Thesis Title	Thermomechanical Treatment to Enhance Protein Functionality of Sacha Inchi Press Cake
Author	Piyawan Phonphimai
Degree	Master of Science (Innovative Food Science and Technology)
Advisor	Associate Professor Natthawuddhi Donlao, Ph. D.
Co-Advisors	Professor Yukiharu Ogawa, Ph. D. Professor Jaspreet Singh, Ph. D.

ABSTRACT

Sacha inchi oil residue/press cake (SP) is a protein-rich by-product of oil extraction with strong potential as a sustainable plant-based ingredient. However, its utilization in food applications is limited by restricted protein accessibility, the presence of antinutritional factors, suboptimal techno-functional properties, and undesirable sensory attributes such as bitterness and astringency. This thesis aimed to enhance the protein content and overall functionality of Sacha inchi press cake through cyclone-based dry fractionation and thermal–mechanical processing, and to elucidate the relationships between production yield, protein recovery, structural characteristics, functionality, digestibility, and application performance.

In the first part of the study, cyclone-based dry fractionation following grinding and sieving (≤250 μm) was applied, yielding four fractions (F1–F4). These fractions were characterized in terms of physicochemical composition, nutritional quality, amino acid profile, bioactive compounds, and techno-functional properties. Protein enrichment was modest, increasing from 53.06% in the starting material (SP) to 56.62% in fraction F3 and 57.12% in fraction F4. Fraction F3 exhibited the highest yield (32.56%), whereas F4 showed a substantially lower yield (8.91%). Protein solubility was strongly pH-dependent, ranging from 19.96% at pH 2 to 95.76% at pH 12. Functional properties varied among fractions: F3 showed a reduction in foaming capacity (from 11.38% to 3.95%) but improved foam stability (from 88.89% to 96.83%). Emulsifying activity and stability slightly decreased (from 87.70% and

78.87% to 76.52% and 70.09%, respectively), while gelation properties improved, as indicated by a decrease in the least gelation concentration from 18% to 10%. Bioactive compound content remained largely unchanged, with total phenolic content ranging from 28.31 to 29.14 mg GAE/g and ferric reducing antioxidant power increasing from 64.04 to 79.94 μmol $FeSO_4$/g. The low amino acid score (0.04) indicated limited protein quality. Overall, dry fractionation resulted in only minor improvements, highlighting trade-offs between yield, protein enrichment, and functionality, and underscoring the need for complementary processing strategies.

In the second part of the study, the effectiveness of autoclaving (AC) followed by colloid milling (CL) was investigated as an integrated thermal–mechanical approach to improve the physicochemical, functional, nutritional, and sensory properties of SP. The SP was autoclaved at 121 °C and subsequently subjected to colloid milling, after which the resulting materials were evaluated using the same analytical framework as in Part 1, along with application testing in cracker formulations. This combined processing significantly reduced bulk density from 0.65 to 0.25 g/cm³ and increased protein solubility at pH 2 from 19.29 to 31.79 mg/g. Water- and oil-holding capacities increased from 3.81 to 4.89 g/g and from 6.67 to 9.01 g/g, respectively, while emulsifying activity improved from 50.0% to 62.5%. *In vitro* gastrointestinal digestion demonstrated higher protein solubility for colloid-milled samples during both the gastric (9.34 mg/g) and intestinal (11.02 mg/g) phases compared with autoclaved samples. Amino acid analysis revealed a well-balanced essential amino acid profile, with an essential amino acid index of 108 and a predicted biological value of 106. Crackers formulated with cassava starch and autoclaved–colloid-milled press cake at a 90:10 cassava starch press cake ratio achieved the highest overall sensory acceptability score (8.62).

Overall, these findings demonstrate that while cyclone-based dry fractionation alone provides limited improvements, the combination of autoclaving and colloid milling is an effective strategy for valorizing SP into a functional plant protein ingredient with enhanced nutritional quality, techno-functional performance, digestibility, and sensory acceptability, supporting its potential application in sustainable food systems.

Keywords: Sacha Inchi Press Cake, Plant Protein Valorization, Dry Fractionation, Autoclaving, Colloid Milling, Protein Functionality, *In Vitro* Digestibility