Unlocking Hidden Value in Canola By-Products: A Proteomics-Driven Discovery of Bioactive Peptides

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Abstract

Global canola seed meal (CSM) production reached approximately 49.2 million metric tons in 2023, underscoring its significance as a protein-rich by-product of oil extraction with increasing potential for valorization in food, feed, and pharmaceutical applications. With Australia alone projected to produce 5.5 million metric tons of canola by 2025, yielding around 696,000 tons of CSM—there is growing interest in exploring this underutilized resource.

This study investigated the impact of two extraction solvents and enzymatic digestion strategies on the number of identified proteins with potential bioactive peptides (BAPs) in CSM, aiming to assess its value as a sustainable source of functional biomolecules. A discovery proteomics approach was used, combined with predictive bioinformatics tools. Proteins were extracted using a chaotropic denaturant (urea) or a food-grade mild alkaline solution (alkaline water), followed by digestion with trypsin or chymotrypsin. Mass spectrometry was performed using data-dependent acquisition on a SCIEX 6600 TripleTOF.

Urea-based extraction yielded significantly higher protein and peptide recovery compared to alkaline water. A total of 541 proteins were identified using urea with trypsin, 192 with alkaline water and trypsin, 194 with urea and chymotrypsin, and 90 with alkaline water and chymotrypsin, all at a global 1% FDR. The corresponding numbers of potential BAPs identified from protein-derived fragments were 113 (urea + trypsin), 111 (urea + chymotrypsin), 90 (alkaline + trypsin), and 100 (alkaline + chymotrypsin) based on 80 percent sequence homology to known BAPs bioactive peptides. Potential antibacterial peptides were the most abundant, followed by peptides associated with blood-brain barrier permeability, and antifungal, antiviral, antihypertensive, and anticancer activities.

A significant portion of the identified BAPs, over 75%, originated from defensin-like proteins, small cysteine-rich peptides that are part of the plant's innate immune system. These proteins are well known for their antimicrobial properties, particularly their ability to disrupt microbial membranes, making them a promising source of naturally derived antimicrobial agents.

Further screening with Bioranker predicted 13 non-toxic peptides with 90% confidence, based on sequence features, and structural properties, showing strong potential for anti-inflammatory and anticancer applications.

This study highlights the significant potential of canola seed meal for valorization, demonstrating its use as a rich source of bioactive peptides that could be harnessed for the development of high-value ingredients in food, health, and nutrition applications.