

GreenNutriFood

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BOOK OF ABSTRACTS

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SUSTAINABLE FOOD, GREEN CHEMISTRY
AND HUMAN NUTRITION

Dubrovnik riviera, Croatia

University of Zagreb, Faculty of Food Technology and Biotechnology

Universitat De València, Faculty of Pharmacy and Food Sciences

**1st International Congress on Sustainable Food, Green Chemistry and Human
Nutrition**

GREENNUTRIFOOD2025

Book of Abstracts

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ELECTROPHORETIC ANALYSIS AND DIGESTIBILITY OF RAPESEED PROTEINS ISOLATED BY DIFFERENT EXTRACTION METHODS

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Abstract

Lab-on-a-chip electrophoresis was used to analyze the protein profile of isolated rapeseed proteins, which mainly consist of two dominant proteins: cruciferin and napin. Cruciferin, a 12S globulin, is present at 55–85% and consists of two trimers with a total molecular mass of 300–350 kDa. Each monomer contains α (~30 kDa) and β (~20 kDa) polypeptides linked by disulfide bonds, with additional stability from non-covalent interactions. Napin, a 2S albumin, is present at 15–45% and consists of two polypeptides (~4 kDa and ~9 kDa) connected by disulfide bridges, with a total mass of 12–16 kDa.

In this study, proteins isolated by alkaline extraction (ALK12) and proteins obtained using deep eutectic solvents (DES) – choline chloride:urea (DES1) and betaine:citric acid (DES2) – were analyzed. Electrophoretic analysis revealed that the ALK12 isolate contained seven dominant fractions with molecular masses of 15, 61, 94, 132, 177, 199, and 229 kDa. The fractions at 132 kDa (38%) and 61 kDa (23%) correspond to cruciferin subunits, while the high-molecular-weight fractions (177–229 kDa) may represent oligomers formed under alkaline conditions. The presence of the 15 kDa fraction suggests the possible presence of napin or oleosin. The DES1 isolate showed two main fractions at 57 kDa (57%) and 145 kDa (43%), whereas the DES2 protein distribution was similar, with 60 kDa (66%) and 149 kDa (34%).

The *in vitro* digestibility of the isolated proteins was assessed using the standardized INFOGEST protocol, and the molecular weight distribution after digestion was analyzed by electrophoretic methods. During digestion, cruciferin breaks down into smaller fragments with masses of 20–40 kDa, while napin hydrolyzes into peptides smaller than 10 kDa. The results revealed significant differences in digestibility among the analyzed isolates. After digestion, ALK12 had a high proportion of peptides below 10 kDa, indicating extensive degradation. In the DES1 isolate, high-molecular-weight proteins were partially degraded into fractions of 10–50 kDa, while the DES2 isolate exhibited the highest susceptibility to enzymatic hydrolysis, with the lowest proportion of proteins above 50 kDa after digestion.

The increased presence of napin in digests may enhance absorption and contribute to bioactive peptide release. Native cruciferin is harder to digest, but partial hydrolysis improves its peptide bioavailability. Extraction and processing methods directly affect rapeseed protein *in vitro* digestibility, ranging from 70% to 100%. Hydrolysis-resistant fragments may reduce digestibility, while hydrolyzed proteins show improved absorption and nutritional value.

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Keywords: Lab-on-a-chip electrophoresis, protein, alkaline extraction, DES