formulation of functional foods. For the production of bioactive peptides, hemp must also be considered, a cheap and sustainable undervalued plant, whose seeds' protein is of high quality.

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