THERMOMECHANICAL TREATMENT TO ENHANCE PROTEIN FUNCTIONALITY OF SACHA INCHI PRESS CAKE

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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

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

AC	Autoclaved press-cake meal
CL	Colloid-milled press-cake meal
DF	Dilution factor
DSC	Differential scanning calorimetry
F1-F4	Cyclone-fractionated samples (Fractions 1–4)
FTIR	Fourier transform infrared spectroscopy
OHC	Oil-holding capacity
RM	Raw material
RM-SP	Raw Sacha inchi press cake
SC-RM	Size-classified raw material
SEM	Scanning electron microscopy
SI	Sacha inchi
SIS	Sacha Inchi seeds
SP	Sacha inchi press cake
TPC	Total phenolic content
TFC	Total flavonoid content
TGA	Thermogravimetric analysis
WHC	Water-holding capacity

CHAPTER 1

INTRODUCTION

1.1 Background

The increasing global demand for sustainable, plant-based protein ingredients has intensified interest in the valorization of agricultural and agro-industrial by-products. Oilseed press cakes, generated as residues after oil extraction, represent abundant yet underutilized resources rich in plant proteins, dietary fiber, and other valuable components. The incorporation of such by-products into food systems not only contributes to protein security but also supports the development of sustainable and circular food production models.

Sacha inchi (*Plukenetia volubilis* L.) is an oilseed crop cultivated primarily in tropical regions and is widely recognized for the high nutritional quality of its oil. Oil extraction generates substantial quantities of press cake as a by-product, which has been reported to contain a high protein content and a favorable amino acid composition. Despite this nutritional potential, the utilization of Sacha inchi press cake as a food ingredient remains limited, and its value is often restricted to low-value applications or waste streams.

The limited application of Sacha inchi press cake in food systems is mainly attributed to its unfavorable physicochemical and techno-functional properties. Proteins are entrapped within a dense and heterogeneous matrix composed of residual oil, fiber, and cell wall components, which restrict protein accessibility and solubility. These structural constraints negatively affect key functional attributes, including water- and oil-holding capacity, emulsifying ability, and dispersion behavior, while also limiting protein digestibility. As a result, Sacha inchi oil residue cannot be directly utilized as a functional protein ingredient without targeted processing interventions.

This thesis was therefore designed as a sequential study comprising two experimental phases aimed at improving the utilization of protein-rich Sacha inchi press cake. In the first phase (Part 1), cyclone-based dry fractionation was employed to establish baseline compositional, structural, and functional characteristics of press cake fractions

with different particle size distributions. The results of Part 1 highlighted the inherent heterogeneity of the press cake matrix and provided fundamental insights into protein distribution, enrichment potential, and functional limitations. Building on these findings, the second phase (Part 2) focused on the application of integrated thermal and mechanical processing specifically autoclaving and colloid milling to further modify the press cake structure with the aim of enhancing protein accessibility, techno-functional performance, digestibility, and suitability for food applications.

1.2 Research Problem and Rationale

Protein enrichment and modification strategies are widely employed to improve the nutritional quality and techno-functional performance of plant-based protein ingredients. Conventional wet fractionation methods are effective in producing high-purity protein isolates; however, they typically require large volumes of water, involve intensive energy input, and generate significant processing costs. In addition, these approaches often suffer from low overall protein recovery yields, limiting their industrial feasibility and sustainability, particularly for the valorization of oilseed press cakes.

Dry fractionation techniques offer a more sustainable alternative, as they operate without solvents, reduce environmental impact, and largely preserve native protein structures. Despite these advantages, the degree of protein enrichment achieved through dry fractionation is generally modest. Moreover, the intact matrix structure of oilseed press cakes often restricts protein accessibility, resulting in limited improvements in functional and nutritional performance when dry fractionation is applied as a standalone process.

Preliminary investigations in this study demonstrated that cyclone-based dry fractionation alone was insufficient to overcome the functional and digestive limitations of Sacha inchi oil residue. Exploratory trials employing mild wet fractionation yielded only marginal increases in protein content, while substantially reducing yield and increasing processing costs. These findings indicate that protein purity alone does not

determine suitability for food applications and underscore the importance of considering yield, matrix structure, functionality, digestibility, and processing feasibility in parallel.

Physical modification techniques, such as thermal and mechanical treatments, have been shown to enhance protein accessibility and techno-functional properties by disrupting protein–matrix interactions without relying on extensive chemical processing. In particular, autoclaving can promote controlled protein unfolding and reduce antinutritional constraints, while colloid milling can modify particle size distribution, surface characteristics, and hydration behavior. Integrating these approaches with dry fractionation therefore represents a practical and scalable strategy for improving the functional performance of oilseed press cake proteins.

Based on these considerations, it is evident that although dry fractionation provides a sustainable route for initial protein enrichment of Sacha inchi press cake, it alone is insufficient to address limitations related to protein accessibility, functionality, and digestibility. Furthermore, mild wet fractionation approaches are constrained by low yield and poor industrial viability. Consequently, a systematic and sequential processing strategy that integrates dry fractionation with autoclaving and colloid milling is required to simultaneously enhance protein accessibility, functional performance, and digestibility of Sacha inchi press cake while maintaining processing efficiency and sustainability.

1.3 Research Objectives

The overall objective of this thesis was to enhance the protein content and composition of Sacha inchi oil residue through sustainable processing strategies and to evaluate the effects of these treatments on structural, functional, nutritional, digestibility, and application-related properties. The specific objectives of this study were to:

1.3.1 Investigate the effects of cyclone-based dry fractionation on protein enrichment, composition, and techno-functional properties of Sacha inchi oil residue in Part 1.

1.3.2 Evaluate the limitations of mild wet fractionation in terms of protein yield,

processing efficiency, and industrial feasibility.

1.3.3 Examine the effects of thermal treatment (autoclaving) and mechanical modification (colloid milling) on protein structure, particle size distribution, and functional performance in Part 2.

1.3.4 Assess the impact of processing treatments on protein digestibility using an *in vitro* gastrointestinal digestion model.

1.3.5 Evaluate the applicability of the modified Sacha inchi oil residue as a food ingredient through product formulation and sensory evaluation.

1.4 Scope and Limitations of the Study

This study focused on the valorization of Sacha inchi oil residue/press cake obtained after oil extraction, with an emphasis on processing strategies that are compatible with sustainable food systems. The scope of the research encompassed two main approaches: (i) cyclone-based dry fractionation following grinding and sieving to evaluate protein enrichment potential and functionality, and (ii) integrated thermal–mechanical processing involving autoclaving and colloid milling to enhance protein accessibility, structural characteristics, techno-functional performance, digestibility, and sensory quality. Comprehensive characterization was conducted to assess physicochemical composition, microstructure, functional and hydrocolloid-related properties, nutritional quality, and in vitro digestibility, along with limited application testing in food formulations.

Mild wet fractionation was examined only at a preliminary level and was not further optimized due to relatively low protein recovery yields and higher processing costs compared with the modest improvements in protein enrichment achieved. Protein digestibility was assessed using an in vitro gastrointestinal digestion model; therefore, the results may not fully reflect in vivo digestive behavior or protein utilization. Furthermore, application studies were restricted to selected model food systems, and large-scale processing, long-term storage stability, and industrial-scale validation were beyond the scope of this thesis.

1.5 Research Framework

This thesis employed a stepwise research framework designed to balance protein enrichment, functional performance, digestibility, and processing feasibility for the valorization of Sacha inchi oil residue/press cake. In the first stage, cyclone-based dry fractionation was applied to assess protein enrichment potential and to establish a baseline for protein separation efficiency, yield, and techno-functional performance. This stage provided insight into the limitations of dry fractionation as a stand-alone approach, particularly in terms of protein recovery, functionality, and nutritional quality.

Based on these findings, an integrated thermal–mechanical modification strategy was subsequently implemented. Autoclaving was applied to disrupt the native protein–matrix structure, reduce antinutritional constraints, and enhance protein accessibility, followed by colloid milling to further modify particle size, surface properties, and hydration and interfacial behavior. The effects of these processing steps were systematically evaluated through comprehensive physicochemical, structural, hydrocolloid-related functional, and nutritional analyses, including in vitro gastrointestinal digestion, to elucidate structure–process–property relationships.

The final stage of the research framework focused on food application and sensory evaluation using selected model food systems to assess the practical suitability and consumer acceptability of the modified Sacha inchi press cake as a functional plant protein ingredient. Overall, this integrated framework provides both scientific insight and practical guidance for selecting and combining sustainable processing strategies to upgrade oilseed by-products into value-added protein ingredients for food applications.

CHAPTER 2

LITERATURE REVIEW

2.1 Sacha Inchi Press Cake

Until now, Sacha Inchi is a rather unknown plant in Europe, and research about the chemical composition of the seeds and the characterization of the oil is limited. In Figure 2.1. Sacha Inchi fruit, including the seeds, is shown. Sacha Inchi (*Plukenetia volubilis* L.), also known as the "Inca peanut", "wild peanut", "mountain peanut" or "Inca inchi", is a plant belonging to the Euphorbiaceae family and grows at altitudes between 200 and 1500 m in the Amazonian forest (Gutiérrez et al., 2011; Maurer et al., 2012). Currently grown extensively in Peru and the southern region of Colombia, this plant is thought to be a promising new crop (Gutiérrez et al., 2011). The plant has been a component of the food of many native tribal groups for thousands of years since it was discovered by the local indigenous people. Certain tribal women combine flour and oil to make a skin lotion, and they also utilize the mixture to make a variety of dishes and drinks (Guillén et al., 2003; Pies et al., 2010). Plants of *Plukenetia Volubilis* flower approximately five months after planting. For the entire growing season, they are in flower and bearing fruit. The male flowers are small, white, and grouped, while the female flowers are situated near the foot of the racemose inflorescence. The fruits are capsules that contain one seed per lobe and four to seven lobes (Cai et al., 2011). The plant produces lenticular, 15–20 mm long, 7–8 mm thick seeds that taste bitter and are heat-labile (Guillén et al., 2003). Both the smell and taste of Sacha Inchi oil are slightly similar to those of beans.

Source Anonym et al. (2012)

Figure 2.1 Sacha Inchi plant

2.2 Proximate Composition

Table 2.1 displays the proximate composition data for both fresh and roasted seeds from SI. When the harvested sample material was almost completely dry before examination, there was little moisture in the fresh seeds. The value was somewhat greater than the raw seed value reported by Gutiérrez et al. (2011). However comparable to the worth reported by Hidalgo et al. (2019). As a result of roasting, the moisture content of the roasted seeds naturally decreased. Comparable to that discovered, the protein values obtained ranged from 21 to 30 percent by Kodahl et al. (2020). Compared to SI roasted (49.8%), fresh SI (56.2%) had a higher fat content, and both values were greater than those reported by Gutiérrez et al. (2011) and Hidalgo et al. (2019). Nevertheless, the overall fatty acid content obtained from the analysis of fatty acid profiles yielded a comparable value of 42% (Table 2.2). The disparity across various sources could be attributed to the utilization of distinct chemicals in the process of oil extraction (Gutiérrez et al., 2011). The roasted SI has a greater fiber content (23.88%) compared to the fresh SI (13.03%) and the raw seeds reported by (Hidalgo et al., 2019) (18%). The ash concentration in both fresh SI (2.59%) was lower than the value reported by Gutiérrez et al. (2011) but comparable to that of Hidalgo et al. (2019). The existing information on the carbohydrate content is minimal (Kodahl et al., 2020). The values for both fresh and roasted samples differed from but were lower than, the value reported by Gutiérrez et al. (2011). Nevertheless, Ruiz et al. (2013) approximated the value to

be 12.1%. According to the data in Table 2.1, the energy content of fresh samples (643.63 kcal/100g) and roasted samples (635.25 kcal/100g) were comparable.

Table 2.1 Proximate composition of Sacha inchi seeds

Component	SI Fresh*	SI Roasted*	(Gutiérrez et al., 2011; Hidalgo et al., 2019)
Moisture (%)	6.75 ± 0.08	0.73 ± 0.03	3.30 – 6.72
Crude protein (%)	23.63 ± 0.72	25.46 ± 0.54	24.70 – 29.78
Crude fat (%)	56.20 ± 0.83	49.77 ± 0.46	42.00 – 42.03
Crude fiber (%)	13.03 ± 1.92	23.88 ± 2.65	ND - 18.00
Ash (%)	2.59 ± 0.02	2.68 ± 0.05	2.90 - 4.00
Carbohydrate (%)	10.83 ± 0.13	21.36 ± 0.1	ND – 30.90
Energy (kcal/100g)	643.64 ± 4.42	635.25 ± 2.22	ND

Note *Data from this study; values in this study are expressed as mean ± standard deviation (n = 3; ND - No data)

Source Gutiérrez et al. (2011) and Hidalgo et al. (2019)

2.2.1 Sacha inchi oil

SI kernels contain 35-60% oil, 25-30% protein, vital amino acids, minerals, and vitamin E (Gutiérrez et al., 2017; Vanegas-Azuero et al., 2018). SI kernels are commonly utilized for oil extraction due to their high oil content and important fatty acid concentration (-linolenic, ~50% and linoleic, ~35%) (Gutiérrez et al., 2019). The growing demand for polyunsaturated oils has led to the expansion of the SI oil industry. As a result, the primary by-products of oil processing, the shell and oil press-cake (SIPC), can account for up to 70% of raw seeds (Gutiérrez et al., 2019).

2.2.2 Fatty acid composition

Table 2.2 displays the significant fatty acids in the International System of Units (SI). The roasted seeds had a fatty acid content ranging from 33.4% to 54.3%, as reported by Wang et al. (2018). The levels of saturated fatty acids (SFA) obtained in this

study exceeded the range (6.8-9.1%) estimated by Kodahl et al. (2020) due to the elevated measurement of stearic acid (12.89%). The previously documented values for stearic acid fell within the range of 3% to 4%, as reported by Kodahl et al. (2020), Hidalgo et al. (2019) and Souza et al. (2013). Furthermore, the levels of monounsaturated fatty acids (MUFA) were found to be low in comparison to the typical values, likely due to the absence of oleic acids. Previous research by Kodahl et al. (2020) estimated the percentage of this fatty acid to be between 8.4% and 10.7%. However, Hidalgo et al. (2019) found a higher value of 17.12%. These findings indicate that the extent of oleic acid variations remains uncertain, and the underlying cause has not yet been identified. Possible factors that may have contributed include plant variation, soil composition, climate conditions, and crop management practices. The amount of palmitic acid was consistent with the range of values reported by other researchers. The levels of polyunsaturated acids (PUFA), specifically linoleic and alpha-linolenic acids, were frequently found to be elevated in SI, as reported by Kodahl et al. (2020). The values obtained in this investigation fell within the range published by Kodahl et al. (2020). However, the percentage of omega-6 fatty acids (44.82%) was higher than that of omega-3 fatty acids (35.89%), which differs from earlier studies (Follegatti et al., 2009; Hamaker et al., 1992; Gutiérrez et al., 2011; Carrillo et al., 2018; Betancur et al., 2016; Cisneros et al., 2014; Hidalgo et al., 2019; Souza et al., 2013).

Table 2.2 Fatty acid composition of Sacha inchi seeds

Component	SI Roasted*	Souza et al. (2013), Hidalgo et al. (2019) and Kodahl et al. (2020)
Total Fatty Acid (g/100g)	41.60 ± 3.02	42.03 - 48.52
SFA (% in fat)	18.56 ± 1.47	6.80 – 9.10
MUFA (% in fat)	0.19 ± 0.08	8.52 – 17.12
PUFA (% in fat)	81.26 ± 1.55	77.50 – 84.40
Palmitic (C16:0) (% in fat)	4.64 ± 0.12	4.21 – 5.70
Stearic (C18:0) (% in fat)	12.89 ± 1.97	2.99 – 3.94
Oleic (C18:1 n-9) (% in fat)	0.00 ± 0.00	8.40 – 17.12
Linoleic (C18:2) (% in fat)	44.82 ± 0.89	33.40 – 41.00
α-Linolenic (C18:3) (% in fat)	35.89 ± 0.8	35.20 – 50.80

Note *Values in this study are expressed as mean ± standard deviation (n = 3; ND – No data)

Source Souza et al. (2013), Hidalgo et al. (2019) and Kodahl et al. (2020)

2.3 Mineral Composition

Kim et al. (2019) compared the mineral composition of roasted SI to that of roasted seeds (Table 2.3). The majority of the data exhibited similarity, except for the quantities of calcium, sodium, and phosphorus. The potassium concentration reached its peak at 5178.87 mg/kg, which aligns with the findings of previous studies conducted on raw seeds by Gutiérrez et al. (2011) and Kyaw et al. (2019). Kim et al. (2019) found that phosphorus had the highest concentration at 3867.83 mg/kg. Compared to the roasted seeds studied by Kim et al. (2019), the calcium concentration was lower (1142.23 mg/kg), but it was almost the same as the measurement for raw seeds (1263.2 mg/kg) in the same study. They deduced that the roasting procedure could be the cause of the disparity. However, their investigation revealed that even with the

roasting process, the concentration remained lower. Kim and Joo et al. (2019) study yielded no measurement for sodium. However, this current study reports a much greater concentration of sodium (39.83 mg/kg) compared to the study conducted by Gutiérrez et al. (2011) (15.4 mg/kg). Additionally, the roasted SI contained detectable amounts of selenium.

Table 2.3 Mineral composition of Sacha inchi seeds

Component (mg/kg)	**SI Roasted***	**Roasted seeds****
Calcium (Ca)	1142.23 ± 27.89	2291.70 ± 7.0
Iron (Fe)	44.37 ± 2	47.80 ± 0.6
Potassium (K)	5178.87 ± 171.22	4789.10 ± 14.0
Magnesium (Mg)	3438.70 ± 215.43	3667.50 ± 32.1
Sodium (Na)	39.83 ± 15.31	0.00 ± 0.00
Manganese (Mn)	11.20 ± 0.5	11.90 ± 0.2
Phosphorus (P)	3867 ± 173.35	5442.70 ± 42.8

Note *Data from the roasted seeds

Source Gutiérrez et al. (2011) and Kim and Joo et al. (2019)

2.3.1 Nutrient comparison of sacha inchi seeds

SI contains a significant quantity of polyunsaturated fatty acids (PUFA), ranking second only to walnuts according to Table 2.5. The omega-6 level of walnuts is higher than their omega-3 amount. On the other hand, SI provides a greater quantity of omega-3 (4.2 g) in a 28 g serving size. Additionally, it contains a substantial amount of omega-6 fatty acids (5.2 g). SI has a lower carbohydrate content compared to certain other nuts, yet it is rich in protein. Additionally, it possesses a greater amount of dietary fiber in comparison to other options. Underutilized crops possess the capacity to alleviate food and nutritional insecurity, such as "hidden hunger" (Jacobsen et al., 2015), by enhancing diets with a wide range of nutritionally diverse meals, while also diversifying and enhancing the appeal of food and food culture. In Table 2.4, we have compared roasted SI with Bambara groundnut and lablab bean (Yao et al., 2015; Ademola & Abioye 2017; Hossain et al., 2016), which are categorized as underused crops. Both crops have recently attracted attention because of their capacity to enhance nutritional accessibility and biodiversity. The protein content is comparable among all three

crops. The fat concentration in SI is over 50 times greater than in the other recording, resulting in a higher energy level. The higher fat content in SI is also responsible for the increased presence of polyunsaturated fatty acids (PUFA), specifically linoleic and α-linolenic acids. These acids have been found to have a beneficial impact on human health, particularly in terms of preventing cardiovascular diseases and high blood pressure (Hamaker et al., 1992; Guillén et al., 2003; Carrillo et al., 2018). Additionally, SI contains elevated levels of important minerals such as calcium (1142.23 ± 27.89 mg/kg), magnesium (3438.70 ± 215.43 mg/kg), and phosphorus (3867.83 ± 173.35 mg/kg) in comparison to other crops.

Table 2.4 Nutrient comparison of Sacha inchi (SI) and some com mercially available nuts /seeds (Serving size: 100g)

Nut/ Seed	Sacha*	Almond[1]	Brazil Nut[1]	Cashew Nut[1]	Chest Nut[1]	Hazel Nut[1]	Macadamia[1]	Pea Nut[1]	Pecan[1]	Pistachio[1]	Walnut[1]
Energy (kcal)	643.6	578.6	667.9	560.7	250.0	635.7	728.6	575.0	700.0	567.9	653.6
Carbohydrate (g)	11.1	21.4	11.8	30.7	53.6	16.8	13.9	16.4	13.9	27.5	13.6
Protein (g)	23.9	21.1	14.6	18.6	3.2	15.0	7.9	26.1	9.3	20.4	15.4
Fat (g)	56.1	50.0	67.9	44.3	2.1	61.4	76.8	50.0	72.9	46.1	65.4
Fiber (g)	12.9	12.5	7.5	3.2	5.4	10.0	8.6	8.6	9.6	10.7	6.8
SFA (g)	7.9	3.9	16.4	7.9	0.4	4.6	12.1	6.4	6.4	6.1	6.1
MUFA (g)	0.0	31.4	24.3	24.3	0.7	46.1	59.6	24.6	41.4	23.6	8.9
PUFA (g)	33.9	12.5	24.6	7.9	1.1	8.2	1.4	15.7	21.8	14.6	47.1
Omega-3 (g)	15.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	1.1	0.4	8.9
Omega-6 (g)	18.6	12.5	24.6	7.9	0.7	7.9	1.4	15.7	21.1	14.3	38.2

Source Joseph et al. (2020)

2.4 Protein Composition

2.4.1 Protein and carbohydrate content of Sacha Inchi seeds (SIS)

Sacha Inchi contains a full protein that meets adults' amino acid requirements (Sathe et al., 2002). The protein content of Sacha Inchi seeds (SIS) is approximately 27%, and it is rich in amino acids such as cysteine, tyrosine, threonine, and tryptophan (Maurer et al., 2012). This content is similar to those from sesame seeds (approximately 25%), sunflower (24%), and peanuts (23%) (Gutiérrez et al., 2011). Except for histidine, Sacha Inchi has all of the essential amino acids in adequate amounts when compared with the FAO/WHO recommended amino acid pattern. (Gutiérrez et al., 2011) showed that the total carbohydrate value of the seeds is relatively low (30.9%)

because of the high content of crude oil and protein. SIS has a caloric value of 576 kcal/100 g sample, calculated from the contents of fat, protein, and carbohydrate (Gutiérrez et al., 2011).

2.5 Oxidative Stability of Sacha Inchi Oil

Because of the high content of unsaturated fatty acids, Sacha Inchi oil is very susceptible to peroxidation under mild environmental conditions. Furthermore, composition differences play a crucial role in oxidation rates (Maurer et al., 2012). Fanali et al. (2011) showed that γ-Tocopherol, which is one parameter making the oil more stable to oxidation, turned out to be the most abundant among the tocopherols found in Sacha Inchi oil (more than 50% of the entire tocopherol content). Furthermore, the polyphenolic content is a parameter for the oxidative stability of polyunsaturated fatty acids. Fanali et al. (2011) found 21 phenolic compounds, 15 of which belonged to phenyl alcohol, flavonoid, secoiridoid, and lignin classes, were positively identified. Maurer et al. (2012) measured the peroxide value of different oils (corn, high oleic sunflower, linseed, and Sacha Inchi). In table 2.4, the oxidative value, free fatty acid, and FA composition for the different oil samples, investigated during an oxidative stability test, are shown. Compared with the other oils, except for linseed oil, Sacha Inchi oil was more oxidative unstable, because of the high amounts of polyunsaturated fatty acids. Sunflower oil had the highest oxidative stability. Corn had high levels of peroxide formation at the end of the stability test, although corn oil showed the least oxidation in the first few days. This could be explained through the present natural antioxidants, α-tocopherols. High oleic sunflower oil had the largest amount of saturated and monounsaturated fatty acids and was therefore the most stable oil (Maurer et al., 2012).

2.6 Composition of Press Cakes from Selected Plants

According to Ferchau et al. (2000), cold pressing produces press cakes that are more valuable in residual oil and therefore more useful for animal food proposals than warm pressing or normal industrial extraction with solvents. Raw press cakes, rich in

fat, can serve as valuable protein fodder. Agriculture widely uses press cakes, which reach their highest value when used as animal fodder. For farmers, it is important to produce the press cakes by themselves, to go in the direction of self-supply and self-control, and to get an additional income. The cold-pressed cakes of local oil seeds have the potential to replace imported soybean meal (Ferchau et al., 2000).

2.6.1 Soy

Soybean press cakes are by-products of cold and hot press oil production. Using a short technological process, the soy press cakes achieve a composition of 50% protein, 6% residual oil, and 90% inactivation of trypsin inhibitors by the extrusion-expelling method. Press cakes are mainly used as animal feed, and the most important indicator of anti-nutritional factor destruction for soybean press cake is the urease enzyme content and activity (Nelson et al., 1987; Tucu et al., 2007). Soybean oil is the most valuable plant oil. The protein of soybean contains a considerable quantity of lysine, and the value of protein is limited by the low content of methionine and cysteine (Banaszkiewicz et al., 2011). Press cakes can also be utilized in the form of meals for animal feeding. These meals are mainly used in poultry and pig nutrition. In Table 2.4, the quality of meals obtained by different methods is compared. Many nutritionists recommend dehulled soybean meal instead of protein soybean meal for poultry feeding because of the higher level of balanced protein, lower fiber level, and higher energy level (Tucu et al., 2007).

Soy hulls have a content of about 11% protein, 9–11% galactomannans, 10-12% acidic polysaccharides, 9–10% xylan hemicellulose, and about 40% cellulose. Researchers have identified four proteins in soy hulls: peroxidase (SBP), a hydroxyproline-rich glycoprotein, a glycine-rich protein, and a Bowman-Birk type protease inhibitor (BBI). The baking industry, the chemical industry, and the medical diagnostics industry can utilize SBP (Sessa et al., 2003).

2.6.2 Peanut

According to a study by Riaz et al. (2009) and Cheewapramong et al. (2009), approximately 50% of the original oil in the peanuts could be removed by dry extrusion. In this study, extrudates were pressed immediately after extrusion by using a continuous screw press. After that, the peanut press cakes had a composition of 8% oil, 47% protein

and 6% moisture. The protein dispersibility index (PDI) dropped to 66% in the extrudates and press cakes through heat treatment during extrusion and pressing. The heat treatment did not affect the reaction of the trypsin inhibitor, but 26% of the trypsin inhibitor activity in the press cakes was destroyed only by dry extrusion and screw pressing (Riaz et al., 2009; Cheewapramong et al., 2009). If aflatoxin can be kept away from the press cake, the cakes may be used for animal feed or ground into flour for human consumption. When they are unacceptable, they are used as fertilizer (Dean et al., 2011).

2.6.3 Sunflower

The sunflower press cake is a high-protein source, obtained after pressing sunflower oil by cold extraction. It is used in the production of combined fodders with a great feeding value, which makes it possible to introduce a smaller amount of press cake into combined fodder. The quality of proteins is rather higher than the quality of grain crops and with a fat content of seven to 10% (up to 15%), it has high feeding and energetic value. Sunflower press cake has a raw protein content of up to 38% and a raw fiber content of up to 18% (Anonym et al., 2012). Furthermore, it has a high content of vitamin E, 8.3 mg/100 g α-tocopherol and 0.5 mg/100 g β-tocopherol, high content of phospholipids, vitamins of group B and β-carotene (Franke et al., 2010).

2.7 Plant-based Diets

2.7.1 Plant-based diets and its functional properties

Plant-based diets are eating patterns that focus on foods primarily from plants and avoid the consumption of animal products, such as meat, eggs, and dairy products. This includes fruits, vegetables nuts, seeds, oils, whole grains, legumes, and beans (Lea et al., 2006; Ostfeld et al., 2017). There are several reasons for people to choose a plant-based diet. Regarding concerns of animal welfare, health benefits, environmental or personal preference. There is a high awareness of the potential benefits of eating a plant-based diet, particularly health-related benefits such as decreased saturated fat intake, increased fiber intake, and disease prevention (Lea et al., 2006). It is evident that plant-based diets are beneficial for health owing to the high consumption of plant foods with

lesser meat consumption (Key et al., 2003; Li et al., 1999). Plant-based diets are potent in preventing type 2 diabetes and have been associated with lower rates of obesity, hypertension, hyperlipidemia, cardiovascular mortality, and certain types of cancer (Dinu et al., 2017). According to an analysis of data from 4.1 million person-years of follow-up, the results revealed that those most adherent to the healthful plant-based dietary index had a 34% lower risk of developing diabetes compared with those least adherent (Satija et al., 2016). It is also found that substituting 5% of energy intake from animal protein with vegetable protein was associated with a 23% reduced risk of type 2 diabetes (Malik et al., 2016). Lots of evidence supports plant-based diets for the reduction of cardiovascular risk factors. It is found that the prevalence and incidence of hypertension are significantly lower in people consuming plant-based diets compared with omnivores (Appleby et al., 2002; Orlich & Fraser 2014; Pettersen et al., 2012). The study discovered that plant-based diets are linked to a 35 percent reduction in serum LDL cholesterol and have been proven to lower blood pressure in interventional trials (Yokoyama et al., 2014).

A plant-based diets are typically containing legumes, whole grains, fruits, root and green leafy vegetables, and nuts (Aune et al., 2013; Cooper et al., 2012). Legumes have also been proven to help with insulin resistance and metabolic syndrome prevention (Jenkins et al., 2012). Most plant-based diets are naturally high in carbohydrates and fiber and low in fat, this is often associated with weight loss. All of these elements also have been found to be protective against diabetes. The improvement of glycemic control therefore remains unclear whether it is due to changes in diet or weight loss (McMacken & Shah, 2017). According to previous research, these functions have been associated with the availability of phytochemicals, the physiologically active chemical substances derived from plants. These phytochemicals can be divided into six categories, namely flavonoids, phenolic acids, saponins, alkaloids, polysaccharides, and others.

The plant-based meat industry is poised to play a significant role in the future of food, driven by current trends towards sustainability, health-conscious eating, and technological innovation. To succeed, the industry must invest in research and development, prioritize sustainability, ensure quality and safety, and remain adaptable and transparent by meeting these needs and embodying these qualities, the plant-based meat

industry can not only meet the demands of today's consumers but also contribute to a more sustainable and ethical food system for the future.

2.8 Overview of Plant-based Food Products

As global demand for sustainable food sources intensifies, plant-based food products have emerged as an innovative solution (He et al., 2020). Among these, Sacha Inchi (*Plukenetia volubilis* L.) press cake, a nutrient-rich by-product of oil extraction, presents a promising avenue for valorization in the food industry (Kyriakopoulou et al., 2019). This residue, composed of approximately 56.82% protein, 30.60% carbohydrates, and bioactive compounds such as phenolics, embodies the potential for novel applications in functional and plant-based food development (Kumar et al., 2017). The food industry increasingly seeks alternative protein sources to meet consumer demands for environmentally sustainable, nutritionally superior, and functional ingredients (He et al., 2020). Sacha Inchi press cake aligns with this vision by offering high-quality proteins with a complete amino acid profile (Kyriakopoulou et al., 2019). Moreover, its phenolic compounds enhance the antioxidant properties of derived products, providing health-promoting benefits that are highly sought after by modern consumers (Kumar et al., 2017). Processing technologies such as mild wet fractionation and cyclone separation enable the production of value-added fractions from Sacha Inchi press cake (Kyriakopoulou et al., 2019). These include protein-enriched powders suitable for meat analogs, dairy substitutes, and protein-enhanced beverages, as well as fiber-rich fractions for use in bakery and snack products (He et al., 2020). These developments capitalize on the intrinsic functional properties of Sacha Inchi proteins, such as water and oil absorption capacities, gelation, and emulsification, which are crucial for diverse food applications (Kumar et al., 2017). Despite its potential, commercial utilization of Sacha Inchi press cake in plant-based food products faces challenges (Kyriakopoulou et al., 2019). Its bitter taste, associated with residual polyphenols, requires attention during product formulation (He et al., 2020). Furthermore, optimizing extraction processes to maintain bioactive integrity and ensuring sensory acceptability through flavor masking techniques are critical for its success in the market (Kumar et al., 2017). Sacha Inchi

press cake exemplifies the transition toward a circular bioeconomy by transforming an agricultural by-product into a high-value ingredient for plant-based foods (Kyriakopoulou et al., 2019). Future research should focus on enhancing processing techniques, studying the functional properties of derived fractions, and developing innovative applications to unlock its full potential (He et al., 2020). As the plant-based food market continues to grow, Sacha Inchi press cake offers a unique opportunity to contribute to sustainable food innovation (Kumar et al., 2017).

2.9 The Process of Oil Extraction

Proteins The extraction of oil from Sacha Inchi (*Plukenetia volubilis* L.) seeds involves a series of processes aimed at maximizing oil yield while maintaining the nutritional and functional qualities of the residue. The process is critical for producing edible oil and protein-enriched press cake, which has emerging applications in the food industry as a sustainable ingredient. Oil extraction typically starts with mechanical pressing or expeller processing, where the seeds are mechanically compressed to extract oil (Rosenthal et al., 1996). This step is often followed by solvent extraction to recover residual oil, with hexane being the most commonly used solvent due to its efficiency in achieving oil yields above 95% (Rosenthal et al., 1996). However, concerns about hexane's volatile organic compound (VOC) emissions, which contribute to environmental pollution and health risks, have driven the development of alternative methods such as aqueous and enzymatic extractions (Rosenthal et al., 1996). Aqueous extraction uses water as the medium to separate oil and proteins from the press cake, presenting a safer and more environmentally friendly option compared to traditional hexane-based methods (Rosenthal et al., 1996). The use of enzymes, including proteases and carbohydrases, can further enhance oil release by hydrolyzing cell wall components and protein-lipid membranes (Rosenthal et al., 1996). These enzymatic treatments significantly increase oil recovery while preserving the protein's functional properties. Another innovative approach combines pressing with mild wet fractionation techniques, which minimize thermal damage and preserve bioactive compounds in the press cake (Rosen-

thal et al., 1996). This integrated process results in a high-quality protein-rich by-product, suitable for incorporation into functional food products such as protein isolates and concentrates. The choice of extraction method impacts the quality of the extracted oil and the usability of the press cake. Aqueous and enzymatic methods are gaining traction due to their ability to produce cleaner, safer, and more sustainable food ingredients while addressing consumer demands for eco-friendly processes (Rosenthal et al., 1996) . The evolution of oil extraction techniques for Sacha Inchi seeds reflects the food industry's commitment to innovation and sustainability. Optimizing these methods will enable efficient utilization of Sacha Inchi press cake, enhancing its potential as a valuable resource in the plant-based food market.

The Sacha inchi (*Plukenetia volubilis*), has gained recognition for its oil-rich seeds, which are a valuable source of omega-3 and omega-6 fatty acids, tocopherols, phytosterols, and phenolic compounds (Chirinos et al., 2013). Despite the extensive utilization of Sacha inchi oil, its press cake, a by-product of oil extraction, remains underutilized. This by-product holds significant promise as an ingredient in functional foods and other food industry applications due to its nutritional and functional properties. Rich in proteins, dietary fiber, and residual lipids, Sacha inchi press cake has been identified as a potential source of bioactive compounds such as antioxidants, polyphenols, and essential amino acids (Chirinos et al., 2013). The proteins in the press cake exhibit an amino acid profile suitable for human nutrition, including high levels of cysteine, tyrosine, threonine, and tryptophan, which can enhance the nutritional quality of food products (Hamaker et al., 1992). Moreover, its fiber content can improve gut health and contribute to the development of high-fiber food products. The oil residues in the press cake retain significant amounts of bioactive lipids, including omega-3 fatty acids and tocopherols, which offer antioxidative and anti-inflammatory benefits (Fanali et al., 2011). These properties make the press cake a viable candidate for functional food applications aimed at cardiovascular health and chronic disease prevention. Additionally, the phenolic compounds in Sacha inchi press cake contribute to its antioxidant capacity, enhancing its potential as a natural preservative in food formulations (Chirinos et al., 2013). Potential applications of Sacha inchi press cake in the food industry include its use as a plant-based protein supplement, a source of natural antioxidants, and a functional ingredient for gluten-free and high-protein food formulations.

Its incorporation into baked goods, snack bars, and beverages can cater to growing consumer demands for sustainable and health-oriented products. Furthermore, the press cake's bioactive compounds could be extracted for use in nutraceuticals, enhancing its economic value.

2.10 Protein Content and Functional Properties of Plant Proteins

Proteins are one of the most important macronutrients in food systems due to their nutritional value and their critical role in determining food structure and quality (Damodaran & Parkin, 2017). In plant-based materials, proteins contribute significantly to functional performance, including solubility, water- and oil-holding capacity, emulsifying ability, foaming capacity, gelation behavior, and digestibility (Day, 2013; Lam et al., 2018). Therefore, when plant-derived by-products are proposed as alternative protein sources, both protein quantity and protein functionality must be systematically evaluated.

2.10.1 Protein content and distribution in plant-based materials

Protein content is commonly used as a primary indicator of nutritional quality and suitability of plant materials as protein sources (Sá et al., 2020). Oilseed by-products and press cakes are often characterized by elevated protein levels as a result of oil removal, making them attractive raw materials for protein recovery and valorization (Pelgrom et al., 2015). However, proteins in plant matrices are not uniformly distributed and are frequently associated with non-protein components such as lipids, starch, and dietary fiber through physical entrapment and non-covalent interactions (Kornet et al., 2021; Lam et al., 2018). These interactions can limit protein extractability and accessibility, particularly in dry processing systems where extensive solubilization steps are avoided (Pelgrom et al., 2015). Consequently, protein-rich fractions obtained via dry fractionation may differ substantially in composition and functionality from conventional protein isolates produced by wet extraction.

2.10.2 Functional properties of proteins

Functional properties describe the behavior of proteins in food systems under specific environmental conditions such as pH, ionic strength, and temperature (Damodaran & Parkin, 2017). Protein solubility is considered a key prerequisite for most functional attributes, as it directly influences emulsifying, foaming, and gelation properties (Day, 2013). Structural modifications induced by processing, including denaturation, unfolding, aggregation, and reassociation, can either enhance or impair protein functionality depending on their extent and reversibility (Loveday et al., 2019).

Water-holding and oil-holding capacities are particularly relevant for protein-rich fractions intended for bakery, snack, and meat analogue applications, as these properties influence texture, mouthfeel, and yield (Kyriakopoulou & et al., 2022). Emulsifying and foaming properties are governed by the ability of proteins to adsorb at oil–water or air–water interfaces and to form cohesive, viscoelastic interfacial films (Lam et al., 2018). These properties are strongly affected by protein molecular flexibility, surface hydrophobicity, and particle size distribution.

2.10.3 Protein digestibility and structural accessibility

In addition to functional performance, protein digestibility is a critical indicator of nutritional quality. Digestibility is closely linked to protein structure and matrix accessibility, as tightly folded proteins or proteins embedded within complex plant matrices may hinder enzyme penetration during digestion (Carbonaro et al., 2012). Mechanical and thermal treatments have been reported to enhance protein digestibility by disrupting cellular structures, reducing particle size, and partially unfolding protein molecules, thereby increasing enzyme accessibility (Carbonaro et al., 2012). Nevertheless, excessive thermal treatment may lead to protein aggregation or the formation of Maillard reaction products, which can reduce enzymatic hydrolysis and negatively affect digestibility (Dupont et al., 2010). Therefore, the balance between structural modification and protein damage is a critical consideration in the design of protein processing strategies.

2.10.4 Relationship between protein structure, functionality, and processing

Processing-induced structural changes play a central role in determining protein functionality and digestibility. Moderate unfolding may expose functional groups that enhance solubility and interfacial activity, whereas extensive aggregation may decrease

protein mobility and accessibility (Loveday et al., 2019). In dry fractionation systems combined with thermomechanical treatments, protein behavior is further influenced by interactions with residual lipids and polysaccharides, which can lead to protein entrapment within the plant matrix (Pelgrom et al., 2015). Understanding the relationship between processing conditions, protein structure, functional properties, and digestibility is therefore essential for the development of value-added protein ingredients from plant-based by-products.

2.10.5 Research gap

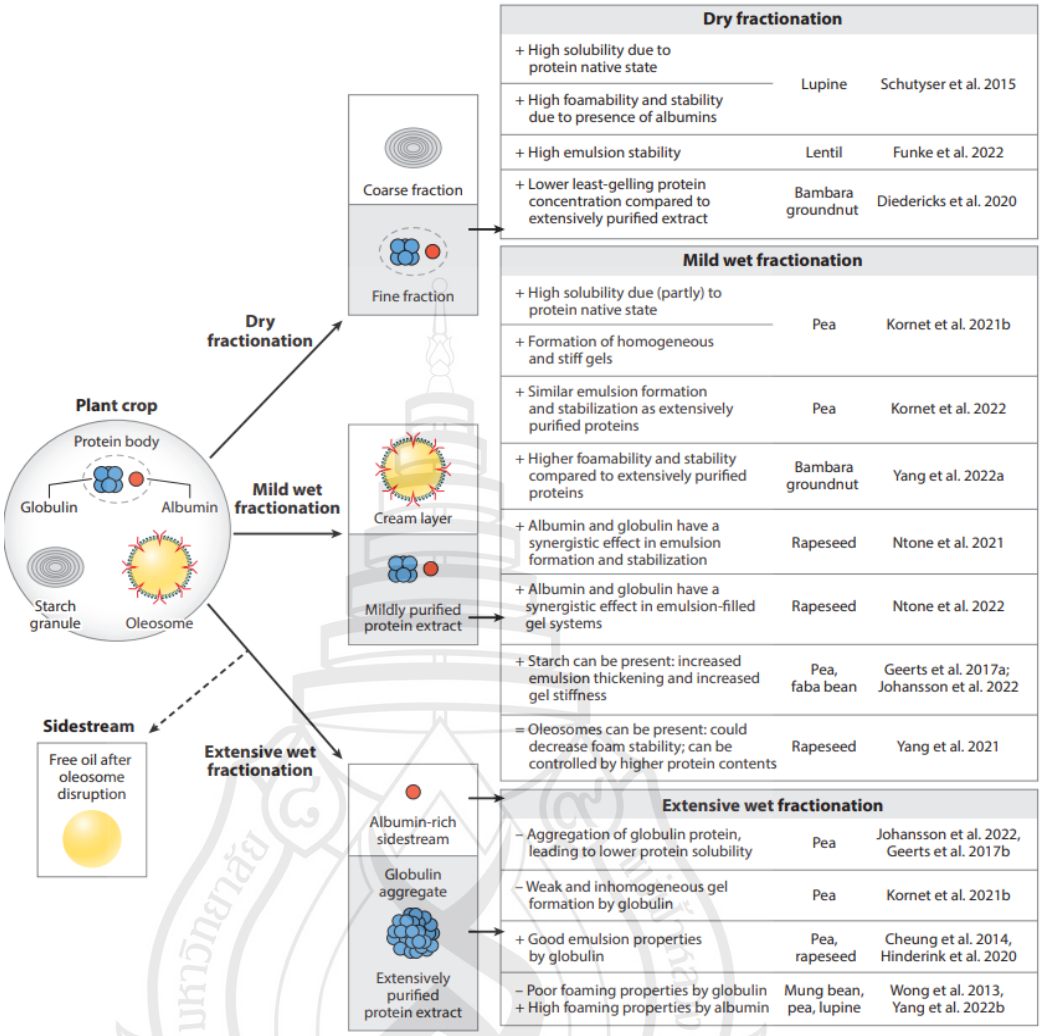
Although numerous studies have investigated protein extraction and functionality in plant materials, limited information is available on how combined mechanical and thermal treatments influence protein content, functional properties, and digestibility within dry-fractionated systems (Kornet et al., 2021; Kyriakopoulou et al., 2022). In particular, a comprehensive evaluation linking protein quantity, structural modification, functional behavior, and nutritional quality remains scarce for underutilized oilseed press cakes. This knowledge gap highlights the need for systematic investigation of protein-rich fractions produced through sustainable thermomechanical and dry fractionation approaches.

2.11 Methods for Purification of Proteins

Proteins are large molecules composed of one or more polypeptide chains that exhibit both hydrophilic and hydrophobic properties. They are present in various creatures such as plants, mammals, fungi, and algae (Silva et al., 2022). A protein molecule, similar to traditional surfactants, possesses both hydrophobic and hydrophilic areas. Protein molecules possess amphiphilic properties, allowing them to act as surfactants or surface-active agents. They can adsorb to the interface between oil, air, and water, resulting in a decrease in interfacial tension (Li et al., 2019). The amphiphilic character of proteins, derived from the acid-base properties of amino acids, enables them to stabilize emulsions and foams. These big molecules can form strong, stretchy films at the edges of substances. This makes the emulsion more stable through electrostatic interaction, hydrogen bonding, and hydrophobic interaction. Furthermore, they can create a

protective layer that stops oil droplets from sticking together and merging by creating an effect of steric and electrostatic repulsion among the droplets at the same time (Li et al., 2022d). A comprehensive overview of the various applications of meat, fish, milk, egg, and plant proteins as biosurfactants. These proteins demonstrate robust emulsifying properties that are similar to those of traditional surfactants, such as animal proteins like bovine serum albumin. Proteins from various sources were studied by Wan et al. (2019), including whey protein (Chang et al., 2020; Fan et al., 2021), casein/sodium caseinate (Qi et al., 2022), lactoglobulin (Wang et al., 2022a), myofibril (Jia et al., 2022; Yu et al., 2022), pompano (Sun et al., 2022), shrimp (Li et al., 2022a), and egg (Zhao et al., 2023a) proteins.

Physical methods can achieve the purification of proteins from Sacha-Inchi (*Plukenetia volubilis*). Sacha-Inchi seeds are known to contain high nutritional value proteins, so purifying them can be beneficial for various applications. The following is a comprehensive method for purifying proteins from Sacha-Inchi using physical techniques.



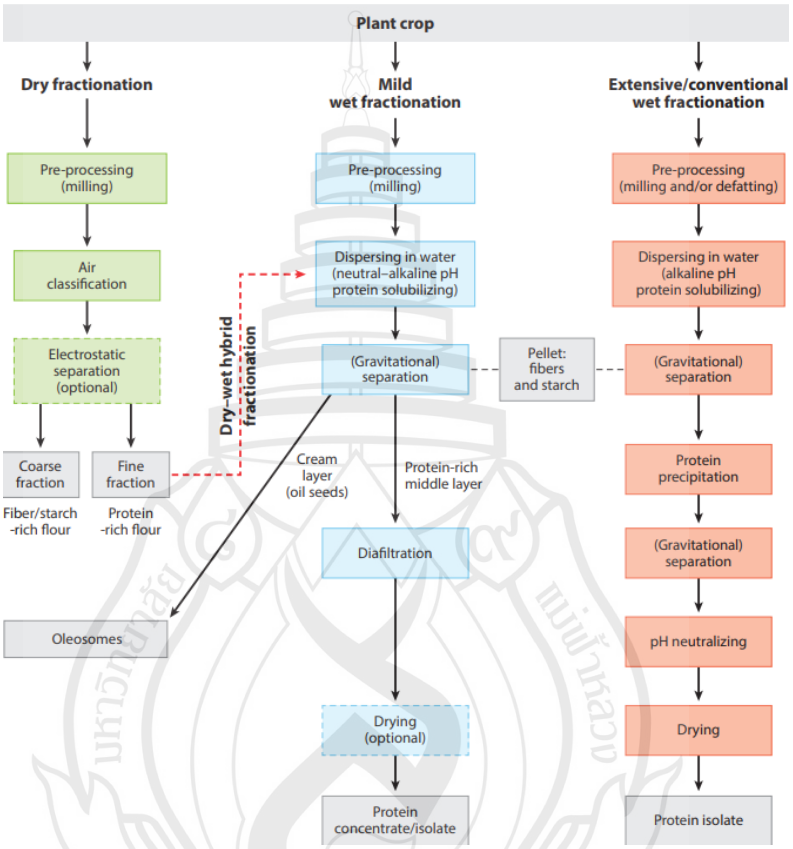
Source Lie-Piang et al. (2023)

Figure 2.2 Overview of fractions that can be obtained with dry fractionation, mild wet fractionation, and extensive wet fractionation methods and their functional properties, such as solubility, foaming, or gelation.

Conventional wet separation techniques entail the dissolution of undesirable substances or the precipitation of desired components such as protein. (Figure 2.3) This procedure has the capability to eliminate antinutritional elements, while it has the potential to modify the characteristics of proteins. In order to alleviate this issue, alternative methods such as maintaining a neutral pH or employing dry fractionation might be taken into account (Rodríguez-Ambriz et al., 2005; Swamylingappa & Srinivas, 1994).

The term "mild fractionation" for food is very new and has been used in a limited number of Scopus articles. In contrast, dry fractionation has a longer-established

history. Dry fractionation, which originated in 1984, initially aimed to separate fats but later transitioned to the manufacturing of sustainable ingredients starting in 2011. Varied definitions result in inconsistent research coverage by Barakat and Mayer (2017), Boye et al. (2010), Schutyser et al. (2015), and Zhu et al. (2021a) discuss alternative fractionation methods.



Source Lie-Piang et al. (2023)

Figure 2.3 This diagram provides a clear summary of the dry fractionation, mild wet fractionation, and extensive or traditional wet extraction techniques used to create plant-based ingredients such as protein- and starch-rich substances, oleosomes, or protein isolates.

2.11.1 Sieving and air classification

Dry fractionation separates flour into protein, starch, and fiber-rich fractions through milling and particle separation. Proper milling is crucial for optimal separation, creating individual components like starch granules and proteosomes. Crops high in oil

content require defatting before dry fractionation. The milling process needs crop-specific optimization for effective separation (Politiek et al., 2022). The process of separating milled flour into fractions can be achieved using the methods of sieving, elutriation, and air classification. These techniques facilitate the segregation of flour particles according to their size. Dry fractionation guarantees the preservation of microbiological stability, enabling the utilization of all fractions for subsequent purposes (Srinivasan & Singh, 2008). For many years, air classification has been employed to enhance the quality of flours, particularly those derived from legumes that contain starch, such as field peas and lentils (Tyler et al., 1981). Pseudo-cereals and oil-bearing crops have also been effectively fortified using this method. The legume cotyledons contain starch granules of bigger size, which enables them to be separated based on their size using air classification methods. This separation process is carried out after the starch granules are released through impact or pin milling. Several studies have investigated this technique, including those by King and Dietz, (1987), Vasanthan and Bhatty, (1995), Wu and Stringfellow, (1995), and Wu et al., (1994). Since 2011, there has been a renewed interest in the scientific literature due to the recognition of crops as highly promising protein sources. The dry fractionation techniques used for crops such as barley, oat, corn, cottonseed, wheat, soybean, lupine, and pea result in the separation of components that are supplemented with specific nutrients. Dry procedures are more energy-efficient than wet processes because they do not necessitate the use of water (Pelgrom et al., 2015b; Schutyser et al., 2015).

2.11.2 Electrostatic separation

Air classification is a method that separates different components by considering their particle size and density. However, it has difficulties when dealing with components that have similar characteristics. Previous papers have highlighted the possibilities of electrostatic separation for such flours. The technique entails the selective charging of particles and their subsequent separation through the application of an electric field (Barakat & Mayer, 2017; Wang et al., 2016; Zhu et al., 2021a). Electrostatic separation is a novel technique used in the separation of food ingredients. Examples comprise the augmentation of plant materials such as lupine, soybean, navy bean, rapeseed, oat bran, and wheat bran. Ingredients obtained from legumes have a high protein content, while ingredients derived from oats and wheat bran are rich in β-glucans and

arabinoxylans. Triboelectric separation is a technique employed in the mining and waste recycling industries. It is utilized for treating coal fly ash and for the beneficiation of minerals such as iron and phosphate (Bittner et al., 2014). Studies on yellow pea and lentil fractionation have shown that air classification followed by electrostatic separation can achieve protein concentrations of up to 65%. The protein content of pea flour was increased from 57.1% to a range of 63.4-67.6% using electrostatic separation. The yields of the ingredients obtained ranged from 15.8% to 4.0%. The combination of a magnetic field and an electric field resulted in a greater separation of proteins, leading to an increase in content from 59.7% to 72.1%, with a yield of 9.2% for the ingredients (Zhu et al., 2021b). The protein concentration in yellow peas is limited to approximately 75% due to the nature of proteosomes. The limited production of protein concentrate is ascribed to the utilization of small-scale electrostatic separation machines. Dry fractionation enables the exploitation of all fractions based on their specific functionality, such as thickening or water binding (Plant & Moore 1983).

2.11.3 Mild wet fractionation

Boye et al. (2010) examined the process of separating plant proteins using wet approaches, such as extracting and precipitating proteins through the use of salt to enhance solubility. The water-only extraction approach produces worse results compared to methods that take into account modified pH or ionic strength. The protein concentration varies between 54% and 67%. The process of yellow pea processing entails the separation of fractions that are rich in starch and protein. Ultrafiltration and diafiltration are used to concentrate proteins following wet fractionation. Möller et al. (2022) achieved a protein yield of 87% by employing washing and ultrafiltration techniques. Kornet et al. (2020) employed a method of extracting albumins by increasing the pH and using ultrafiltration, without the need for precipitation. This approach allowed them to recover albumins with distinct properties that are useful for emulsion, foam stabilization, and gelation. Efforts are underway to create less intense methods for extracting oil from crops, with the aim of avoiding problems related to protein solubility and oxidation, in addition to addressing protein and starch concerns. Conventional techniques such as hot pressing and solvent-based extraction might not effectively use the full potential of raw materials. The use of mild wet oil extraction is being investigated as a comprehensive method to extract oils, proteins, and other components at the same time.

This strategy aims to overcome the concerns associated with the use of organic solvents in food processing (Mosenthin et al., 2016). Scientists investigated the extraction of proteins and oleosomes using a higher pH and centrifugation technique, which helps to maintain the oils within their native vesicles. Oleosomes are intracellular structures found in plant cells that consist of oils enclosed by a phospholipid membrane. They have the ability to disperse in water. The studies have shown that these approaches can be used to extract oleosomes and proteins at the same time. The use of water with high ionic strength proved to be successful in extracting oleosomes. However, the stability of the emulsion varied depending on the specific cation utilized (Romero-Guzmán et al., 2020c). Drying methods have the ability to modify attributes of fractions, such as protein solubility. Substituting liquid concentrate for drying can enhance material characteristics and minimize environmental consequences (Van der Goot et al., 2016).

Sacha inchi (*Plukenetia volubilis*), a perennial plant native to the Amazon region, has gained prominence for its high nutritional value, particularly its protein-rich press cake a byproduct of oil extraction (Geerts et al., 2018; Lie-Piang et al., 2023). Utilizing this cake for protein fractionation offers a sustainable pathway to meet the growing demand for functional plant-based food ingredients (Lie-Piang et al., 2023). This study explores the feasibility of employing mild wet fractionation, a sustainable and innovative processing technique, to isolate and enhance the functionality of sacha inchi proteins for the food industry (Lie-Piang et al., 2023). Mild wet fractionation is a gentle, aqueous-based process designed to preserve the native state of proteins, ensuring better functionality while minimizing environmental impact (Lie-Piang et al., 2023). Unlike traditional fractionation methods, which often involve harsh chemical solvents, this approach employs water or buffered solutions to selectively solubilize and precipitate proteins, maintaining their native properties (Geerts et al., 2018; Lie-Piang et al., 2023). The technique not only reduces energy consumption and chemical waste but also caters to consumer demand for clean-label ingredients (Geerts et al., 2018; Lie-Piang et al., 2023). The process begins with the dispersion of defatted sacha inchi press cake in water, followed by pH adjustment to enhance protein solubility (Geerts et al., 2018). Centrifugation separates proteins from fibers and insoluble components, yielding a protein-rich fraction and a fibrous residue (Lie-Piang et al., 2023). The protein fraction can be further purified using ultrafiltration or diafiltration to

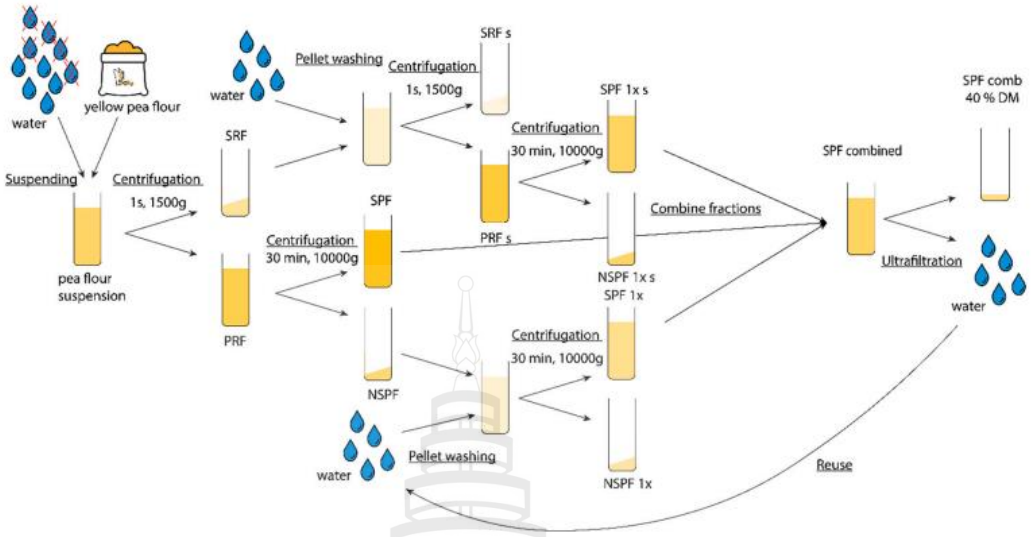
achieve the desired concentration and functionality (Geerts et al., 2018; Lie-Piang et al., 2023). Such methods have been successful in other plant matrices, such as soy and legumes, where functional properties like solubility, water-holding capacity (WHC), and emulsification were optimized (Geerts et al., 2018; Lie-Piang et al., 2023). The investigate the specific functional properties of sacha inchi proteins extracted via mild wet fractionation, focusing on their application in food formulations (Lie-Piang et al., 2023). Parameters such as WHC, gelation, and emulsification capacity will be characterized to tailor the fractions for various food products (Geerts et al., 2018). The anticipated outcomes include high-yield protein fractions with superior functionality, suitable for meat analogs, beverages, and bakery applications (Lie-Piang et al., 2023; Geerts et al., 2018). In addition to its technical benefits, mild wet fractionation aligns with sustainable processing goals by minimizing resource use and reducing the carbon footprint of ingredient production (Lie-Piang et al., 2023). This approach offers an efficient use of the sacha inchi press cake, contributing to circular economy models in the food industry (Geerts et al., 2018; Lie-Piang et al., 2023).

2.11.4 Hybrid dry-wet fractionation

Following the air classification and/or electrostatic separation process, the resulting fractions may consist of concentrates rather than isolates. Washing the target fraction using a gentle wet process can effectively eliminate any remaining fiber, starch, and other substances. This has been proven in the separation of pea protein using a wet suspension and selective centrifugation in water, without the need for pH correction. We concentrate the protein in the uppermost layers and then subject it to microfiltration and diafiltration processes to obtain a highly pure native protein concentrate (Pelgrom et al., 2015a; Schutyser et al., 2015). The hybrid method can provide purities comparable to isolates but with a somewhat reduced yield. The protein solubility in quinoa was evaluated, along with yellow pea and mung bean. We found that adding salt enhanced the protein solubility in quinoa. A hybrid fractionation approach enhanced the protein recovery of faba bean (Dumoulin et al., 2021).

The objective of mild fractionation of crops for ingredient synthesis is to eliminate the use of pesticides and minimize water and energy consumption in the food production process. The resource usage efficiency of several mild fractionation path-

ways for yellow peas has been explored and compared to typical wet fractionation techniques (Geerts et al., 2018). Concentrates enhanced in protein or starch are prepared from yellow peas using both dry (Pelgrom et al., 2013) and moderate wet fractionation (Geerts et al., 2017a). The purities of the fractions obtained via dry fractionation are lower than those obtained from mild wet fractionation and are also less pure than isolates derived from traditional fractionation. Dry fractionation exhibits the highest rational exergy efficiency, followed by mild wet fractionation and typical wet fractionation. The efficiencies are primarily affected by material loss (Geerts et al., 2018). The study conducted by Moller et al. (2021) examined the efficacy of moderate fractionation processes by comparing the purity performance of mild wet fractionation and dry fractionation methods on yellow peas. The results showed that the higher purities achieved in moderate wet fractionation are due to the further separation of the flour particles when they are dispersed in water. The study revealed that the soluble components in the pieces of pea flour were definitely solubilized, leading to improved separation. Therefore, it was deduced that water serves as a potentially advantageous supplementary factor in moderate fractionation. The authors of the study Moller et al. (2021) suggested that washing the pellets during moderate wet fractionation could result in increased purities in the corresponding fractions. In order to conduct a more thorough examination of the function of water in mild wet fractionation and to enhance the process in terms of both yield and purity (as shown in Figure 2.4).



Source adopted from Geerts et al. (2017b)

Figure 2.4 Graphic representation of the mild wet fractionation process with adaptations and additions, including one washing step of the starch rich fraction (SRF) and the non-soluble protein fraction (NSPF) and a subsequent ultra filtration step of the soluble protein fraction (SPF). PRF is the protein rich fraction, 1× indicates obtained after one washing step, the small 's' indicates the fractions origin from the starch fraction.

2.11.5 Cyclone separators

The press cake is an underutilized resource with potential for enrichment into functional plant-based proteins suitable for the food industry. One promising method to achieve this is the use of cyclone separators, a technology traditionally employed for gas-solid separation, now being adapted for fine particle classification and fractionation in food systems (Wang et al., 2023). The cyclone separator operates on the principle of centrifugal force, which separates particles based on their size, density, and shape when introduced into a rapidly rotating air stream (Wang et al., 2023). In the context of sacha inchi press cake, dry-milled material can be fed into the cyclone separator, where denser protein-rich particles are separated from lighter, fibrous components. The process does not require solvents, aligning with consumer demand for clean-label and environmentally friendly processing (Geerts et al., 2018; Wang et al., 2023). Protein enrichment using cyclone separators relies on optimization of operating parameters such as airflow velocity, inlet geometry, and cyclone dimensions, which influence separation efficiency

and particle classification (Wang et al., 2023). Research in similar systems, such as those used for legume and cereal protein enrichment, has demonstrated that careful tuning of these parameters can yield high-purity protein fractions while maintaining their functional properties (Schutyser et al., 2015). These principles can be adapted to enhance the protein content and functionality of sacha inchi-derived fractions. The enriched protein fractions are expected to exhibit functional properties such as solubility, water-holding capacity, and emulsification, making them suitable for incorporation into plant-based foods, including meat analogs, dairy alternatives, and bakery products (Pelgrom et al., 2015). By employing cyclone separation, the enrichment process also minimizes energy consumption and waste, contributing to the circular economy and sustainability goals in the food industry (Lie-Piang et al., 2023; Wang et al., 2023). The application of cyclone separators for enriching sacha inchi press cake proteins, aiming to optimize the process parameters and evaluate the functional and nutritional properties of the resulting fractions. The anticipated outcomes include high-yield protein isolates that meet industrial standards for plant-based food formulations while advancing sustainable processing practices.

2.12 Thermal and Mechanical Modification

2.12.1 Principles of thermal treatment on proteins

Thermal treatment is widely used to modify protein structure and functionality. Heat induces protein denaturation by disrupting hydrogen bonds and hydrophobic interactions, leading to partial unfolding of protein molecules. This structural modification weakens protein–matrix interactions and can enhance protein accessibility and solubility (Loveday et al., 2020).

However, excessive thermal treatment may promote protein aggregation, which can reduce solubility and functional performance. Therefore, careful control of processing conditions is essential to achieve beneficial structural modification while minimizing adverse effects (Brodkorb et al., 2019; Minekus et al., 2014).

2.12.2 Autoclaving (AC) as a pretreatment strategy

Autoclaving is a hydrothermal treatment that applies high temperature and pressure in the presence of moisture. Unlike chemical treatments, autoclaving does not require the addition of reagents and is considered a food-grade physical pretreatment method. The process disrupts cell wall structures, weakens protein–matrix associations, and facilitates protein accessibility.

Autoclaving has been reported to improve protein digestibility by enhancing enzymatic accessibility during gastrointestinal digestion (Brodkorb et al., 2019; Minekus et al., 2014). In oilseed-derived materials, hydrothermal treatment has been shown to modify protein structure and improve techno-functional properties such as water-holding and emulsifying capacities (Njoroge et al., 2025; Vogelsang-O'Dwyer et al., 2020).

Recent studies focusing on the valorization of Sacha inchi press cake and related by-products demonstrated that thermal processing significantly alters physicochemical properties and enhances functional performance, supporting the application of autoclaving as an effective pretreatment strategy (Njoroge et al., 2025; Vogelsang-O'Dwyer et al., 2020).

2.12.3 Applications of autoclaving in protein-rich materials

2.12.3.1 Legume proteins

Autoclaving has been extensively applied to legume proteins as a hydrother mal treatment to reduce antinutritional factors and improve protein digestibility. Heat and pressure disrupt hydrogen bonding and protein–fiber interactions, resulting in enhanced enzymatic accessibility during gastrointestinal digestion (Minekus et al., 2014).

Previous studies on legumes have demonstrated that autoclaving significantly improves *in vitro* protein digestibility and modifies protein structure, leading to increased susceptibility to digestive enzymes (Brodkorb et al., 2019). Structural disruption of legume cell matrices has also been reported to enhance protein solubility and functional properties following thermal treatment (Loveday et al., 2020).

In addition, autoclaving has been shown to weaken matrix rigidity and red-

uce physical barriers that limit protein accessibility in plant-based materials (Njoroge et al., 2025). Similar effects have been observed in protein-rich oilseed materials, supporting the applicability of autoclaving across different plant protein sources (Njoroge et al., 2025; Vogelsang-O'Dwyer et al., 2020).

2.12.3.2 Oilseed meals

In oilseed meals, proteins are often tightly associated with fiber and residual lipids, which restrict protein accessibility and functional performance. Autoclaving has been reported to disrupt protein–matrix interactions and alter protein conformation, thereby improving solubility and functionality (Njoroge et al., 2025).

Thermal processing of oilseed-derived materials has been shown to enhance water-holding capacity and emulsifying properties by modifying protein structure and reducing matrix integrity (Vogelsang-O'Dwyer et al., 2020). These effects are attributed to heat-induced denaturation and partial unfolding of protein molecules, which increase exposure of functional groups (Loveday et al., 2020).

Furthermore, hydrothermal treatment has been associated with improved protein digestibility in oilseed meals by facilitating enzymatic hydrolysis (Brodkorb et al., 2019; Minekus et al., 2014). Reviews on oilseed by-product valorization emphasize that autoclaving represents an effective physical pretreatment strategy to enhance protein functionality while maintaining food-grade processing conditions (Njoroge et al., 2025).

2.12.3.3 By-products

Protein-rich by-products from food and agricultural processing are increasingly investigated as alternative protein sources. However, their utilization is often limited by structural rigidity and low protein accessibility. Autoclaving has been reported to effectively disrupt cell wall structures and weaken protein–matrix interactions in such by-products, improving protein availability (Njoroge et al., 2025).

Studies on plant-based by-products have shown that hydrothermal treatment enhances protein solubility and digestibility by altering matrix integrity and protein conformation (Brodkorb et al., 2019; Minekus et al., 2014). Similar structural modifications have been observed in oilseed press cakes following thermal processing, leading to improved functional performance (Njoroge et al., 2025; Vogelsang-O'Dwyer et al., 2020).

The application of autoclaving as a physical pretreatment method is there f-ore considered a practical and scalable strategy for valorizing underutilized protein-rich by-products in food systems (Loveday et al., 2020).

2.13 Mechanical Size Reduction and Modification

2.13.1 Colloid milling and high-shear milling

Colloid milling and high-shear milling are mechanical processing techniques widely used to reduce particle size and modify the physical structure of plant-based materials. These techniques operate by applying intense shear, friction, and impact forces, which disrupt agglomerated particles and break down structural components within the matrix. As a result, fine particles with increased surface area and improved uniformity are produced, which are essential for enhancing dispersion and functional performance in food systems (Loveday et al., 2020).

Mechanical size reduction has been shown to significantly influence hydration behavior and protein accessibility. Increased surface area generated by colloid milling enhances protein–water and protein–oil interactions, facilitating improved solubility and dispersion in complex food matrices (Phonphimai et al., 2025). Such effects are particularly important for protein-rich plant materials in which proteins are embedded within fibrous or lipid-containing matrices.

In addition, high-shear milling has been reported to improve techno-functional properties, including water-holding capacity, oil-holding capacity, and emulsifying stability, by promoting exposure of functional protein groups and improving interfacial adsorption behavior (Vogelsang-O'Dwyer et al., 2020). These functional improvements contribute to better processing performance and product quality in food formulations.

Mechanical processing also plays a role in enhancing protein digestibility. Reduced particle size and increased surface exposure facilitate enzymatic accessibility during gastrointestinal digestion, leading to improved protein hydrolysis and bioaccessibility (Brodkorb et al., 2019; Minekus et al., 2014). Similar trends have been reported

for plant protein materials and protein-rich by-products subjected to mechanical modification, supporting the general applicability of colloid milling across different protein sources (Njoroge et al., 2025).

Collectively, previous studies indicate that colloid milling and high-shear milling are effective mechanical strategies for improving protein accessibility, functionality, and digestibility in plant-based materials. These characteristics make mechanical size reduction a suitable complementary approach to thermal pretreatment in the valorization of Sacha inchi press cake proteins.

2.13.2 Effects of mechanical size reduction

2.13.2.1 Particle size distribution

Mechanical size reduction leads to a narrower particle size distribution which enhances dispersion stability and reduces sedimentation in food systems. Particle size distribution strongly influences hydration behavior and interaction between protein particles and surrounding phases (Loveday et al., 2020).

Studies on mechanically processed plant proteins have demonstrated that reduced particle size improves dispersion and uniformity, resulting in enhanced functional performance (Phonphimai et al., 2025). In addition, finer particle size has been associated with improved enzymatic accessibility during digestion due to increased surface exposure (Minekus et al., 2014).

Similar trends have been reported for protein-rich by-products and flours, w-here particle size reduction enhanced functional and nutritional properties (Brodkorb et al., 2019; Njoroge et al., 2025; Vogelsang-O'Dwyer et al., 2020).

2.13.2.2 Surface area

Reduction in particle size results in a significant increase in surface area, w-hich enhances protein–water and protein–oil interactions. Increased surface area improves hydration behavior and facilitates functional interactions in food systems (Loveday et al., 2020).

Studies on dry-fractionated and mechanically processed plant proteins have shown that increased surface area contributes to improved protein accessibility and solubility (Phonphimai et al., 2025). Enhanced surface exposure also promotes enzymatic hydrolysis during digestion by increasing contact between proteins and digestive enzymes (Minekus et al., 2014).

In oilseed press cakes and other protein-rich materials, increased surface area following mechanical processing has been associated with improved techno-functional properties and digestibility (Brodkorb et al., 2019; Njoroge et al., 2025; Vogelsang-O'Dwyer et al., 2020).

2.13.2.3 Techno-functional properties

Particle size reduction has been widely reported to influence techno functional properties of plant-based protein materials. Reduced particle size enhances water-holding and oil-holding capacities by increasing surface exposure and improving protein–phase interactions (Vogelsang-O'Dwyer et al., 2020).

Mechanical processing has also been shown to improve emulsifying properties by promoting protein adsorption at oil–water interfaces and stabilizing emulsion systems (Loveday et al., 2020). In addition, finer particles generally exhibit improved dispersion and stability in food formulations (Phonphimai et al., 2025).

Improvements in techno-functional properties following mechanical size reduction have been linked to enhanced protein accessibility and digestibility (Brodkorb et al., 2019; Minekus et al., 2014; Njoroge et al., 2025).

2.13.3 Applications of colloid milling in plant protein materials

Colloid milling has been applied to a wide range of plant protein materials and flours to improve particle size uniformity and functional performance. High-shear forces generated during colloid milling disrupt agglomerates and enhance dispersion behavior in food systems (Loveday et al., 2020).

In studies on oilseed press cakes, colloid milling has been shown to enhance functional properties when applied as a post-treatment following protein enrichment or thermal pretreatment (Phonphimai et al., 2025). Improvements in hydration behavior, emulsifying capacity, and processing performance have been reported (Vogelsang-O'Dwyer et al., 2020).

Colloid milling has also been associated with improved digestibility of plant proteins by increasing surface area and enzymatic accessibility (Brodkorb et al., 2019; Minekus et al., 2014). Reviews on sustainable protein processing emphasize that colloid milling is an effective mechanical strategy for valorizing plant protein materials and by-products (Njoroge et al., 2025).

2.14 *In vitro* Gastrointestinal Digestion

The simulated static *in vitro* gastrointestinal digestion model source of energy and necessary components such as macro- and microelements are coming from the digestion steps. The form of the nutritional components released during digestion depends on both initial food properties as well as transformations during the digestive mechanistic and chemical processes (Somaratne et al., 2020). Essentially, *in vitro* digestive models offer a valuable substitute for animal and human models in quickly evaluating dietary ingredients. An optimal *in vitro* digestion approach should yield precise outcomes within a brief timeframe (Coles et al., 2005), making it a valuable tool for swiftly evaluating foods or delivery systems with varying compositions and structures. Practically, it is impossible for any *in vitro* procedure to attain the same level of precision (Coles et al., 2005; Fuller et al., 1991). Therefore, it is necessary to find a balance between the accuracy and simplicity of use of any *in vitro* digestion model. In recent years, food and animal scientists have employed various *in vitro* digestion models to examine the structural and chemical alterations that take place in different foods under simulated gastrointestinal conditions. Although none of these methods has yet been widely accepted. Generally, digestion can be described as a complex process in which nutrients from food are subject to processes lead to using them for energy, growth, and cell repair. Therefore, *in vitro* digestion models were developed based on human physiology to investigate structural modifications, digestibility, and release of food constituents under simulated gastrointestinal conditions (Brodkorb et al., 2019). To generate the gastrointestinal conditions the ionic strength and pH, as well as addition of enzymes, bile salts and even fermentation reactions to simulate colon conditions, have to be adjusted (Brodkorb et al., 2019). The processes take place in the digestive tract, which occurs in a twisting tube that starts at the mouth and ends at the anus. The tract can be divided into several parts, each of which is necessary for proper digestion including the mouth, esophagus, stomach, small intestine, large intestine, and anus (Wojtunik-Kulesza et al., 2020a).

Table 2.5 Digestive processes in the human digestive tract

Part of the digestive tract	**pH**	**Substrates (Nutrient)**	**Enzymes**	**Digestion Product**
Mouth	Neutral	Starch, fats	Salivary amylase	Maltose and dextrin, non-esterifiec fatty acids
Esophagus	Neutral	Moving food to the stomach after initial enzymatic and mechanistic processes in the mouth		
Stomach	15-2.0	Peptides, emulsified lipids casein	Pepsin, lipase rennet	Amino acids, glycerol, fatty acids, glycerides, curdle casein
Part of digestive tract	pH	Substrates (Nutrient)	Enzymes	Digestion Product
Small intestine	Light alkaline, approx. 8	Polypeptides, starch, sucrose, fats, proteins, starch/ glycogen	Aminopeptidase, amylase, sucrose, lipase, chymotrypsin, pancreatic amylase	Amino acids, maltose and dextrin, glucose and fructose, glycerol and fatty acids, amino acids, maltose and isomaltose
Large intestine	Neutral	Absorption of water and salts, production and absorption of vitamins propelling feces for elimination from the organism		

Source adapted from Wojtunik-Kulesza et al. (2020a)

2.14.1 Mechanism and the models of *in vitro* gastrointestinal digestion

The two main stages of the digestive process simulation are the stomach and small intestine digestion simulations. The circumstances and methods for research of the digestibility and bioaccessibility of phytochemicals have been modified according to this concept, however, the physiological settings selected vary greatly between various static *in vitro* experiments. In essence, static modeling of stomach digestion is carried out by pepsin hydrolysis of homogenized materials over time at constant pH and temperature. Typically, the temperature of the human body (37 °C) is utilized. Usually, dynamic activities like mechanical forces or ongoing pH and secretion flow rate variations that occur during human digestion are not replicated. The presence or absence of phospholipid vesicles, the presence or absence of lipase, an incubation period between 0.5 and 2 hours, a pH range between 1.7 and 2.5, and the ratio of pepsin to substrate are the main variations among the methods used to replicate the gastric phase of digestion. Static models are less effective in studies of complete digestion but are more beneficial when there is restricted digestion (such as the gastric or intestinal phases). In order to increase the nutritional value of foods and enhance food design, these techniques can be used to assess the impact of digestion conditions and conduct studies on the impact of food structure and composition, dietetic factors, and food processing on nutrient and bioactive compound bioaccessibility. For digestion of basic meals and isolated or purified food components, static models are primarily employed (Wojtunik-Kulesza et al., 2020b). Starting at the mouth stage of *in vitro* digestion, food is put through a variety of chemical, biological, and mechanical processes. pH, ionic strength, temperature, the activity of different digestive enzymes (particularly lingual lipase, amylase, and protease), interactions with biopolymers in the saliva (mucin), interactions with sensory receptors of the tongue and mouth, and reduction of particle size in bolus by chewing are just a few of the possible changes that food components may go through (Mc Clements & Li, 2010). The next step of digestion is the gastric stage, which is a complicated process involving both mechanical activities and the activity of gastric fluids. Hydrochloric acid, pepsinogens, lipase, mucus, electrolytes, and water are all components of gastric juice. After eating, the rate of gastric juice production increases to between 1 to 10 mL/min from between 1 to 4 mL/min during fasting. Protein denaturation results

from hydrochloric acid's contribution of activated pepsin. Healthy human beings' fasting stomach pH falls between the ranges of 1.3 and 2.5. In general, eating a meal raises the pH to above 4.5, depending on the food's ability to act as a buffer. The shift in stomach pH is only taken into account in dynamic models, as most static *in vitro* research has been carried out at a pH below 2.5, which is a pH comparable to the human fasting condition rather than to actual meal digestion (Blanquet-Diot & Alric, 2009). pH, temperature, time, and pancreatic juice, which contains electrolytes, bile salts, and enzymes, are all factors in the *in vitro* small intestine digestion of food. The duodenum's pH in the fed condition can range from 5.4 to 7.5 (Clarysse et al., 2009). up to 7.0 to 7.5 in the ileum and to 5.3 to 8.1 in the jejunum. Along with other digestive enzymes like maltase, lactase, α-dextrinase, and peptidases, pancreatic enzymes such as proteases, amylases, and lipases help break down food. The types of biliary acids utilized (bile salt mixes, actual fresh bile, or individual bile salts) and the types of enzymes employed (pancreatin or specific enzymes) are the main distinctions between the approaches.

2.15 Summary and Research Gap

The reviewed literature confirms that Sacha inchi press cake is a protein-rich by-product with considerable potential for food applications; however, its utilization is limited by matrix integrity, restricted protein accessibility, and suboptimal functional and digestibility properties (Loveday et al., 2020; Njoroge et al., 2025).

Dry fractionation offers a sustainable approach for protein enrichment but provides only moderate improvements due to matrix constraints (Phonphimai et al., 2025). Mild wet fractionation can increase protein purity but is limited by low yield and high processing cost (Njoroge et al., 2025). Thermal and mechanical processing strategies, particularly autoclaving and colloid milling, have demonstrated potential for improving protein accessibility, functionality, and digestibility in plant-based materials (Brodkorb et al., 2019; Minekus et al., 2014; Vogelsang-O'Dwyer et al., 2020).

Despite these advances, limited information is available on integrated processing strategies that combine dry fractionation with autoclaving and mechanical size

reduction to optimize protein content, functionality, and digestibility simultaneously. This research gap forms the basis of the present study. However, no study has systematically compared dry fractionation and hydrothermal–mechanical processing within the same Sacha inchi press-cake system.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw materials

Sacha inchi (*Plukenetia volubilis* L.) press cake, obtained as a by-product from cold-press oil extraction, was used as the primary raw material in this study. The press cake was supplied by Tai C.M.S. Standard Industrial Co., Ltd. (Chiang Rai, Thailand). At the time of receipt, the material contained approximately 5% residual moisture.

To maintain sample integrity prior to processing, the press cake was portioned into 1 kg batches, vacuum-sealed in polyethylene bags, and stored at −20 °C under dry conditions. These storage conditions were selected to minimize moisture uptake, lipid oxidation, and biochemical changes before further treatments and analyses.

Figure 3.1 Sacha inchi (*Plukenetia volubilis* L.) seeds and press cake used as raw materials in this study

3.1.2 Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade unless otherwise specified. Hydrochloric acid (HCl), sodium hydroxide (NaOH), methanol, petroleum ether, and other solvents employed for extraction and analytical procedures were purchased from Merck (Darmstadt, Germany).

Reagents required for protein analysis, including Kjeldahl digestion chemicals, were obtained from standard commercial suppliers and prepared according to established protocols. For antioxidant analyses, Folin–Ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Ferric reducing antioxidant power (FRAP) reagents, including ferric chloride and 2,4,6-tripyridyl-s-triazine (TPTZ), were prepared freshly prior to analysis.

Enzymes used for *in vitro* gastrointestinal digestion included pepsin (from porcine gastric mucosa) and pancreatin (from porcine pancreas), which were obtained from Sigma-Aldrich (St. Louis, MO, USA). All buffer solutions and simulated digestive fluids were prepared using distilled water and adjusted to the required pH values prior to use.

3.1.3 Equipment and instruments

Sample processing and analyses were carried out using standard laboratory equipment and analytical instruments appropriate for each experimental procedure. Drying of press-cake samples was performed using a cabinet tray dryer (Model BP-80, KluayNamThaiTowOp, Bangkok, Thailand), while particle size reduction was achieved using a hammer mill fitted with a 2 mm screen. Sieving was conducted using a mechanical sieve shaker (Endecotts Ltd., London, UK) to obtain uniform particle size fractions.

Autoclaving treatments were conducted using a laboratory autoclave operated at controlled temperature and pressure conditions (121 °C, 30 min). Colloid milling was performed using a bench-scale colloid mill to generate high shear forces for particle disintegration and slurry homogenization.

Centrifugation steps were carried out using a refrigerated centrifuge capable of operating at speeds up to 10,000 × g, with temperature control set at 4 °C. Drying of solid fractions after processing was performed in a hot-air oven with adjustable temperature settings.

For compositional and physicochemical analyses, pH measurements were conducted using a digital pH meter (AQUASEARCHER AB33, OHAUS, Shanghai, China), while water activity was determined using a water activity meter (AquaLab

3TE, Decagon Devices, Pullman, WA, USA). Color parameters were measured using a HunterLab colorimeter equipped with a D65 illuminant and a 10° standard observer.

Structural characterization was performed using a scanning electron microscope (Vega 3 XMU, TESCAN, Brno, Czech Republic) after sample coating with a sputter coater (Quorum SC7620, Quorum Technologies Ltd., East Sussex, UK). Fourier-transform infrared (FTIR) spectra were collected using an FTIR spectrometer (Nicolet iS5, Thermo Fisher Scientific, Madison, WI, USA) equipped with an attenuated total reflectance (ATR) accessory.

Thermal properties were analyzed using differential scanning calorimetry (DSC 3, Mettler-Toledo AG, Greifensee, Switzerland) and thermogravimetric analysis (TGA 2, Mettler-Toledo AG, Greifensee, Switzerland). Particle size distribution measurements were conducted using a laser diffraction particle size analyzer (LA-960, HORIBA Scientific, Longjumeau, France).

3.2 Preparation of Sacha Inchi Press Cake Samples

3.2.1 Sample preparation

Prior to thermal and mechanical treatments, the Sacha inchi press cake was prepared to obtain a uniform powder suitable for subsequent processing. The frozen press-cake material was first allowed to equilibrate to ambient temperature before drying. Drying was carried out in a cabinet tray dryer at 60 °C for 6 h to reduce the moisture content to approximately 4–5%, thereby improving milling efficiency and storage stability.

The dried press cake was subsequently subjected to size reduction using a hammer mill equipped with a 2 mm screen. To ensure uniformity of the starting material and minimize variability during downstream processing, the milled material was sieved using a mechanical sieve shaker to obtain particles with diameters ≤250 μm. Oversized particles were discarded, and only the fine powder fraction was collected for further treatments.

The resulting press-cake powder was immediately vacuum-sealed in polyethylene bags to prevent moisture absorption and oxidative deterioration. Prepared samples were stored at −20 °C under dry conditions until autoclaving and colloid milling treatments were performed. All sample preparation steps were conducted under controlled laboratory conditions to ensure reproducibility and consistency across experimental replicates.

3.2.2 Sample coding and experimental design

To ensure clarity, consistency, and traceability throughout the experimental workflow, all samples were assigned specific codes according to their processing history and experimental scope. Samples investigated in this study comprised materials obtained from two sequential research parts, namely dry fractionation (Part 1) and thermal–mechanical processing (Part 2).

Samples from Part 1 were derived from dry fractionation of Sacha inchi press cake using cyclone separation and were included as reference materials. The unfractionated raw material used as cyclone feed was designated as SC-RM, while the cyclone-separated fractions were coded as F1, F2, F3, and F4, representing materials with different particle size distributions and protein enrichment levels. These fractions were used primarily for comparison of compositional, structural, and techno-functional characteristics.

Samples from Part 2 were obtained from thermal and mechanical processing of Sacha inchi press cake. The untreated press cake was designated as SP. Press cake subjected to autoclaving treatment was coded as AC, while press cake processed by combined autoclaving followed by colloid milling was designated as CL. These samples were evaluated for physicochemical properties, structural characteristics, techno-functional behavior, *in vitro* gastrointestinal protein digestibility, and food application performance.

The experimental design followed a sequential and comparative approach, enabling evaluation of (i) dry fractionation effects using SC-RM and F1–F4, and (ii) thermal and mechanical processing effects using SP, AC, and CL. Unless otherwise specified, all experiments were conducted using at least three independent replicates. This structured coding system allowed direct comparison across processing strategies while

maintaining clear distinction between reference materials and samples representing the core novelty of the present study.

3.3 Protein Fractionation Procedures

3.3.1 Dry fractionation by cyclone separation

Dry fractionation of Sacha inchi press cake meal was performed using a laboratory-scale multi-stage cyclone separation system to obtain protein-enriched fractions based on differences in particle size, density, and aerodynamic behavior. The experimental setup and airflow pathway of the cyclone system are illustrated in Figure 3.2.

Prior to fractionation, the raw Sacha inchi press cake meal (SC-RM) was dried to a moisture content below 5% to minimize particle agglomeration and ensure stable pneumatic transport. The dried material was milled using a hammer mill and sieved to obtain a uniform particle size suitable for air classification. The prepared powder was then fed continuously into the cyclone separator at a controlled feed rate.

The cyclone separation system consisted of a series of interconnected cyclone units operating under negative pressure generated by an induced-draft fan (Figure 3.2). As the material entered the cyclone chamber, particles were subjected to centrifugal forces, causing denser and coarser particles to migrate toward the cyclone wall and be collected at the underflow outlet, while finer and lighter particles were carried upward with the air stream and recovered at the overflow outlet. By sequentially connecting multiple cyclone stages, fractionation was achieved across progressively finer particle size ranges.

Four cyclone fractions (F1–F4) were collected sequentially from the system, along with the unfractionated raw material (SC-RM) serving as the reference sample. Each fraction was collected separately, sealed in polyethylene bags, and stored at −20 °C prior to further physicochemical, structural, techno-functional, and nutritional analyses. The collected fractions were used as baseline samples for comparison with subsequent thermal and mechanical processing applied in Part 2 of this study.

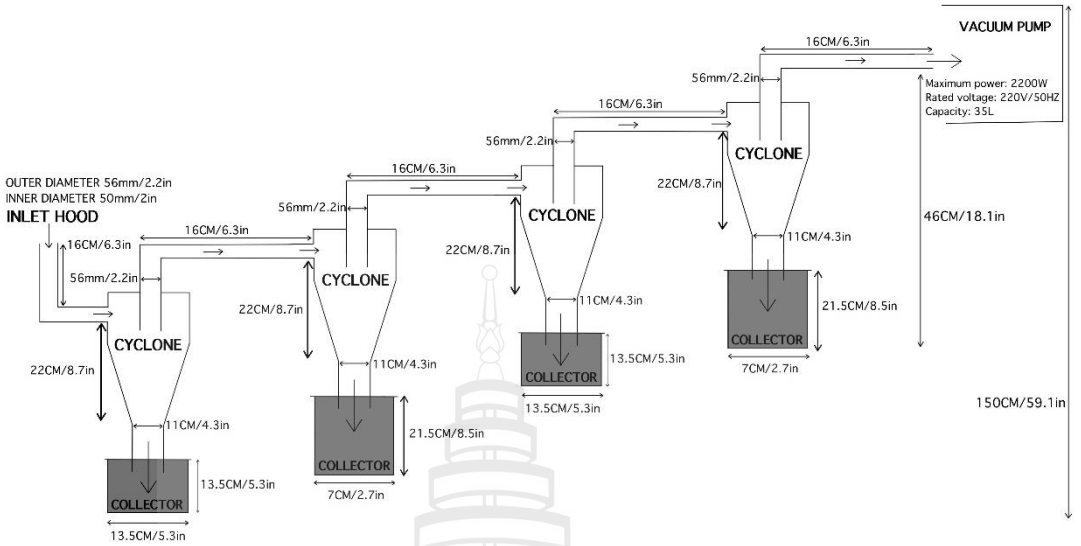


Figure 3.2 Schematic representation of the multi-stage cyclone separator system employed for protein fractionation of Sacha inchi press cake meal (SC-RM), illustrating the airflow pathway and sequential separation stages.

3.3.2 Mild wet fractionation (preliminary study)

A mild wet fractionation approach was preliminarily evaluated to investigate the protein solubilization behavior of Sacha inchi press cake under aqueous conditions prior to the application of thermal and mechanical treatments. This preliminary study aimed to assess the influence of dilution ratio, soaking time, and mild heating on protein distribution, rather than to establish a fully optimized extraction process.

Sacha inchi press-cake powder was dispersed in distilled water at different solid-to-liquid ratios ranging from 1:5 to 1:25 (w/v). The dispersions were gently mixed to ensure uniform hydration and subjected to soaking under two conditions: (i) room temperature (approximately 25 °C) for short-term (10 min) and extended (21 h) durations, and (ii) mild heating at 60 °C for 1 h. No pH adjustment or chemical extraction agents were applied in order to preserve native protein characteristics and to minimize chemical modification.

After soaking, the suspensions were separated by filtration to obtain a liquid fraction and an insoluble residue. The protein concentration in the liquid fraction was determined using the Biuret method and expressed as mg/mL. The effects of dilution

ratio and soaking condition on protein solubilization were assessed by comparing protein concentrations across treatments.

This preliminary evaluation provided comparative insights into protein solubilization behavior under mild aqueous conditions. However, due to limitations related to dilution requirements, protein recovery efficiency, and practical feasibility, mild wet fractionation was not further optimized as a standalone process. Instead, the outcomes of this preliminary study were used to inform the selection of subsequent autoclaving and colloid milling treatments, which were expected to induce more effective structural modification and enhanced protein accessibility in the present study.

3.4 Thermal and Mechanical Treatments

3.4.1 Autoclaving treatment (AC)

Autoclaving was applied as a thermal treatment to induce structural modification of the Sacha inchi press-cake matrix and enhance protein accessibility prior to mechanical processing and *in vitro* gastrointestinal digestion. The treatment was designed to promote protein unfolding, weaken protein–polysaccharide interactions, and facilitate subsequent particle disintegration.

Prepared press-cake powder was dispersed in distilled water at a solid-to-liquid ratio of 1:5 (w/v). Specifically, 160 g of sample was mixed with 800 mL of distilled water in a heat-resistant media bottle and agitated until a uniform suspension was obtained. The suspension was then hydrated at 4 °C for 24 h to allow sufficient water penetration into the press-cake matrix and ensure homogeneous thermal response during processing.

Following hydration, the suspension was equilibrated to room temperature. To prevent pressure buildup during heating, the bottle cap was loosened slightly before autoclaving. Thermal treatment was conducted at 121 °C for 30 min under saturated steam conditions. After completion of the autoclaving cycle, the treated suspension was allowed to cool naturally to room temperature.

The autoclaved suspension was subsequently filtered through cheesecloth to separate the liquid phase from the solid residue. Gentle manual pressing was applied to

maximize phase separation without inducing additional mechanical disruption. The solid fraction obtained after autoclaving was designated as AC and retained for further mechanical processing and analytical characterization, while the liquid fraction was collected for compositional analysis.

3.4.2 Colloid milling treatment (CL)

Colloid milling was employed as a mechanical treatment following autoclaving to further disrupt the thermally softened press-cake matrix and reduce particle size through high-shear forces. This step was intended to enhance dispersion uniformity, increase surface area exposure, and improve protein accessibility for subsequent physicochemical, functional, and *in vitro* digestion analyses.

The solid residue obtained after autoclaving (AC) was remixed with distilled water at a solid-to-liquid ratio of 1:4 (w/v). Specifically, 200 g of the autoclaved solid fraction was combined with 800 mL of distilled water and gently pre-mixed to form a homogeneous slurry prior to milling. The slurry was then processed using a colloid mill operated under continuous flow conditions for 15 min to ensure consistent mechanical shear throughout the sample.

Following colloid milling, the processed suspension was divided into three replicates and transferred into separate beakers. Each replicate was allowed to stand at room temperature to permit gravitational phase separation, and the time required for visible separation was recorded at 5, 10, and 15 min. After standing, the suspensions were filtered through cheesecloth to separate the liquid phase from the final solid residue.

The solid fraction obtained after colloid milling was designated as CL and subjected to further drying, grinding, and analytical characterization. The corresponding liquid fraction was collected for evaluation of protein distribution and moisture content. This combined thermal–mechanical processing strategy enabled systematic assessment of the effects of colloid milling on particle size reduction, microstructural modification, and protein functionality relative to autoclaving alone.

3.5 Physicochemical and Structural Analyses

3.5.1 Proximate composition analysis

The proximate composition of Sacha inchi press cake meal and its cyclone-fractionated samples was determined according to the Official Methods of Analysis of AOAC (2000), as previously reported in our published study (Phonphimai et al., 2025). Moisture content was determined by oven drying the samples at 105 °C until a constant weight was obtained (AOAC Method 925.10). Crude protein content was analyzed using the Kjeldahl method (AOAC Method 978.04), and the nitrogen content was converted to protein using a conversion factor of 6.25. Crude fat content was determined by Soxhlet extraction using petroleum ether as the solvent (AOAC Method 920.39). Ash content was measured by incineration of the samples in a muffle furnace at 550 °C until constant weight (AOAC Method 942.05). Crude fiber content was analyzed using acid–alkali digestion following AOAC Method 991.43. Total carbohydrate content was calculated by difference, using the following equation:

$$\text{Carbohydrate (\%)} = 100 - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash})$$

All results were expressed on a dry weight basis. The analyses were performed in triplicate.

3.5.2 Protein composition and dietary fiber analysis

Protein composition and dietary fiber content of the autoclaved press cake subjected to colloid milling (CL) were determined by Central Laboratory (Thailand) Co., Ltd., an ISO/IEC 17025–accredited analytical service provider, to ensure analytical accuracy and data reliability.

Amino acid composition was determined following acid hydrolysis of sample proteins. Briefly, CL samples were hydrolyzed with hydrochloric acid under controlled temperature conditions to liberate constituent amino acids. The hydrolyzed samples were subsequently derivatized and analyzed using an automated amino acid analyzer

based on chromatographic separation and post-column detection. Individual amino acids were identified and quantified by comparison with certified amino acid standards, and results were expressed as milligrams of amino acid per gram of protein.

Dietary fiber content was determined using an enzymatic–gravimetric method. The CL samples were sequentially treated with heat-stable α-amylase, protease, and amyloglucosidase to remove digestible starch and protein fractions. The remaining residue, representing total dietary fiber, was recovered, dried, and weighed. Dietary fiber content was expressed as a percentage of dry sample weight.

Protein content of CL was additionally determined using the Kjeldahl method to support amino acid composition calculations and nutritional evaluation. Total nitrogen content was measured following acid digestion, distillation, and titration, and protein concentration was calculated using a nitrogen-to-protein conversion factor of 6.25. All analytical procedures were conducted according to internationally recognized standard methods, and results were reported on a dry weight basis.

3.5.3 Color measurement and total color difference (ΔE)

Color characteristics of Sacha inchi press-cake samples were evaluated to determine the effects of dry fractionation and thermal–mechanical processing on visual appearance. Color parameters were measured using a HunterLab colorimeter equipped with a D65 standard illuminant and a 10° standard observer. The instrument was calibrated prior to measurement using a standard white calibration tile.

Color values were recorded in the CIE L*a*b* color space, where L* represents lightness (0 = black, 100 = white), a* represents the red–green axis (+a* = red, −a* = green), and b* represents the yellow–blue axis (+b* = yellow, −b* = blue). Measurements were performed at room temperature, and each sample was measured at multiple positions to minimize surface heterogeneity. The results were expressed as mean values with corresponding standard deviations.

The total color difference (ΔE) was calculated to quantify overall color changes induced by processing. ΔE was defined in the CIE L*a*b* color system as the Euclidean distance between the color coordinates of a sample and those of a reference sample. In this study, the untreated press cake was used as the reference sample, and the mean L*, a*, and b* values of the reference were applied as baseline values. The total color difference was calculated using the following equation:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

Where L^*, a^*, b^* are the color parameters of the processed samples, and L_0^*, a_0^*, b_0^* are the corresponding mean values of the reference sample. ΔE values were calculated for each replicate, and the results were expressed as mean ± standard deviation.

3.5.4 Particle size distribution analysis

Particle size distribution of Sacha inchi press-cake samples was determined to evaluate the effects of thermal and mechanical treatments on particle disintegration and dispersion behavior. Measurements were conducted for raw material press cake (SP), autoclaved press cake (AC), and autoclaved press cake followed by colloid milling (CL).

Prior to analysis, each sample was dispersed in distilled water to obtain a stable suspension suitable for laser diffraction measurement. The suspensions were gently stirred to minimize particle agglomeration while avoiding excessive shear that could alter particle size. Particle size distribution was measured using a laser diffraction particle size analyzer (LA-960, HORIBA Scientific, Longjumeau, France), operating in wet measurement mode.

The refractive indices of the dispersed phase and the dispersant were set according to manufacturer recommendations for plant-based materials. Background measurements were performed prior to each analysis to ensure signal stability. Particle size distributions were recorded as volume-based distributions, and characteristic diameters, including the median particle size (D50) and the 10th and 90th percentile diameters (D10 and D90), were calculated automatically by the instrument software.

All measurements were conducted in triplicate, and results were expressed as mean values with corresponding standard deviations. Particle size distribution data were used to assess the extent of particle fragmentation induced by autoclaving and colloid milling and to support interpretation of subsequent microstructural, functional, and *in vitro* digestibility results.

3.5.5 Microstructural analysis

Microstructural characteristics of Sacha inchi press-cake samples were examined using scanning electron microscopy (SEM) to evaluate morphological changes induced by thermal and mechanical processing. Analyses were conducted for raw material press cake (SP), autoclaved press cake (AC), and autoclaved press cake followed by colloid milling (CL).

Prior to imaging, samples were gently mounted on aluminum stubs using double-sided carbon adhesive tape to ensure stable positioning. To improve surface conductivity and minimize charging effects during observation, the mounted samples were coated with a thin layer of carbon using a sputter coater (Quorum SC7620, Quorum Technologies Ltd., East Sussex, UK).

SEM observations were performed using a Vega 3 XMU scanning electron microscope (TESCAN, Brno, Czech Republic) operated at appropriate accelerating voltages. Micrographs were captured at different magnifications (500×, 2,000×, and 5,000×) to visualize surface morphology, particle integrity, and structural disruption across multiple length scales.

Representative images were selected to illustrate differences in particle compactness, surface roughness, and degree of fragmentation among samples. SEM micrographs were used to support interpretation of particle size distribution, techno-functional properties, and *in vitro* protein digestibility results.

3.5.6 Fourier-transform infrared (FTIR) analysis

Fourier-transform infrared (FTIR) spectroscopy was employed to investigate molecular structural changes and functional group modifications of Sacha inchi press-cake samples induced by thermal and mechanical processing. Analyses were conducted for raw material press cake (SP), autoclaved press cake (AC), and autoclaved press cake followed by colloid milling (CL).

FTIR spectra were collected using an FTIR spectrometer (Nicolet iS5, Thermo Fisher Scientific, Madison, WI, USA) equipped with an attenuated total reflectance (ATR) accessory. Prior to analysis, samples were equilibrated to room temperature and placed directly onto the ATR crystal. A uniform contact pressure was applied to ensure consistent spectral acquisition.

Spectra were recorded over the mid-infrared region from 4,000 to 650 cm^{-1} at a resolution of 4 cm^{-1}, with 32 scans averaged for each spectrum to improve signal-to-noise ratio. Background spectra were collected before each measurement and automatically subtracted. All spectra were baseline-corrected and normalized prior to interpretation.

Characteristic absorption bands associated with protein secondary structures (amide I and amide II regions), polysaccharide components, and other functional groups were analyzed. FTIR spectra were used to assess changes in molecular interactions and conformational features resulting from autoclaving and colloid milling, and to support interpretation of observed variations in functional properties and *in vitro* protein digestibility.

3.5.7 Thermal analysis (DSC and TGA)

Thermal properties of Sacha inchi press-cake samples were evaluated using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) to assess changes in thermal behavior and stability induced by thermal and mechanical processing. Analyses were conducted for raw material press cake (SP), autoclaved press cake (AC), and autoclaved press cake followed by colloid milling (CL).

DSC measurements were performed using a differential scanning calorimeter (DSC 3, Mettler-Toledo AG, Greifensee, Switzerland). Approximately 5–8 mg of each sample was accurately weighed and sealed in aluminum pans, with an empty pan used as a reference. Samples were scanned from 25 to 250 °C at a heating rate of 10 °C/min under a nitrogen atmosphere to prevent oxidative degradation.

Thermal transitions, including onset temperature (T_o), peak temperature (T_p), and denaturation enthalpy (ΔH), were determined from the resulting thermograms using the instrument software. DSC data were used to evaluate protein denaturation behavior and structural modifications resulting from autoclaving and colloid milling.

Thermal stability and degradation behavior were further examined using a thermogravimetric analyzer (TGA 2, Mettler-Toledo AG, Greifensee, Switzerland). Approximately 5–10 mg of each sample was placed in a platinum pan and heated from 25 to 600 °C at a heating rate of 10 °C/min under a continuous nitrogen flow.

Weight loss profiles and derivative thermogravimetric (DTG) curves were recorded to identify thermal degradation stages and residual mass at high temperatures.

TGA data were used to assess the effects of thermal and mechanical treatments on the thermal stability and decomposition characteristics of press-cake components.

All thermal analyses were conducted in triplicate, and representative thermograms were selected for presentation.

3.6 Techno-functional Properties

Techno-functional properties of Sacha inchi press-cake samples were evaluated to determine the effects of dry fractionation (Part 1) and thermal–mechanical processing (Part 2) on their functional performance in food systems. Analyses were conducted for cyclone-derived fractions (SC-RM, F1–F4) and thermally and mechanically processed samples (SP, AC, and CL), as appropriate for each functional test. All measurements were performed in triplicate unless otherwise specified.

3.6.1 Protein solubility

Protein solubility of Sacha inchi press cake meal and its cyclone-fractionated samples was determined according to the method described by Ketnawa and Ogawa (2019), as previously reported in our published study (Phonphimai et al., 2025). Briefly, protein dispersions were prepared at a concentration of 1% (w/v) in buffer solutions with pH values ranging from 2 to 12. The dispersions were stirred at room temperature for 30 min to allow protein solubilization and then centrifuged at 10,000 × g for 15 min. The protein content in the supernatant was determined using the Biuret method, while the total protein content of the sample was determined prior to solubility analysis. Protein solubility was calculated using the following equation:

$$\text{Protein solubility (\%)} = \frac{Protein\ content\ in\ the\ supernatant}{Total\ protein\ content\ in\ the\ sample} \text{ x } 100$$

All measurements were performed in triplicate, and the results were expressed as percentage protein solubility.

3.6.2 Water-holding capacity (WHC)

Wettability and dispersibility of Sacha inchi press cake meal and its cyclone-fractionated samples were determined according to the method described by Malomo et al. (2014), as previously reported in our published study (Phonphimai et al., 2025).

For wettability measurement, 1 g of sample was gently sprinkled onto the surface of distilled water at room temperature without agitation. The wettability time was recorded as the time required for the sample particles to become completely wetted and sink below the water surface.

Dispersibility was evaluated by dispersing 1 g of sample in 100 mL of distilled water, followed by gentle stirring. After standing for a fixed period, the dispersed portion was carefully decanted, dried, and weighed. The dispersibility was expressed as the percentage of dispersed solids relative to the initial sample weight.

Equation

Wettability

$$\text{Wettability (s)} = t$$

where t is the time (seconds) required for complete wetting of the sample particles.

Dispersibility

$$\text{Dispersibility (\%)} = \frac{Wd}{Wi} \text{ x } 100$$

where W_d is the weight of the dispersed solid, and W_i is the initial sample weight.

All measurements were conducted in triplicate, and the results were reported as mean values.

3.6.3 Oil-holding capacity (OHC)

Water-holding capacity (WHC) and oil-holding capacity (OHC) of Sacha inchi press cake meal and its cyclone-fractionated samples were determined according to the method described by Malomo et al. (2014), as previously reported in our published study (Phonphimai et al., 2025). Briefly, 1 g of sample was mixed with 10 mL of distilled water or vegetable oil in a centrifuge tube and allowed to stand at room temperature for 30 min with occasional stirring to ensure adequate interaction between the sample and the liquid phase. The mixture was then centrifuged at 3,000 × g for 15 min, and the supernatant was carefully decanted. The residue was weighed to determine the amount of water or oil retained by the sample.

Equation

$$\text{WHC or OHC (g/g)} = \frac{Wi - Wi}{Wi}$$

where W_r is the weight of the residue after centrifugation and W_i is the initial weight of the dry sample. The water- and oil-holding capacities were expressed as grams of water or oil retained per gram of dry sample. All measurements were performed in triplicate.

3.6.4 Emulsifying properties

Emulsifying activity (EA) and emulsion stability (ES) of Sacha inchi press cake meal and its cyclone-fractionated samples were determined according to the method described by Malomo et al. (2014), as previously reported in our published study (Phonphimai et al., 2025). Briefly, protein dispersions (1%, w/v) were prepared in distilled water. The dispersion was homogenized with vegetable oil at a ratio of 2:1 (v/v) using a high-speed homogenizer for 1 min to form an oil-in-water emulsion. Immediately after homogenization, an aliquot of the emulsion was transferred to a graduated centrifuge tube and centrifuged at 1,300 × g for 5 min to separate the emulsified and aqueous phases. Emulsifying activity was expressed as the percentage of the emulsified layer relative to the total volume. Emulsion stability was evaluated by measuring the emulsified layer after heating the emulsion at 80 °C for 30 min, followed by cooling to room temperature and centrifugation.

Equation

Emulsifying activity (EA)

$$EA(\%) = \frac{V_e}{V_t} \times 100$$

Emulsion stability (ES)

$$ES(\%) = \frac{V_e}{V_{e0}} \times 100$$

where V_e is the volume of the emulsified layer after centrifugation, V_t is the total volume of the emulsion, and V_{e0} is the initial

volume of the emulsified layer before the stability test. All measurements were performed in triplicate, and the results were expressed as percentages.

3.6.5 Foaming properties

Foaming capacity (FC) and foam stability (FS) of Sacha inchi press cake meal and its cyclone-fractionated samples were determined according to the method described by Malomo et al. (2014), as previously reported in our published study (Phonphimai et al., 2025). Briefly, protein dispersions (1%, w/v) were prepared in distilled water and homogenized using a high-speed homogenizer for 2 min to incorporate air and form foam. The total volume of the dispersion was recorded before and immediately after homogenization to determine foaming capacity. Foam stability was evaluated by measuring the foam volume after standing at room temperature for 30 min.

Equation

Foaming capacity (FC)

$$FC(\%) = \frac{V_f - V_i}{V_i} \times 100$$

Foam stability (FS)

$$FS(\%) = \frac{V_{ft}}{V_f} \times 100$$

where V_i is the initial volume of the protein dispersion before homogenization, V_f is the foam volume immediately after homogenization, and V_{ft} is the foam volume after a standing time of 30 min. All measurements were carried out in triplicate, and the results were expressed as percentages.

3.6.6 Least gelation concentration (LGC)

The gelation properties of Sacha inchi press cake meal and its cyclone-fractionated samples were evaluated by determining the least gelation concentration (LGC) according to the method described by Malomo et al. (2014), as previously reported in our published study (Phonphimai et al., 2025). Briefly, protein suspensions were prepared at different concentrations ranging from 2 to 20% (w/v) in distilled water. The suspensions were heated in a boiling water bath at 95 °C for 1 h, followed by rapid cooling

under running tap water. The samples were then allowed to stand at room temperature for 2 h. Gel formation was assessed by inverting the test tubes, and the least gelation concentration was defined as the lowest protein concentration at which the sample did not flow upon inversion. The least gelation concentration was reported as percentage (% w/v), and all measurements were conducted in triplicate.

3.7 *In vitro* Gastrointestinal Digestion

3.7.1 Static *in vitro* gastrointestinal digestion model

In vitro gastrointestinal protein digestion was performed to evaluate the effects of thermal and mechanical processing on protein digestibility of Sacha inchi press-cake samples. This analysis was conducted exclusively for samples obtained in Part 2, namely SP, AC, and CL.

A static *in vitro* digestion model was employed to simulate human gastric and intestinal digestion under controlled laboratory conditions. Sample dispersions were prepared at a concentration of 5% (w/v) in distilled water. The initial pH of the dispersions was adjusted to 2.0 using 1 M HCl to simulate gastric conditions.

Gastric digestion was initiated by the addition of pepsin solution, followed by incubation at 37 °C for 2 h with constant shaking to ensure uniform enzyme–substrate interaction. After completion of the gastric phase, aliquots were collected for analysis. The pH of the remaining digesta was then adjusted to 7.0 using 1 M NaOH to simulate intestinal conditions.

Intestinal digestion was initiated by the addition of pancreatin solution, and the mixture was further incubated at 37 °C for 2 h under continuous agitation. Enzyme concentrations, incubation temperature, and digestion times were kept constant for all samples to allow direct comparison among SP, AC, and CL.

At the end of each digestion phase, enzymatic activity was terminated by heating the samples at 95 °C for 10 min, followed by rapid cooling to room temperature. Digested samples were subsequently centrifuged to separate soluble and insoluble fractions.

3.7.2 Determination of protein digestibility

Protein digestibility was evaluated by quantifying the soluble protein released during the gastric and intestinal digestion phases. The supernatants obtained after centrifugation were analyzed for soluble protein content using the Biuret method.

Protein digestibility was expressed as the percentage of soluble protein relative to the total protein content of the undigested sample. Digestibility values were calculated separately for the gastric digestion stage and the combined gastrointestinal digestion stage. All digestion experiments and analytical measurements were conducted in triplicate, and results were reported as mean values with corresponding standard deviations.

3.8 Food Application and Sensory Evaluation

3.8.1 Preparation of cassava starch–press-cake crackers

Crackers were formulated to evaluate the sensory impact of incorporating autoclaved press-cake meal followed by colloid milling (CL) as a protein-enriched ingredient in a cassava starch–based snack product. Cassava starch was selected as the base material due to its neutral flavor, light color, and common use in expanded snack products. Four cracker formulations were prepared based on different substitution ratios of cassava starch and CL. The formulations consisted of:

(i) Control, containing 100 g of cassava starch without CL;

(ii) 90:10, containing 90 g of cassava starch and 10 g of CL;

(iii) 80:20, containing 80 g of cassava starch and 20 g of CL; and

(iv) 70:30, containing 70 g of cassava starch and 30 g of CL.

For all formulations, sodium chloride (0.5 g) and ground black pepper (0.5 g) were added to the dry ingredients. The dry components were thoroughly mixed to ensure homogeneity prior to dough preparation. Water was gradually added, and the mixture was kneaded until a uniform dough was obtained. The dough was subsequently shaped into thin sheets and cut into standardized cracker pieces. The prepared crackers were fried in hot oil under controlled conditions until full expansion and a crisp texture

were achieved. After frying, the crackers were drained to remove excess oil and allowed to cool to room temperature before sensory evaluation.

3.8.2 Sensory evaluation

Sensory evaluation was conducted to assess consumer acceptance of crackers formulated with varying levels of CL substitution. The study was carried out in accordance with ethical principles for research involving human participants and was approved by the Institutional Review Board of Mae Fah Luang University (Ethics Approval No. COE No. 126/2025, Protocol No. EC 25138–14). Written informed consent was obtained from all participants prior to participation.

A total of 52 untrained panelists, aged 21–60 years, were recruited from students and staff of Mae Fah Luang University, Chiang Rai, Thailand. Sensory attributes evaluated included appearance, aroma, color, texture, flavor, and overall acceptability. All attributes were rated using a 9-point hedonic scale, where 1 indicated "dislike extremely" and 9 indicated "like extremely."

Cracker samples were coded with three-digit random numbers and served to panelists in a randomized order to minimize order bias. Drinking water was provided to cleanse the palate between samples. Sensory evaluation was conducted in a controlled sensory testing environment under standardized lighting and ambient conditions to ensure consistency of panelist responses.

3.9 Statistical Analysis

All experimental data were statistically analyzed to determine significant differences among samples resulting from dry fractionation and thermal–mechanical processing. Results were expressed as mean values ± standard deviation of at least three independent replicates unless otherwise specified.

Data were subjected to one-way analysis of variance (ANOVA) to evaluate the effect of sample type on measured parameters. When significant differences were detected, mean comparisons were performed using Duncan's multiple range test at a significance level of $p < 0.05$.

For sensory evaluation data, mean hedonic scores were analyzed using one-way ANOVA, followed by Duncan's multiple range test to identify significant differences among cracker formulations.

All statistical analyses were conducted using IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview of Experimental Results

This chapter presents the experimental results and their interpretation regarding the effects of dry fractionation (Part 1) and subsequent thermal–mechanical processing (Part 2) on the physicochemical properties, structural characteristics, techno-functional performance, protein digestibility, and food application potential of Sacha inchi press cake.

Results from Part 1 are first used as reference data to establish baseline differences among cyclone-derived fractions (SC-RM and F1–F4), particularly with respect to particle size distribution, compositional variation, and structural heterogeneity. These data provide a comparative framework for understanding how subsequent processing steps influence material properties. Results from Part 1 are used as a conceptual framework to support interpretation of structural and functional changes observed in Part 2.

The primary focus of this chapter is placed on samples obtained from Part 2, namely untreated press cake (SP), autoclaved press cake (AC), and autoclaved press cake followed by colloid milling (CL). Comparative evaluation among these samples is used to elucidate the individual and combined effects of thermal treatment and high-shear mechanical processing on protein redistribution, matrix structure, functional behavior, and *in vitro* gastrointestinal protein digestibility.

The results are discussed in a sequential manner, beginning with processing yield and basic physicochemical characteristics, followed by compositional changes, particle size distribution and microstructural alterations, and molecular and thermal properties. Subsequent sections address changes in techno-functional properties relevant to food systems, protein digestibility under simulated gastrointestinal conditions, and the applicability of CL as a protein-enriched ingredient in starch-based cracker formulations.

Throughout this chapter, experimental observations are interpreted in relation to underlying structural and molecular mechanisms and are compared with recent literature to highlight similarities, differences, and emerging trends. Particular emphasis is placed on linking processing-induced structural modifications to functional performance and digestibility outcomes, providing an integrated understanding of how combined autoclaving and colloid milling enhance the nutritional and technological value of Sacha inchi press cake.

Accordingly, the present chapter emphasizes protein-related structural, functional, and digestibility outcomes, while analyses not directly aligned with these objectives are discussed in the context of overall interpretation.

4.2 Processing Yield and Physicochemical Characteristics

Processing yield and physicochemical characteristics of raw press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid milling (CL) are presented in Table 4.1. Results are discussed in relation to the baseline behavior observed in Part 1 (cyclone-based dry fractionation) to elucidate the effects of subsequent thermal and mechanical processing on structural and compositional constraints inherent to Sacha inchi press cake (Loveday, 2020; Njoroge et al., 2025; Vogelsang-O'Dwyer et al., 2020).

4.2.1 Processing yield and protein recovery

Autoclaving of Sacha inchi press cake resulted in a high production yield of 89.38%, whereas subsequent colloid milling reduced the yield to 83.94%. The relatively high yield after autoclaving indicates that hydrothermal treatment alone did not induce substantial material loss, consistent with previous reports on hydrothermally treated oilseed meals where thermal softening primarily alters matrix structure rather than promoting extensive solubilization (Njoroge et al., 2025).

The additional reduction in yield observed after colloid milling reflects enhanced mechanical disintegration of the thermally softened matrix, leading to the release of fine soluble components during aqueous separation. Similar yield trade-offs

have been reported for oilseed and legume proteins subjected to combined hydrothermal treatment and high-shear or high-pressure homogenization, where increased functional performance is accompanied by partial loss of fine solids (Loveday, 2020; Vogelsang-O'Dwyer et al., 2020).

Protein recovery values exceeded 100% analytical recovery for both AC (105.38%) and CL (112.16%). In this study, "protein recovery (%)" refers to analytical recovery based on Kjeldahl nitrogen determination, whereas "protein recovery yield (%)" represents mass-based recovery relative to initial protein content. These values represent apparent (analytical) protein recovery, rather than true mass balance recovery. Comparable observations have been reported for hydrothermally processed plant protein matrices, where disruption of protein–polysaccharide and protein–lipid associations enhances nitrogen accessibility during Kjeldahl analysis (Loveday, 2020; Vogelsang-O'Dwyer et al., 2020). In the present study, the higher apparent protein recovery observed for CL compared with AC suggests that colloid milling further amplified protein accessibility following thermal softening, rather than increasing absolute protein content. Therefore, protein recovery values exceeding 100% should be interpreted as indicators of enhanced analytical nitrogen accessibility resulting from processing, rather than actual protein generation or mass gain.

In contrast, Part 1 demonstrated that cyclone fractionation preserved production yield but achieved only modest protein enrichment due to intact matrix structure. This comparison highlights that dry fractionation improves protein distribution without substantially altering analytical accessibility, whereas hydrothermal–mechanical processing modifies both structure and detectability. This clarification is critical for accurate interpretation of protein recovery values in physically modified plant protein matrices.

4.2.2 Moisture content, water activity, and pH

Thermal and mechanical processing significantly reduced moisture content from 4.16% (SP) to 2.59% (AC) and 2.32% (CL), accompanied by a decrease in water activity from 0.50 to 0.33 and 0.30, respectively. These reductions indicate improved storage stability and reduced susceptibility to microbial growth.

In Part 1, cyclone fractionation did not significantly alter moisture content or water activity among fractions, confirming that particle classification alone is insufficient to modify hydration-related properties. Similar findings have been reported for dry-fractionated oilseed meals, where matrix integrity remains largely preserved (Phonphimai et al., 2025).

Reductions in water activity following hydrothermal and high-shear processing have been widely reported for oilseed and legume proteins and are attributed to structural loosening and more efficient moisture removal during drying (Loveday, 2020; Vogelsang-O'Dwyer et al., 2020). The present results align with these trends, demonstrating that matrix disruption is required to meaningfully modify water-binding behavior.

A gradual increase in pH from 5.33 (SP) to 5.48 (AC) and 6.09 (CL) was observed. This shift likely reflects protein unfolding and exposure of basic amino acid residues, as well as partial removal of acidic low-molecular-weight compounds during aqueous processing. Similar pH increases have been reported for oilseed and legume proteins subjected to hydrothermal treatment and high-pressure homogenization and are generally attributed to structural rearrangement rather than chemical modification (Etzbach et al., 2024; Ketnawa et al., 2022).

4.2.3 Nutritional composition and protein enrichment

Protein content increased progressively from 57.03% (SP) to 60.09% (AC) and 63.96% (CL), corresponding to an approximate 12% relative increase from SP to CL. This magnitude of enrichment is substantially higher than that observed in Part 1, where cyclone fractionation increased protein content from 53.06% (SC-RM) to 57.12% (F4), representing a relative increase of approximately 8%.

The enhanced protein content observed after autoclaving and colloid milling is attributed to matrix restructuring and component redistribution, rather than selective protein extraction. Hydrothermal treatment weakens protein–fiber and protein–lipid interactions, while subsequent mechanical shear promotes finer dispersion and redistribution of protein within the solid phase. Similar compositional shifts have been reported for oilseed and legume proteins subjected to hydrothermal treatment followed by high-shear or high-pressure homogenization (Etzbach et al., 2024; Njoroge et al., 2025).

Ash content decreased from 5.50% (SP) to 3.89% (AC) and 3.15% (CL), indicating partial leaching of soluble minerals during aqueous processing. This trend is consistent with previous reports on hydrothermally treated oilseed meals, where mineral solubilization accompanies matrix disruption (Njoroge et al., 2025). Carbohydrate content also decreased slightly after colloid milling, suggesting removal or redistribution of non-protein components.

Fat content remained relatively stable (≈5%), indicating that the applied processing conditions primarily targeted structural modification rather than lipid removal. Preservation of lipid content is advantageous for maintaining nutritional value and sensory characteristics in food applications.

4.2.4 Color attributes and processing history

Autoclaving resulted in a decrease in lightness (L*) from 74.97 to 67.21 and an increase in total color difference (ΔE = 8.27), reflecting heat-induced browning reactions and pigment concentration. Similar color changes have been widely reported for hydrothermally treated oilseed and legume proteins and are commonly attributed to Maillard reactions and thermal redistribution of colored components (Loveday, 2020).

Subsequent colloid milling partially mitigated these changes, yielding intermediate L* (70.07) and ΔE (6.47) values. Mechanical disruption may enhance light scattering through finer particle size distribution, partially offsetting thermal darkening. From an application perspective, such moderate color changes are generally acceptable in starch- and cereal-based food systems, particularly when accompanied by improved functional performance.

4.2.5 Implications of processing strategy

Comparison between Part 1 and Part 2 clearly demonstrates that dry fractionation alone improves protein distribution but does not sufficiently modify physicochemical constraints, whereas integration with hydrothermal and mechanical processing effectively enhances protein accessibility, compositional uniformity, and storage stability. Similar conclusions have been drawn in recent reviews on oilseed press cake valorization, which emphasize the superiority of combined hydrothermal–shear strategies over single-step fractionation approaches (Etzbach et al., 2024; Loveday, 2020).

Overall, the processing-induced changes in yield and physicochemical characteristics provide a structural and compositional foundation for the functional and digestibility improvements discussed in subsequent sections.

Table 4.1 Production yield, color attributes, physicochemical characteristics, and nutritional composition of raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL).

Properties	**SP**	**AC**	**CL**
Production yield (%, dry basis)			
Production yield	-	89.38±1.02^{a}	83.94±0.41^{b}
Protein recovery	-	105.38±0.00^{b}	112.16±0.00^{a}
Protein recovery yield	-	94.18±1.08^{a}	93.89±0.63^{a}
Color attributes			
L* (Lightness)	74.97±0.34^{a}	67.21±0.61^{c}	70.07±0.65^{b}
a* (Redness/Greenness)	5.33±0.12^{a}	4.93±0.14^{b}	4.47±0.04^{c}
b* (Yellowness/Blueness)	21.06±0.56^{a}	18.27±0.35^{b}	16.94±0.14^{c}
ΔE	0.58±0.25^{c}	8.27±0.55^{a}	6.47±0.50^{b}
Whiteness	66.85±0.26^{a}	62.14±0.53^{c}	65.32±0.53^{b}
Physicochemical characteristics			
pH	5.33±0.01^{c}	5.48±0.00^{b}	6.09±0.01^{a}
Water activity (a_w)	0.50±0.00^{a}	0.33±0.00^{b}	0.30±0.00^{c}
Nutritional composition (%, dry basis)			

Table 4.1 (Continued)

Properties	**SP**	**AC**	**CL**
Moisture	4.16±0.06[a]	2.59±0.15[b]	2.32±0.09[c]
Protein	57.03±0.08[c]	60.09±0.14[b]	63.96±0.26[a]
Fat	5.33±0.31[b]	5.44±0.04[a]	4.98±0.06[c]
Ash	5.50±0.03[a]	3.89±0.01[b]	3.15±0.03[c]
Carbohydrate	24.79±0.11[a]	24.80±0.13[a]	22.40±0.04[b]
Crude fiber	3.19±0.10[a]	3.19±0.35[a]	3.19±0.65[a]
Total dietary fiber	-	-	24.21±0.19
Total starch	0.21±0.03[a]	0.18±0.04[b]	0.15±0.02[c]

Note Values are expressed as mean ± standard deviation (n = 3).

Mean values with different superscript letters within the same row are significantly different ($p < 0.05$)

SP refers to raw press-cake powder, which represents the unprocessed form of the press-cake.

AC refers to autoclaved press-cake meal, which is the press-cake subjected to autoclaving to enhance protein recovery.

CL refers to autoclaved press-cake meal followed by colloid-milled press-cake m -eal, which refers to the press-cake that has undergone both autoclaving and col loid milling to achieve a finer particle size and improved protein extraction.

ΔE represents the color difference, indicating the overall perceptual difference between two colors.

WI, represents the whiteness index, which reflects the degree of whiteness of the sample, with higher values corresponding to greater whiteness.

In addition to basic physicochemical characteristics, changes in bioactive compounds, antioxidant activities, and antinutritional factors induced by processing are summarized in Table 4.2, as these parameters are known to influence protein functionality and digestibility.

Table 4.2 Bioactive compounds, antioxidant activities, and antinutritional factors of raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL).

Properties	**SP**	**AC**	**CL**
Bioactive compounds			
TPC (as mg GAE/g sample, dry basis)	27.95±0.28^{a}	19.08±0.39^{b}	18.77±0.21^{bc}
TFC (as mg OTE /g sample, dry basis)	0.20±0.01^{a}	0.11±0.01^{b}	0.10±0.01^{bc}
Antioxidant activities			
DPPH (as mg AAE equivalents/g sample, dry basis)	5.42±0.22^{a}	4.14±0.18^{b}	3.76±0.13^{c}
ABTS (as mg AAE equivalents/g sample, dry basis)	4.91±0.14^{a}	3.35±0.17^{b}	2.88±0.11^{c}
FRAP (as µmol $FeSO_4$ equivalents/g sample, dry basis)	122.58±0.73^{a}	118.65±0.91^{b}	110.83±0.44^{c}
Antinutritional factors			
Phytic acid (as mg phytic acid equivalents/g sample, dry basis)	0.40±0.00^{a}	0.33±0.00^{b}	0.26±0.00^{c}
Trypsin inhibitor activity (TIU (per mg sample, dry basis)	0.38±0.00^{a}	0.30±0.02^{b}	0.26±0.01^{c}
Tannin acid (as mg tannic acid equivalents/g sample, dry basis)	0.14±0.01^{a}	0.04±0.00^{b}	0.03±0.01^{bc}
Saponin content (as mg saponin equivalents/g sample, dry basis)	34.50±1.83^{a}	23.98±0.88^{b}	21.41±1.05^{c}

Note Values are expressed as mean ± standard deviation (n = 3).

Mean values with different superscript letters within the same row are significantly different ($p < 0.05$).

SP refers to raw press-cake powder, which represents the unprocessed form of t-

he press-cake.

AC refers to autoclaved press-cake meal, which is the press-cake subjected to autoclaving to enhance protein recovery.

CL refers to autoclaved press-cake meal followed by colloid-milled press-cake meal, which refers to the press-cake that has undergone both autoclaving and colloid milling to achieve a finer particle size and improved protein extraction.

TPC refers to total phenolic content (expressed as mg gallic acid equivalents, GAE, per gram of sample on a dry weight basis);

TFC refers to total flavonoid content (expressed as mg quercetin equivalents, QTE, per gram of sample on a dry weight basis);

DPPH refers to 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (expressed as mg ascorbic acid equivalents, AAE, per gram of sample on a dry weight basis);

ABTS refers to 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity (expressed as mg ascorbic acid equivalents, AAE, per gram of sample on a dry weight basis);

FRAP refers to ferric reducing antioxidant power (expressed as μmol $FeSO_4$ equivalents per gram of sample on a dry weight basis);

TIU refers to trypsin inhibitor units.

4.3 Proximate Composition and Protein Distribution

The proximate composition and protein distribution of Sacha inchi press cake were evaluated to clarify how different processing strategies influence nutrient redistribution and protein enrichment mechanisms. Results from Part 1 (cyclone-based dry fractionation) are first discussed to establish baseline compositional trends associated with particle size–dependent separation, followed by comparison with Part 2 (autoclaving and colloid milling) to elucidate how hydrothermal and mechanical processing induce deeper structural modification and protein redistribution beyond physical fractionation alone (Loveday, 2020; Njoroge et al., 2025; Phonphimai et al., 2025).

4.3.1 Proximate composition of cyclone-fractionated samples (Part 1)

The proximate composition of cyclone-fractionated samples (SC-RM and F1–F4) is presented in Table 4.3, while production yield and particle characteristics are summarized in Table 4.3. Dry fractionation resulted in a gradual increase in protein content from 53.06% in SC-RM to 57.12% in F4, corresponding to an approximate 8% relative enrichment. This trend clearly demonstrates that cyclone separation effectively redistributed protein-rich particles toward finer fractions.

However, the magnitude of protein enrichment remained moderate. Moisture content, pH, and water activity did not differ significantly among fractions, indicating that dry fractionation primarily altered particle distribution without inducing substantial physicochemical modification. Similar observations have been reported for dry-fractionated oilseed and legume materials, where intact cellular structures and residual oil limit the extent of protein liberation despite effective particle size classification (Njoroge et al., 2025).

Carbohydrate content decreased progressively from 33.93% in SC-RM to 30.41% in F4, suggesting partial segregation of carbohydrate-rich components into coarser fractions. Ash content decreased slightly after fractionation, whereas fat content remained relatively constant across fractions. These results indicate that cyclone fractionation mainly induces physical redistribution of macronutrients rather than compositional modification through matrix disruption.

Overall, Part 1 confirms that dry fractionation is an effective, low-impact strategy for modest protein enrichment with minimal compositional alteration, providing a useful baseline for subsequent processing.

4.3.2 Protein redistribution mechanisms: Part 1 versus Part 2

In contrast to Part 1, thermal and mechanical processing (Part 2) resulted in more pronounced changes in protein distribution, as reflected in Table 4.1. Protein content increased from 57.03% in SP to 60.09% in AC and further to 63.96% in CL, representing an approximate 12% relative increase from SP to CL. This level of enrichment exceeds that achieved through cyclone fractionation alone.

The enhanced protein content observed after autoclaving and colloid milling cannot be attributed solely to particle size effects. Instead, it reflects matrix restructuring and protein accessibility enhancement. Hydrothermal treatment disrupts hydrogen

bonding and weakens protein–polysaccharide and protein–lipid interactions, facilitating partial protein unfolding and redistribution. Subsequent colloid milling applies intense shear forces that further break down softened agglomerates, promoting more homogeneous protein dispersion within the solid phase.

Similar redistribution mechanisms have been reported for oilseed and legume proteins subjected to hydrothermal processing combined with high-shear or high-pressure homogenization, where protein enrichment arises from structural modification rather than selective extraction (Etzbach et al., 2024; Loveday, 2020). The present findings therefore support the hypothesis that structural accessibility, rather than purity alone, governs protein enrichment efficiency in press-cake matrices.

4.3.3 Carbohydrate, ash, and fat redistribution

Carbohydrate content showed limited variation among cyclone fractions in Part 1 but decreased noticeably following colloid milling in Part 2. This contrast highlights the limitation of dry fractionation in modifying matrix composition. The reduction in carbohydrate content after hydrothermal–mechanical processing suggests partial removal or redistribution of non-protein polysaccharides during aqueous processing.

Ash content decreased from 5.50% in SP to 3.15% in CL, indicating leaching of soluble mineral components during autoclaving and subsequent washing steps. Comparable mineral losses have been reported for hydrothermally treated oilseed meals and protein-rich by-products and are attributed to diffusion of inorganic ions into the aqueous phase once the matrix integrity is weakened (Njoroge et al., 2025).

Fat content remained relatively stable across all treatments, with values close to 5%, indicating that the applied processing conditions primarily targeted structural modification rather than lipid extraction. Preservation of lipid content is advantageous for maintaining nutritional value and sensory attributes in food applications and contrasts with solvent-based protein isolation approaches, which often result in extensive lipid removal.

4.3.4 Implications for protein quality and functionality

Amino acid composition of cyclone-fractionated samples (SC-RM and F3) presented in Tables 4.5 and 4.6 indicates that dry fractionation did not substantially alter amino acid profiles or protein quality indices. Essential-to-total amino acid ratios (E/T)

and amino acid scores (AAS) remained largely unchanged, confirming that cyclone separation redistributed protein quantity without affecting intrinsic protein quality.

Similarly, the amino acid profile of CL (Table11.) demonstrates that thermal and mechanical processing preserved amino acid composition while enhancing protein accessibility. The maintenance of essential amino acid balance alongside increased protein content suggests that processing-induced enrichment did not compromise nutritional quality.

Collectively, these results indicate that Part 1 processing primarily induces quantitative redistribution of protein, whereas Part 2 processing enables qualitative enhancement of accessibility and functional potential without altering amino acid integrity. This distinction provides a critical foundation for interpreting the functional and digestibility improvements discussed in subsequent sections.

Table 4.3 Production yield, particle size distribution and color attributes, of Sacha inchi press cake meal (SC-RM) and fractions 1-4 (F1-F4) fractionated from the cyclone fractionation.

Properties	SC-RM	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Weight (g)	3,000±0.00^{a}	1,002.67±3.06^{b}	659.33±2.0^{d}	976.67±2.08^{c}	267.33±2.08^{e}
Production yield (%)	-	33.42±0.10^{a}	21.98±0.07^{c}	32.56±0.07^{b}	8.91±0.07^{d}
Bulk density (g/cm³)	1.41±0.5^{bc}	1.43±0.00^{a}	1.42±0.00^{b}	1.40±0.00^{d}	1.38±0.00^{e}
Particle size distribution (μm)					
D10	32.78±0.89^{c}	29.87±0.50^{d}	32.08±0.20^{c}	41.82 ±0.29^{a}	37.36±0.25^{b}
D50	272.83±0.68^{a}	204.38±5.02^{b}	169.35±0.80^{c}	135.15±5.26^{d}	127.52±5.38^{d}
D90	738.58±0.67^{a}	713.61±12.70^{b}	537.31±15.03^{c}	497.55±8.11^{d}	421.12±4.49^{e}
Color attributes					
L* (Lightness)	81.51±0.16^{a}	81.50±0.08^{a}	81.52±0.06^{a}	81.51±0.20^{a}	81.50±0.0^{ba}
a*(Redness/ Greenness)	2.13±0.09^{a}	2.18±0.05^{a}	2.17±0.08^{a}	2.22±0.05^{a}	2.17±0.08^{a}
b*(Yellowness/ Blueness)	16.20±0.12^{a}	16.14±0.06^{a}	16.10±0.10^{a}	16.09±0.10^{a}	16.18±0.09^{a}
ΔE	0.19±0.07^{a}	0.13±0.06^{c}	0.15±0.08^{b}	0.15±0.07^{b}	0.14±0.04^{bc}
Whiteness	75.32±0.10^{a}	75.35±0.08^{a}	75.40±0.07^{a}	75.39±0.16^{a}	75.33±0.08^{a}

Note All values are means ± SD of three replicates. Mean values with different letters in each row are significantly different ($p < 0.05$).

Table 4.4 Physicochemical characteristics, and nutritional composition of Sacha Inchi press cake meal (SC-RM) and fractions 1-4 (F1-F4) fractionated from the c-yclone fractionation.

Properties	**SC-RM**	**Fraction 1**	**Fraction 2**	**Fraction 3**	**Fraction 4**
Physicochemical characteristics					
pH	6.26±0.07^{b}	6.24±0.04^{d}	6.25±0.04^{c}	6.24±0.05^{d}	6.27±0.04^{a}
a_w	0.20±0.01^{a}	0.20±0.01^{a}	0.25±0.01^{a}	0.26±0.01^{a}	0.27±0.00^{a}
Nutritional composition (%, dry basis)					
Moisture	3.05±0.02^{a}	3.05±0.03^{a}	3.04±0.05^{a}	3.05±0.04^{a}	3.05±0.04^{a}
Protein	53.06±0.08^{d}	54.29±0.42^{c}	55.54±0.16^{b}	56.62±0.34^{a}	57.12±0.29^{a}
Fat	4.31±0.15^{a}	4.200.03^{b}	4.22±0.01^{b}	4.24±0.04^{b}	4.24±0.01^{b}
Ash	5.51±0.27^{a}	5.02±0.02^{b}	5.02±0.06^{b}	5.03±0.09^{b}	5.04±0.19^{b}
Carbohydrate	33.93±0.33^{a}	33.30±0.20^{b}	32.04±0.10^{c}	30.92±0.12^{d}	30.41±0.10^{e}
Crude fiber	0.14±0.06^{a}	0.14±0.01^{a}	0.14±0.01^{a}	0.14±0.01^{a}	0.14±0.01^{a}
Total starch	0.42±0.00^{a}	0.36±0.00^{b}	0.32±0.00^{c}	0.28±0.00^{d}	0.23±00^{e}

Note All values are means ± SD of three replicates. Mean values with different letters in each rows are significantly different ($p < 0.05$).

Table 4.5 Essential and non-essential amino acid profiles of SC-RM and Fraction 3 (F3) in comparison with FAO/WHO reference values.

Amino acids (mg/g protein)	SC-RM	F3	FAO/WHO reference pattern			FAO/WHO Suggested pattern	
			Soybean	Egg	PROVISIONAL Suggested pattern	Adults	School children 10-12 years
Essential amino acids (EAA)							
Histidine (His)	4.63±0.19^{ns}	4.64±0.22^{ns}	25.30	22	n/r	n/r	n/r
Isoleucine (IIe)	11.60±0.44^{ns}	11.26±0.90^{ns}	45.40	54	40	18	37
Leucine (Leu)	15.34±0.59^{ns}	14.90±1.18^{ns}	77.80	86	70	25	56
Lysine (Lys)	10.68±0.43^{ns}	9.14±1.20^{ns}	63.80	70	55	22	75
Methionine (Met)	0.53±0.05^{ns}	0.67±0.17^{ns}	12.60	n/r	n/r	n/r	n/r
Phenylalanine (Phe)	5.51±0.20^{ns}	5.66±0.22^{ns}	49.40	n/r	n/r	n/r	n/r
Threonine (Thr)	9.28±0.29^{ns}	8.74±0.63^{ns}	38.60	47	40	13	44
Tryptophan (Trp)	n/a	n/a	12.80	17	10	7	5
Valine (Val)	13.91±0.52^{ns}	13.42±1.10^{ns}	48.00	66	50	18	41
Met+Cys	13.91±0.52^{ns}	13.42±1.10^{ns}	25.90	57	35	24	34
Phe+Tyr	17.10±0.64^{ns}	16.91±1.11^{ns}	80.80	93	60	25	34
Total essential amino acids (EAA)	71.47±2.67^{ns}	68.45±5.54^{ns}	373.70	362.00	265.00	102.50	257.60
Non-essential amino acids (NEAA)							
Alanine (Ala)	8.01±0.40^{ns}	7.70±0.11^{ns}	42.00	n/r	n/r	n/r	n/r
Arginine (Arg)	24.77±0.84^{ns}	24.01±1.45^{ns}	72.30	n/r	n/r	n/r	n/r

Table 4.5 (Continued)

Amino acids (mg/g protein)	SC-RM	F3	FAO/WHO reference pattern			FAO/WHO Suggested pattern	
			Soybean	Egg	PROVISIONAL Suggested pattern	Adults	School children 10-12 years
Aspartic acid (Asp)	25.02±1.38^{ns}	22.27±2.43^{ns}	117.00	n/r	n/r	n/r	n/r
Cysteine (Cys)	1.43±0.20^{ns}	1.43±0.24^{ns}	13.30	n/r	n/r	n/r	n/r
Glutamic acid (Glu)	29.96±1.38^{ns}	25.52±2.53^{ns}	187.00	n/r	n/r	n/r	n/r
Glycine (Gly)	24.09±0.86^{ns}	22.69±1.49^{ns}	41.80	n/r	n/r	n/r	n/r
Proline (Pro)	8.67±0.33^{ns}	7.90±0.69^{ns}	54.90	n/r	n/r	n/r	n/r
Serine (Ser)	11.45±0.28^{ns}	10.87±0.48^{ns}	51.20	n/r	n/r	n/r	n/r
Tyrosine (Tyr)	11.07±0.34^{ns}	10.69±0.51^{ns}	31.40	n/r	n/r	n/r	n/r
Total non-essential amino acids (NEAA)	144.47±5.98^{ns}	133.08±9.84 ^{ns}	579.50				
Total amino acids (TAA)	215.94±8.65^{ns}	201.52±15.39 ^{ns}	953.20				
EAA/TAA	0.33±0.00^{ns}	0.34±0.00 ^{ns}	0.39				
AAA	21.21±073^{ns}	20.99±0.95 ^{ns}	118.90				
AXA	31.83±1.26^{ns}	30.99±1.96 ^{ns}	199.70				
HAA	70.55±2.83^{ns}	67.98±4.77 ^{ns}	309.80				
SAA	1.96±0.23 ^{ns}	2.10±0.41 ^{ns}	25.90				

Note *Tryptophan (Trp) was not determined in this study due to analytical limitations, and related calculations (e.g., AAA and AXA) were performed excluding Trp (n/a) refers to not available data: (n/r) refers to no report from the reference sources

AAA = aromatic amino acids: Phe, Trp, Tyr and His;

AXA = antioxidant amino acids: Trp, Tyr, Met, Cys, His, Phe and Pro.

HAA = hydrophobic amino acids: Ala, Val, Ile, Leu, Tyr, Phe, Trp, Pro, Met and Cys

SAA = sulphur amino acids: Met, Cys

Amino acid composition used to calculate the amino acid score (AAS) using egg protein reference pattern ((Millward, 2012b).

FAO/WHO reference amino acid pattern required for adults and school children 10-12 years as defined by FAO/WHO Expert Con sultation (Millward, 2012a).

**All values are expressed as means ± SD from three replicates. Means with different letters in each row are significantly different ($p < 0.05$), while "ns" indicates no significant difference. The FAO/WHO reference values are provided for reference but were not included in the statistical analysis.

Table 4.6 Nutritional profile and protein quality of amino acid compositions of Sacha Inchi press cake meal (SC-RM) and fraction 3 (F3) fractionated from the Cyclone fractionation.

Parameters	**SC-RM**	**F3**	**FAO/WHO reference pattern**
			Soybean
E/T (%)	33.10±0.09^{b}	33.96±0.17^{a}	39.20
EAA (%)	19.74±0.74^{ns}	18.91±1.53 ^{ns}	103.23
EAAI	19.73±0.74^{ns}	18.88±1.51^{ns}	81.15
AAS	0.03±0.00^{ns}	0.04±0.01^{ns}	0.74
PER1	5.90±0.25^{ns}	5.74±0.51^{ns}	32.21
PER2	5.33±0.23^{ns}	5.18±0.48^{ns}	31.56
PER3	0.91±0.19^{ns}	0.99±0.55^{ns}	40.05
p-BV	9.81±0.81^{ns}	8.87±1.65^{ns}	76.75
NI	10.47±0.39^{ns}	10.02±0.80^{ns}	32.46

Note E/T (%) = the proportion of essential amino acids (EAAs) to the total amino acids of protein; AAS is calculated using soybean as defined by FAO/WHO Expert Consultation (1991) (World Health, 1991) (World Health, 1991) (FAO/WHO Expert Consultation, 1991).

Met = first limiting amino acid in this study; PER, Protein efficiency ratio; EAAI, essential amino acid index, p-BV, predicted biological value, NI, nutritional in dex.

*All values are expressed as means ± SD from three replicates. Means with different letters in each row are significantly different ($p < 0.05$), while "ns" indicates no significant difference. The FAO/WHO reference values are provided for reference but were not included in the statistical analysis.

Table 4.7 Amino acid composition of autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) compared with FAO/-WHO reference patterns and selected protein sources.

Amino acids (mg/g protein)	CL	FAO/WHO reference pattern			FAO/WHO Suggested pattern	
		Soybean	Egg	PROVISIONAL Suggested pat-tern	Adults	School children 10-12 years
Essential amino acids (EAA)						
Histidine (His)	13.60	25.30	22	n/r	n/r	n/r
Isoleucine (IIe)	25.56	45.40	54	40	18	37
Leucine (Leu)	43.01	77.80	86	70	25	56
Lysine (Lys)	25.72	63.80	70	55	22	75
Methionine (Met)	6.98	12.60	n/r	n/r	n/r	n/r
Phenylalanine (Phe)	16.64	49.40	n/r	n/r	n/r	n/r
Threonine (Thr)	25.66	38.60	47	40	13	44
Tryptophan (Trp)	24.14	12.80	17	10	7	5
Valine (Val)	37.82	48.00	66	50	18	41
Met+Cys	23.06	25.90	57	35	24	34

Table 4.7 (Continued)

Amino acids (mg/g protein)	CL	FAO/WHO reference pattern			FAO/WHO Suggested pattern	
		Soybean	Egg	PROVISIONAL Suggested pattern	Adults	School children 10-12 years
Phe+Tyr	52.13	80.80	93	60	25	34
Total essential amino acids	219.14	373.70	362.00	265.00	102.50	257.60
Non-essential amino acids (NEAA)						
Alanine (Ala)	17.71	42.00	n/r	n/r	n/r	n/r
Arginine (Arg)	57.66	72.30	n/r	n/r	n/r	n/r
Aspartic acid (Asp)	82.31	117.00	n/r	n/r	n/r	n/r
Cysteine (Cys)	16.08	13.30	n/r	n/r	n/r	n/r
Glutamic acid (Glu)	70.04	187.00	n/r	n/r	n/r	n/r
Glycine (Gly)	71.67	41.80	n/r	n/r	n/r	n/r
Proline (Pro)	24.40	54.90	n/r	n/r	n/r	n/r
Serine (Ser)	41.26	51.20	n/r	n/r	n/r	n/r
Tyrosine (Tyr)	35.49	31.40	n/r	n/r	n/r	n/r
Total non-essential amino acids	416.62	579.50				
Total amino acids (TAA)	635.76	953.20				

Table 4.7 (Continued)

Amino acids (mg/g protein)	CL	FAO/WHO reference pattern			FAO/WHO Suggested pattern	
		Soybean	Egg	PROVISIONAL Suggested pattern	Adults	School children 10-12 years
EAA/TAA	0.34	0.39				
AAA	52.13	118.90				
AXA	113.19	199.70				
HAA	223.68	309.80				
SAA	23.06	25.90				

Note CL refers to autoclaved press-cake meal followed by colloid-milled press-cake meal.

Values are expressed as mg/g protein.

FAO/WHO reference patterns are based on the amino acid requirements for adults and school-age children (10–12 years).

EAA: Essential amino acids; NEAA: Non-essential amino acids; TAA: Total amino acids.

AAA: Aromatic amino acids (Phe + Tyr); SAA: Sulfur-containing amino acids (Met + Cys); HAA: Hydrophobic amino acids; AXA: Acidic amino acids.

n/r indicates not reporte.

4.4 Particle Size Distribution and Microstructural Changes

Particle size distribution and microstructural characteristics of Sacha inchi press cake subjected to different processing strategies are presented in Figures 6–8 This section provides physical evidence supporting the compositional and functional changes discussed in Sections 4.2 and 4.3 and elucidates how hydrothermal and mechanical processing alter matrix architecture beyond particle size classification alone.

4.4.1 Particle size distribution: dry fractionation versus hydrothermal–mechanical processing

Cyclone-based dry fractionation (Part 1) effectively separated press-cake particles according to size, as reflected by a progressive reduction in mean particle diameter from SC-RM to finer fractions (F1–F4). However, despite this size-based separation, protein enrichment remained moderate (Section 4.3), indicating that particle size reduction alone was insufficient to disrupt protein–matrix associations.

In contrast, thermal and mechanical processing in Part 2 induced a more pronounced shift in particle size distribution. Autoclaving resulted in partial softening and swelling of the press-cake matrix, which facilitated subsequent size reduction during colloid milling. Colloid milling produced a substantially finer and more homogeneous particle size distribution compared with both SP and cyclone-fractionated samples. Similar reductions in particle size have been reported for oilseed and legume proteins subjected to hydrothermal treatment followed by high-shear or high-pressure homogenization, where thermal softening enhances shear efficiency and promotes extensive particle breakdown.

Importantly, the observed particle size reduction in CL was accompanied by greater protein enrichment and improved functional properties (Sections 4.3 and 4.6), suggesting that effective particle size reduction must be coupled with matrix disruption to meaningfully influence protein accessibility.

4.4.2 Microstructural changes induced by processing

Scanning electron microscopy images (Figure 4.2) reveal distinct differences in microstructure among samples. The raw press-cake powder (SP) exhibited irregular, compact agglomerates with intact fibrous structures and limited porosity. Similar dense microstructures have been reported for untreated oilseed press cakes and are known to restrict water penetration and enzyme accessibility.

Cyclone-fractionated samples retained comparable microstructural features, with only minor differences in particle morphology attributable to size classification. The persistence of intact cell wall fragments and compact protein–fiber assemblies explains the limited improvements in protein accessibility observed in Part 1.

In contrast, autoclaving induced noticeable structural loosening, characterized by surface roughening, fissures, and partial collapse of fibrous networks. These changes reflect thermal denaturation and weakening of non-covalent interactions within the matrix. Subsequent colloid milling further amplified microstructural disruption, producing fragmented particles with increased surface roughness, higher porosity, and reduced agglomeration. Such microstructural features are characteristic of hydrothermal–shear-processed plant proteins and are associated with enhanced hydration and interaction with other food components.

4.4.3 Structure–function implications

The combined effects of reduced particle size and altered microstructure provide a mechanistic explanation for the improved physicochemical and functional properties observed in CL. Increased surface area resulting from finer particles enhances water–protein and oil–protein interactions, contributing to higher water- and oil-holding capacities. Simultaneously, disruption of compact matrix structures improves diffusion pathways for water and digestive enzymes, facilitating functional performance and digestibility.

Notably, the present results indicate that microstructural disruption, rather than particle size reduction alone, is the critical determinant of processing effectiveness. This distinction explains why cyclone fractionation, despite achieving particle size separation, produced limited improvements compared with hydrothermal–mechanical processing. Similar conclusions have been emphasized in recent studies on plant protein

modification, which highlight the importance of matrix architecture in governing functional behavior.

4.4.4 Integration with compositional changes

Microstructural observations are consistent with the compositional trends discussed in Section 4.3. Redistribution of protein and reduction of carbohydrate and ash contents in CL can be attributed to structural loosening and component diffusion enabled by hydrothermal and mechanical processing. The preservation of lipid content further suggests that processing selectively targeted matrix architecture without inducing extensive lipid extraction.

Collectively, particle size distribution and microstructural analyses provide compelling physical evidence that sequential autoclaving and colloid milling effectively transform the press-cake matrix from a dense, poorly accessible structure into a more open and homogeneous system. These structural modifications underpin the enhanced functional and digestibility properties discussed in subsequent sections.

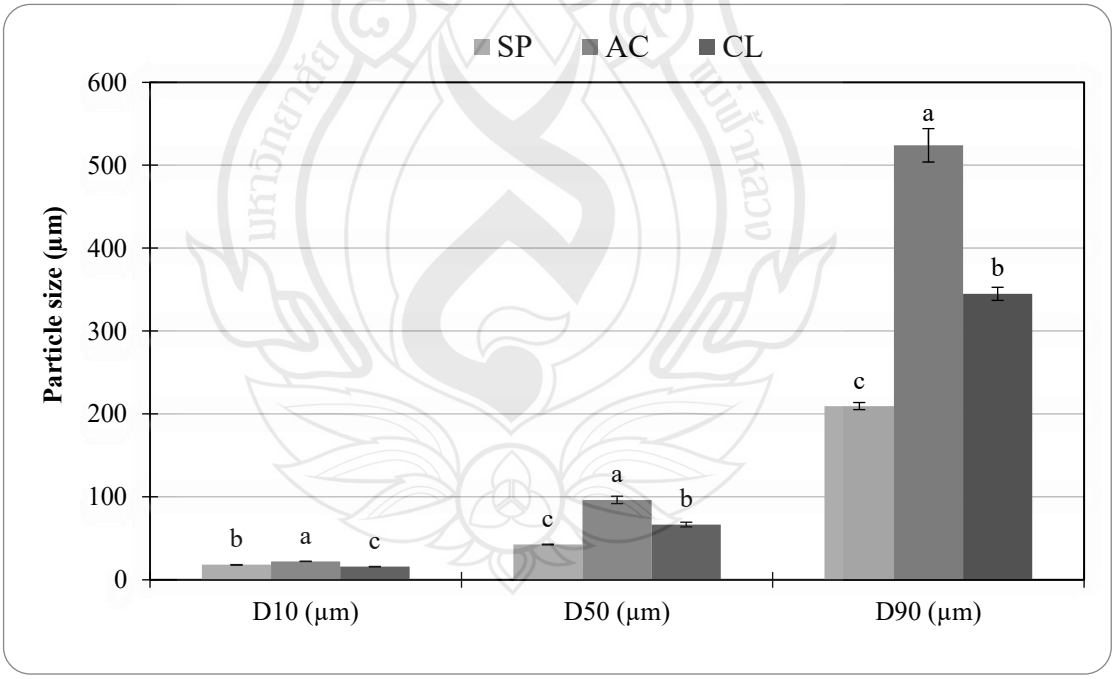


Figure 4.1 Particle size distribution (PSA) of raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL).

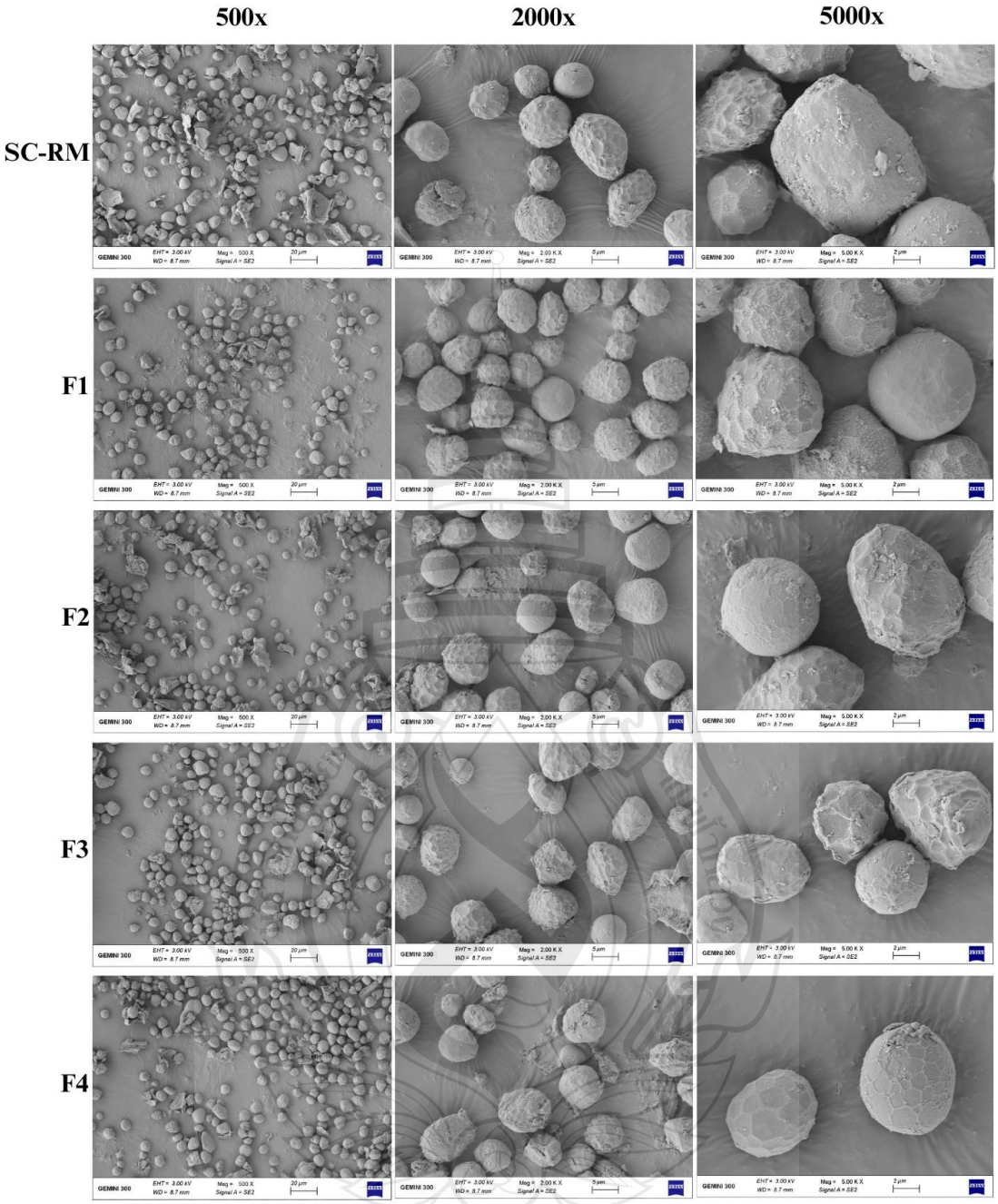


Figure 4.2 SEM microstructural analysis of cyclone-fractionated sacha inchi press cake meal (SC-RM) and fractions (F1–F4) at 500×, 2,000×, and 5,000×.

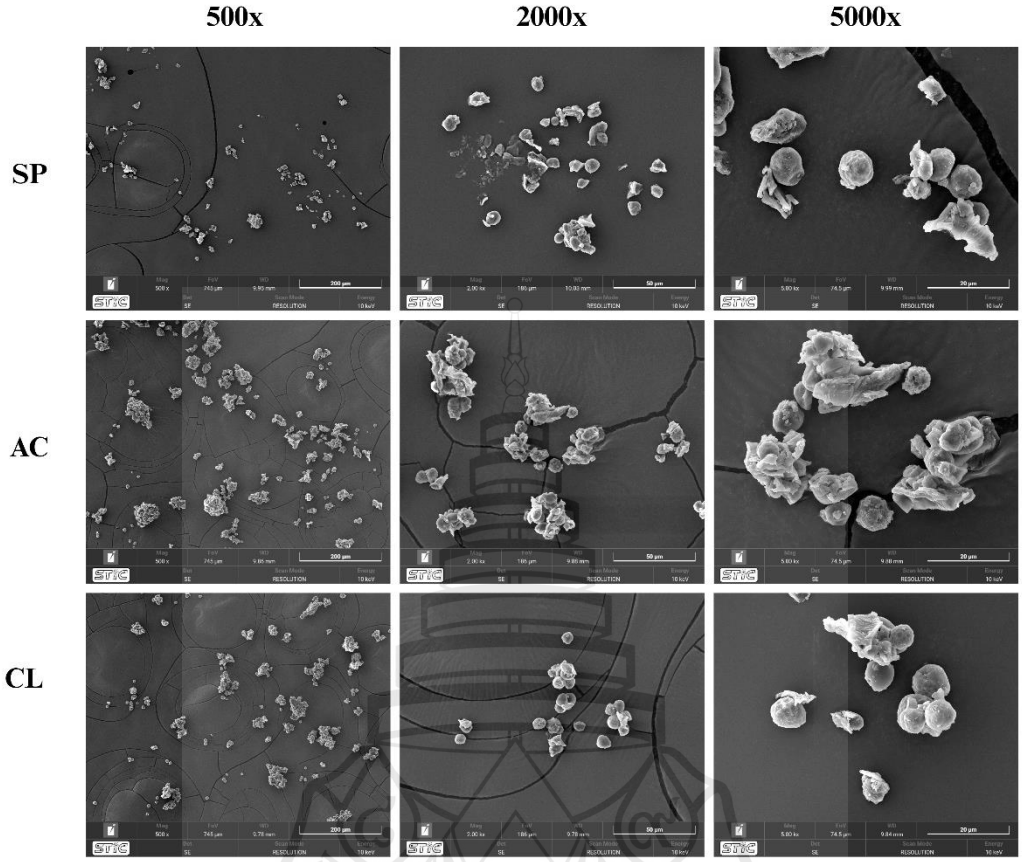


Figure 4.3 Comparative SEM microstructures of of raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal fol lowed by colloid-milled press-cake meal (CL) at 500×, 2,000×, and 5,000×.

4.5 Molecular and Thermal Characteristics (FTIR and DSC/TGA)

All Molecular and thermal characteristics of Sacha inchi press cake subjected to different processing strategies were investigated using Fourier transform infrared spectroscopy (FTIR) and thermal analyses (DSC and TGA). These analyses provide molecular- and energy-level evidence supporting the compositional redistribution (Section 4.3) and microstructural disruption (Section 4.4) induced by autoclaving and colloid milling.

4.5.1 FTIR analysis: molecular interactions and protein structural rearrangement

FTIR spectra of raw press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid milling (CL) are presented in Figure 4.5.a. All samples exhibited characteristic absorption bands typical of plant-based protein–polysaccharide matrices, including broad O–H and N–H stretching vibrations (3200–3400 cm^{-1}), C–H stretching vibrations (2800–3000 cm^{-1}), and prominent amide I (≈1650 cm^{-1}) and amide II (≈1540 cm^{-1}) bands associated with protein secondary structures.

Compared with SP, AC showed noticeable changes in the intensity and shape of the amide I and amide II bands, indicating partial protein denaturation and rearrangement of secondary structures induced by hydrothermal treatment. Heat and pressure applied during autoclaving disrupt hydrogen bonding and hydrophobic interactions stabilizing native protein conformations, resulting in partial unfolding and increased molecular mobility. Similar FTIR modifications have been reported for oilseed and legume proteins subjected to hydrothermal processing, where weakening of protein–matrix interactionsenhances protein accessibility without extensive chemical modification.

Following colloid milling, CL exhibited further band broadening and changes in relative intensity in the amide regions, suggesting increased molecular heterogeneity and disruption of residual protein–polysaccharide associations. High-shear forces generated during colloid milling are known to break non-covalent interactions within thermally softened matrices, promoting exposure of functional protein groups. These molecular-level changes are consistent with the enhanced protein redistribution and functional performance observed in Sections 4.3 and 4.6.

In addition, reduced intensity in the carbohydrate-associated region (1000–1150 cm^{-1}) was observed for CL, supporting partial disruption or redistribution of polysaccharide components. This observation aligns with the decrease in carbohydrate content and ash discussed in Section 4.3, confirming that processing-induced changes occur at both compositional and molecular levels.

4.5.2 DSC analysis: thermal transitions and protein stability

DSC thermograms of SP, AC, and CL are shown in Figure 4.5.b, with key thermal parameters summarized in Table 4.9. The raw press-cake powder exhibited an

endothermic transition associated with protein denaturation, reflecting the presence of partially ordered protein structures within the intact matrix.

After autoclaving, the denaturation peak shifted slightly and exhibited reduced enthalpy (ΔH), indicating partial loss of ordered protein structures and decreased thermal stability. This reduction in denaturation enthalpy reflects prior thermal unfolding during autoclaving, which diminishes the cooperative energy required for further structural transition. Similar reductions in ΔH have been reported for hydrothermally treated oilseed and legume proteins and are commonly interpreted as evidence of protein predenaturation.

In CL, the denaturation peak was further broadened and ΔH decreased to near-zero values, suggesting extensive structural disorder and a predominance of amorphous protein domains. Mechanical shear following hydrothermal softening likely disrupted remaining ordered regions, resulting in a protein system with reduced thermal cooperativity. This progressive decrease in thermal stability supports the conclusion that combined autoclaving and colloid milling induce deeper structural modification than thermal treatment alone.

4.5.3 TGA analysis: thermal degradation behavior and matrix integrity

Thermogravimetric profiles of SP, AC, and CL are presented in Figure 4.5.c–d, with degradation parameters summarized in Table 4.9. All samples exhibited multi-stage weight loss patterns characteristic of plant-based matrices, including initial moisture evaporation (Region I), decomposition of organic components (Region II), and residual char formation (Region III).

Compared with SP, AC exhibited a slight shift in degradation temperatures and reduced weight loss in Region II, indicating altered matrix composition and partial breakdown of thermally labile components during autoclaving. In CL, degradation occurred more gradually, with lower weight loss in Region II and increased residual char formation. This behavior suggests a more homogeneous and disrupted matrix structure, consistent with increased molecular disorder and redistribution of components following combined hydrothermal and mechanical processing.

Similar TGA trends have been reported for mechanically and hydrothermally modified plant protein materials, where matrix disruption alters thermal degradation

pathways and reduces structural resistance to heat. The observed changes in degradation behavior further support the conclusion that processing modified the internal organization of the press-cake matrix rather than merely reducing particle size.

4.5.4 Integrated interpretation and relevance to functional performance

Collectively, FTIR, DSC, and TGA analyses provide strong molecular and thermal evidence that sequential autoclaving and colloid milling fundamentally alter the structural organization of Sacha inchi press cake. FTIR results confirm disruption of protein secondary structures and protein–polysaccharide interactions, while DSC and TGA analyses demonstrate reduced thermal stability and increased molecular disorder.

These molecular-level changes corroborate the compositional redistribution (Section 4.3) and microstructural disruption (Section 4.4) observed after processing and provide a mechanistic basis for the enhanced techno-functional properties and protein digestibility discussed in subsequent sections. Importantly, the combined analytical evidence confirms that improvements observed in Part 2 arise from true structural and molecular modification, rather than superficial particle size effects alone. These molecular and thermal modifications provide a mechanistic basis for the enhanced water-holding, oil-holding, and interfacial properties discussed in the following section.

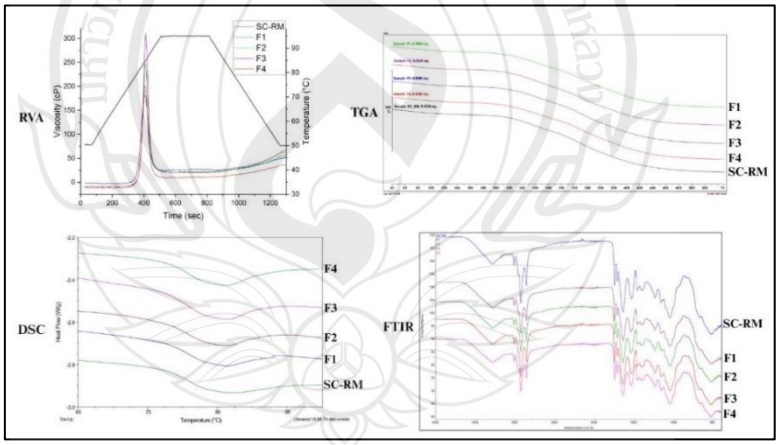


Figure 4.4 Physicochemical characterization of SC-RM and fractions (F1–F4): Analysis of pasting properties (RVA), thermal transitions (DSC), decomposition behavior (TGA), and molecular structure and functional groups by Fourier Transform Infrared Spectroscopy (FTIR) of SC-RM and its individual fractions (F1–F4).

Table 4.8 Viscosity and pasting and thermogravimetric properties of Sacha Inchi press cake meal (SC-RM) and fractions 1-4 (F1-F4) fractionated from the cyclone fractionation.

Properties	SC-RM	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Viscosity and pasting properties by RVA					
Peak (cP)	182.60±0.89^{e}	257.40±1.82^{c}	279.00±1.22^{a}	265.80±1.79^{b}	201.80±1.10^{d}
Trough (cP)	22.40±1.67^{a}	21.20±1.10^{a}	20.80±0.45^{ab}	15.80±1.10^{c}	9.40±1.14^{d}
Final Viscosity (cP)	33.00±1.73^{d}	55.60±1.34^{a}	53.40±1.52^{b}	49.20±1.79^{c}	49.60±0.89^{c}
Setback (cP)	25.20±1.10^{d}	40.40±0.89^{b}	42.60±0.55^{a}	35.60±1.34^{c}	24.20±0.45^{d}
Peak Time (min.)	6.83±0.06^{a}	6.81±0.03^{a}	6.84±0.04^{a}	6.81±0.03^{a}	6.81±0.03^{a}
Pasting Temp (°C)	81.59±0.17^{a}	82.31±0.02^{a}	82.12±0.16^{a}	82.26±0.02^{a}	82.32±0.03^{a}
Thermal properties by DSC					
Endothermic peak (°C)	78.07	78.02	77.08	77.57	78.19
Enthalpy of Transition, ΔH (J/g)	5.79	4.38	5.70	5.30	3.73
Onset Temperature, To (°C)	86.14	85.74	85.64	85.49	85.37
End Temperature, Te (°C)	96.83	92.79	91.85	91.30	91.01

Thermogravimetric parameters by TGA	**Tmax (°C)**	**% weight loss**	**Tmax (°C)**	**% weight loss**	**Tmax (°C)**	**% weight loss**	**Tmax (°C)**	**% weight loss**	**Tmax (°C)**	**% weight loss**
Region I (40-150°C)	79.40	5.16	76.50	5.40	76.50	5.00	76.50	5.00	72.50	5.90
Region II (170-280°C)	226.50	17.73	201.30	17.38	200.50	20.44	317.50	27.36	199.83	18.25
Region III (300-500°C)	433.67	52.09	413.50	50.43	380.50	46.84	437.50	43.17	426.50	51.33
% Residual char	23.91		25.67		26.59		23.33		24.22	

Note *For viscosity and pasting properties by RVA, all values are means ± SD of three replicates. Mean values with different letters in each row are significantly different ($p < 0.05$).

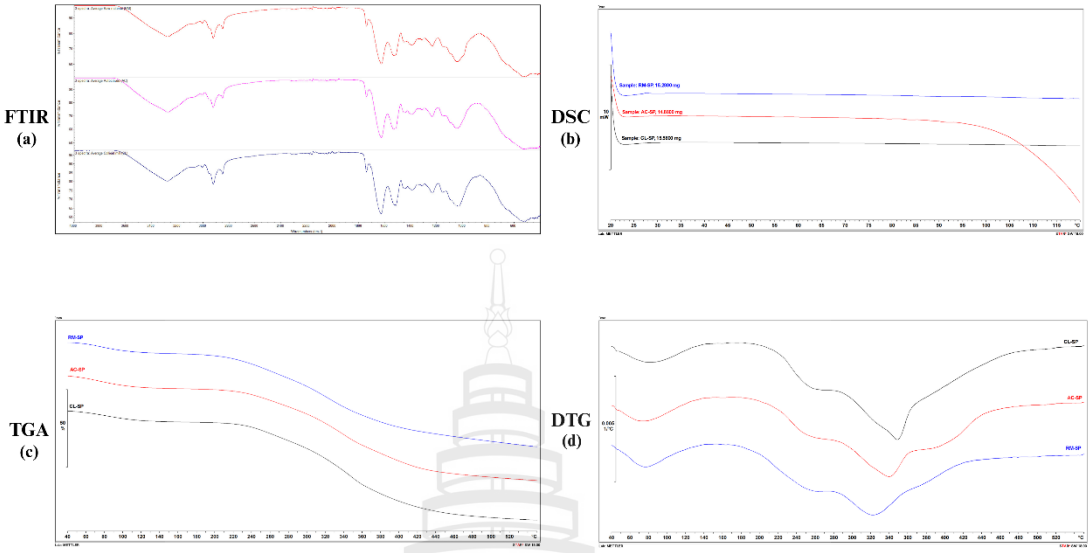


Figure 4.5 Structural and thermal analyses of raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) (a) FTIR spectra, (b) DSC thermos grams, (c) TGA curves, and (d) DTG profiles.

Table 4.9 Particle size distribution and thermal properties of raw Sacha Inchi press cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press cake meal followed by colloid-milled press-cake meal (CL).

Properties	SP	AC	CL
Particle size distribution			
D**10** (μm)	18.14±0.26[b]	22.21±0.06[a]	15.89±0.24[c]
D**50** (μm)	42.58±0.46[c]	96.38±4.46[a]	66.66±2.85[b]
D**90** (μm)	209.58±4.27[c]	524.07±20.14[a]	344.95±7.92[b]
Mean size (μm)	80.08±1.41[c]	189.86±7.36[a]	137.61±3.10[b]
Mode (μm)	31.87±0.02[a]	28.13±0.02[b]	21.41±0.03[c]
Thermal properties			
Endothermic peak (°C)	89.53	91.88	90.92
Enthalpy of Transition, ΔH (J/g)	-0.21	-0.09	-0.07
Onset Temperature, To (°C)	85.26	88.17	86.76
End Temperature, Te (°C)	98.17	98.12	94.72

Thermogravimetric parameters	**Tmax (°C)**	**% weight loss**	**Tmax (°C)**	**% weight loss**	**Tmax (°C)**	**% weight loss**
Region I (40-150°C)	63.14	7.85	68.90	6.12	71.30	5.43
Region II (170-280°C)	301.20	52.31	309.50	48.27	314.80	45.96
Region III (300-500°C)	431.60	21.64	438.20	19.83	445.60	18.12
% Residual char	-	18.20	-	25.78	-	30.49
DTG Tmax, Stage I (°C)	63.4		68.9		71.3	
DTG Tmax, Stage II (°C)	301.2		309.5		314.8	
DTG Tmax, Stage III (°C)	431.6		438.2		445.6	

Note Values are expressed as mean ± standard deviation (n = 3) for particle size distribution parameters.

Thermal parameters obtained from DSC and TGA were determined from single measurements (n = 1). Different superscript letters within the same row indicate significant differences ($p < 0.05$).

T_max values were obtained from the corresponding DTG peaks.

DTG refers to derivative thermogravimetry. Endothermic enthalpy (ΔH) values are reported according to the instrument's sign convention.

SP refers to raw press-cake powder, which represents the unprocessed form of the press-cake.

AC refers to autoclaved press-cake meal, which is the press-cake subjected to autoclaving to enhance protein recovery.

CL refers to autoclaved press-cake meal followed by colloid-milled press-cake meal, which refers to the press-cake that has undergone both autoclaving and col loid milling to achieve a finer particle size and improved protein extraction.

4.6 Techno-functional Properties

Techno-functional properties are critical indicators of the applicability of plant protein ingredients in food systems, as they reflect hydration behavior, interfacial activity, and network-forming ability. The techno-functional properties of Sacha inchi press cake obtained from dry fractionation (Part 1) and thermal–mechanical processing (Part 2) are summarized in Table 4.10 and Table 4.11, respectively, and are discussed in relation to structural and molecular changes described in Sections 4.4 and 4.5. This analysis links processing-induced molecular and structural modifications to practical functional performance relevant to food applications.

4.6.1 Protein solubility

Protein solubility profiles of cyclone-fractionated samples (SC-RM and F3) are shown in Figure 4.6 and summarized in Table 4.10. Protein solubility values in techno-functional analyses were determined using the Bradford assay, whereas solubility during *in vitro* digestion was quantified using the Biuret method due to matrix complexity in Part 1, protein solubility at pH 2 remained low (~20%) and did not differ significantly between SC-RM and F3, while solubility at pH 12 was high (>94%) for both samples. These results indicate that dry fractionation increased protein concentration but did not substantially alter protein solubility behavior. The persistence of low solubility under acidic conditions reflects intact protein–polysaccharide and protein–fiber associations, consistent with the limited microstructural disruption observed in Section 4.4.

In contrast, thermal and mechanical processing in Part 2 significantly improved protein solubility, particularly under acidic conditions (Figure 4.7; Table 4.10). Protein

solubility at pH 2 increased from 19.29 mg/g in SP to 22.48 mg/g in AC and further to 31.79 mg/g in CL. This represents an approximately 65% increase from SP to CL. The enhanced solubility under acidic conditions is particularly important for food applications involving low-pH environments and for gastric digestion.

The improved solubility observed in CL can be attributed to protein unfolding and disruption of protein–matrix interactions, as evidenced by FTIR band broadening and reduced DSC enthalpy (Section 4.5). Similar increases in protein solubility after hydrothermal treatment combined with high-shear processing have been reported for oilseed and legume proteins and are commonly associated with increased exposure of hydrophilic groups and reduced aggregation tendency.

4.6.2 Hydration-related properties: wettability, dispersibility, and bulk density

Dry fractionation (Part 1) resulted in improved wettability and dispersibility of F3 compared with SC-RM (Table 4.10), reflecting finer particle size and increased surface area. Wettability time decreased from 37.54 s (SC-RM) to 25.20 s (F3), while dispersibility increased from 49.26% to 61.04%. However, sinkability remained high (>300 s), indicating that particles still tended to resist rapid hydration due to intact matrix structure.

In Part 2, bulk density decreased markedly from 0.65 g/cm³ (SP) to 0.25 g/cm³ (CL), indicating increased porosity and reduced particle compactness (Table 4.11). Wettability was fastest for CL (16.01 s), and sinkability was also reduced compared with SP and AC, suggesting improved hydration behavior. These changes are consistent with SEM observations showing fragmented, porous structures after colloid milling (Section 4.4).

Overall, these results indicate that while dry fractionation improves surface-related hydration properties to some extent, combined thermal and mechanical processing is required to fundamentally alter matrix structure and hydration dynamics.

4.6.3 Water-holding capacity (WHC) and oil-holding capacity (OHC)

WHC and OHC are key parameters governing texture, mouthfeel, and stability of food products. In Part 1, WHC increased from 3.40 g H_2O/g in SC-RM to 5.08 g H_2O/g in F3, while OHC increased from 2.58 g oil/g to 3.80 g oil/g (Table 4.10). These improvements reflect increased protein content and finer particle size but remain limited by intact internal matrix structure.

In Part 2, WHC increased progressively from 3.81 g H_2O/g (SP) to 4.27 g H_2O/g (AC) and 4.89 g H_2O/g (CL), while OHC increased from 6.67 g oil/g (SP) to 9.01 g oil/g (CL) (Table 4.10). The higher OHC observed in CL indicates enhanced exposure of hydrophobic amino acid residues and increased physical entrapment of oil within a porous matrix.

The simultaneous increase in WHC and OHC in CL reflects a balance between hydrophilic and hydrophobic interactions, which is characteristic of partially unfolded but non-aggregated protein systems. These findings are consistent with molecular evidence from FTIR and DSC analyses, which demonstrated increased molecular flexibility and reduced thermal stability (Section 4.5).

4.6.4 Interfacial properties: foaming and emulsifying behavior

Foaming and emulsifying properties depend on protein solubility, flexibility, and interfacial adsorption behavior. In Part 1, F3 exhibited lower foaming capacity but higher foam stability compared with SC-RM (Table 4.10), suggesting that dry fractionation altered interfacial behavior without improving overall foaming performance. Emulsifying activity and stability decreased slightly in F3, likely due to limited protein flexibility despite increased protein concentration.

In Part 2, foaming capacity increased from 9.07% (SP) to 13.52% (CL), while foam stability decreased slightly (Table 4.10). The increased foaming capacity indicates improved protein adsorption and unfolding at the air–water interface, whereas reduced stability suggests that excessive flexibility may compromise film strength. Similar trade-offs between foaming capacity and stability have been reported for hydrothermally and mechanically modified plant proteins.

Emulsifying activity and stability increased substantially after processing, with CL exhibiting the highest values (62.50% and 74.75%, respectively). Enhanced emulsifying performance can be attributed to increased protein solubility, reduced particle size, and improved interfacial adsorption, all of which were supported by structural and molecular analyses in Sections 4.4 and 4.5.

4.6.5 Least gelation concentration (LGC)

Gelation behavior reflects the ability of proteins to form three-dimensional networks upon heating. In Part 1, the least gelation concentration decreased from 18% (SC-RM) to 10% (F3), indicating improved gel-forming ability due to higher protein

concentration (Table 4.10). However, relatively high concentrations were still required, reflecting limited protein accessibility.

In Part 2, LGC decreased from 18% (SP) to 14% (AC) and further to 8% (CL) (Table 4.10). The substantially lower LGC of CL indicates enhanced network formation at lower concentrations, which is consistent with increased protein accessibility and flexibility following autoclaving and colloid milling. These results suggest that thermal–mechanical processing not only improves hydration and interfacial properties but also enhances structural functionality relevant to gel-based food applications.

4.6.6 Integrated structure–function interpretation

Collectively, the techno-functional results demonstrate that dry fractionation (Part 1) primarily improves surface-related properties through particle size redistribution, whereas thermal and mechanical processing (Part 2) induces deeper structural modification that enables full expression of protein functionality. Improvements in solubility, WHC, OHC, emulsifying activity, and gelation behavior observed in CL are strongly supported by microstructural disruption (Section 4.4) and molecular rearrangement evidenced by FTIR and DSC analyses (Section 4.5).

These findings confirm that enhanced techno-functional performance of CL arises from true matrix restructuring and protein accessibility enhancement, rather than protein enrichment alone. This distinction is critical for understanding the superior food application performance of CL discussed in Section 4.8 and highlights the advantage of integrating dry fractionation with hydrothermal and mechanical processing strategies.

Table 4.10 Techno-functionality of Sacha Inchi press cake meal (SC-RM) and fraction 3 (F3) fractionated from the cyclone fractionation.

Properties	SC-RM	Fraction 3
Protein solubility at pH 2 (%)	19.96±0.18[a]	20.12±0.13[a]
Protein solubility at pH 12 (%)	95.760.82[a]	94.90±0.57[a]
Wettability (Sec.)	37.54±1.09[a]	25.20±1.09[b]
Sinkability (%)	>300±0.00[a]	>300±0.00[a]
Dispersibility (%, w/v)	49.26±1.23[b]	61.04±1.16[a]
WHC (g H_2O/g sample)	3.40±0.40[b]	5.08±0.14[a]
OHC (g oil/g sample)	2.58±0.23[b]	3.80±0.10[a]
Foaming capacity (%)	11.38±0.00[b]	3.95±0.01[a]
Foam stability (%)	88.89±0.00[b]	96.83±1.37[a]
Emulsifying activity (%)	87.70±0.39[a]	76.52±0.30[b]
Emulsion stability (%)	78.87±0.63[a]	70.09±0.84[b]
Gelling concentration (%, w/v)	18	10

Note All values are means ± SD of three replicates. Mean values with different letters in each column are significantly different ($p < 0.05$).

Table 4.11 Techno-functional properties of raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL).

Properties	**SP**	**AC**	**CL**
Protein solubility at pH 2 (mg/g sample, dry basis)	19.29±1.18^{c}	22.48±1.13^{b}	31.79±0.65^{a}
Protein solubility at pH 12 (mg/g sample, dry basis)	108.20±0.68^{b}	107.10±0.66^{c}	109.91±0.65^{a}
Bulk density (g/cm^{3})	0.65±0.03a	0.46±0.02b	0.25±0.03c
Wettability (Sec.)	16.24±0.01^{b}	19.41±0.01^{a}	16.01±0.01^{c}
Sinkability (Sec.)	628.43±0.33^{b}	1164.17±0.31^{a}	444.04±0.04^{c}
Dispersibility (%)	69.90±0.72^{c}	63.07±0.47^{b}	57.26±0.69^{a}
Water-holding capacity (g H_2O/g sample)	3.81±0.17^{c}	4.27±0.13^{b}	4.89±0.21^{a}
Oil-holding capacity (g oil/g sample)	6.67±0.72^{c}	8.36±0.92^{ab}	9.01±0.27^{a}
Foaming capacity (%)	9.07±0.28^{c}	10.38±0.50^{b}	13.52±0.56^{a}
Foam stability (%)	90.32±1.02^{a}	85.65±0.48^{b}	82.71±0.59^{c}
Emulsifying activity (%)	50.00±0.00^{c}	52.50±0.00^{b}	62.50±0.00^{a}
Emulsion stability (%)	52.06±0.45^{c}	62.12±0.30^{b}	74.75±0.65^{a}
Gelling concentration (%, w/v)	18	14	8

Note Values are expressed as mean ± standard deviation (n = 3).

Mean values with different superscript letters within the same row are significantly different ($p < 0.05$).

SP refers to raw press-cake powder, which represents the unprocessed form of the press-cake.

AC refers to autoclaved press-cake meal, which is the press-cake subjected to autoclaving to enhance protein recovery.

CL refers to autoclaved press-cake meal followed by colloid-milled press-cake

meal, which refers to the press-cake that has undergone both autoclaving and colloid milling to achieve a finer particle size and improved protein extraction. Protein solubility was determined using the Bradford assay, with bovine serum albumin (BSA) as the standard.

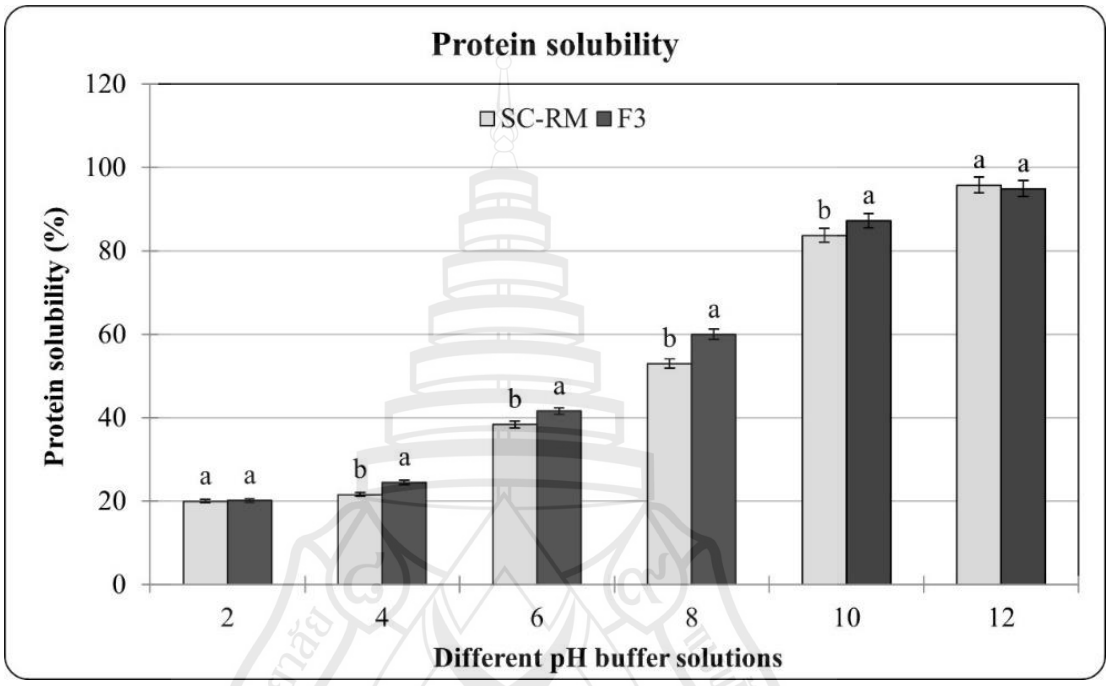


Figure 4.6 Protein solubility (%) of Sacha inchi press cake meal (SC-RM) and Fraction 3 (F3) produced by cyclone separation. Values followed by the same lowercase letter are not significantly different according to statistical analysis ($p > 0.05$).

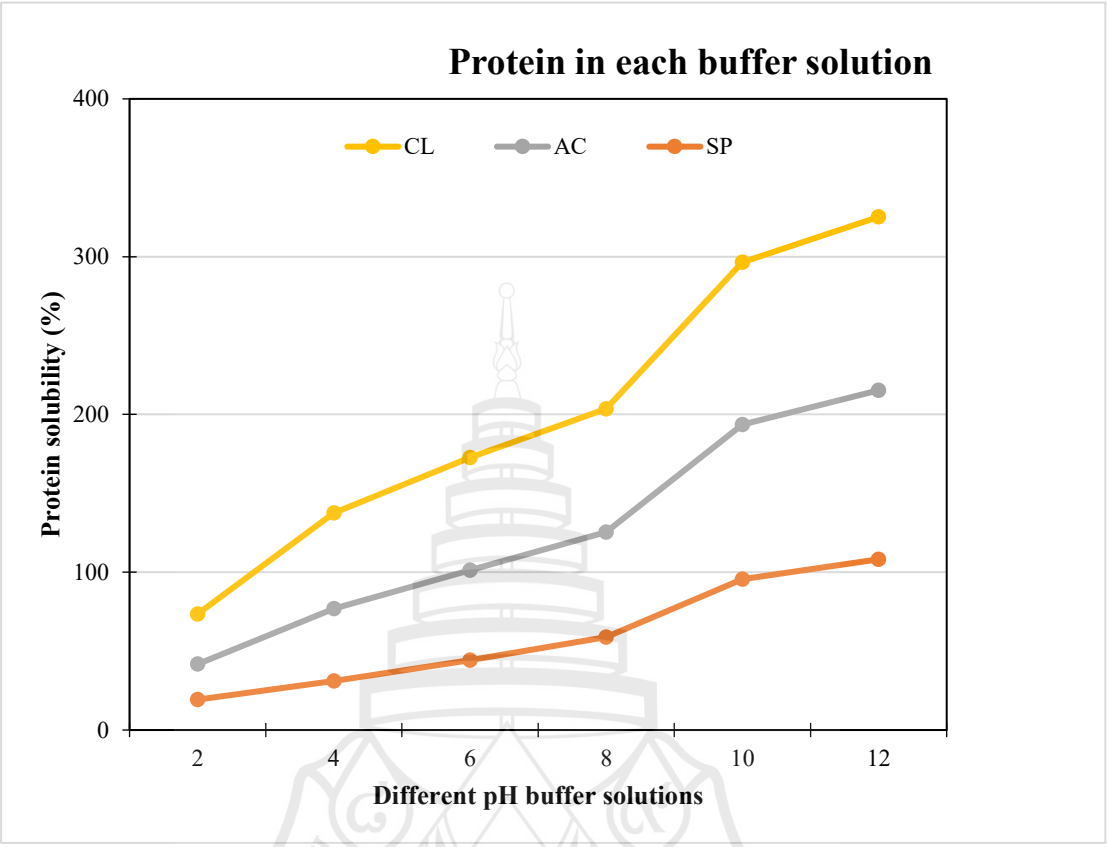


Figure 4.7 Protein solubility profiles of raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) at different pH values.

4.7 *In vitro* Gastrointestinal Protein Digestibility

In vitro gastrointestinal protein digestibility provides insight into the nutritional quality and bioaccessibility of proteins following processing. In the present study, protein digestibility was evaluated for autoclaved press-cake meal (AC) and autoclaved press-cake meal followed by colloid milling (CL), while results from dry fractionation (Part 1) were used as a structural and compositional reference. This evaluation provides insight into how structural accessibility rather than protein content alone governs gastrointestinal protein digestibility.

4.7.1 Effect of processing on protein solubilization before digestion

Before digestion (BF), CL exhibited significantly higher soluble protein content (0.40 ± 0.01 mg/g sample) than AC (0.23 ± 0.01 mg/g sample) (Table 4.12). Although both samples originated from the same raw material, the higher baseline solubility of CL indicates that colloid milling enhanced protein accessibility beyond that achieved by hydrothermal treatment alone.

In Part 1, cyclone fractionation increased protein concentration but did not substantially improve protein solubility under acidic conditions (Section 4.6.1). This confirms that protein enrichment alone is insufficient to enhance digestibility when the protein remains embedded within an intact matrix. Similar observations have been reported for dry-fractionated oilseed and legume proteins, where limited matrix disruption results in low baseline solubility despite increased protein content (Loveday, 2020; Vogelsang-O'Dwyer et al., 2020).

4.7.2 Gastric digestion stage

During the gastric digestion stage (G2), protein solubility increased markedly in both samples; however, CL showed significantly higher solubilization (9.34 ± 0.13 mg/g) compared with AC (5.62 ± 0.11 mg/g). This represents an approximately 66% increase in gastric-phase solubility for CL relative to AC.

The enhanced gastric digestibility of CL can be attributed to combined effects of hydrothermal denaturation and high-shear mechanical disruption. Autoclaving induces partial protein unfolding and weakens protein–polysaccharide interactions, while colloid milling further disrupts the softened matrix and increases surface area available for pepsin action. Previous studies on hydrothermally treated plant proteins and oilseed meals have similarly reported enhanced gastric digestibility due to improved enzyme accessibility following matrix loosening (Brodkorb et al., 2019; Etzbach et al., 2024).

In contrast, high-pressure homogenization and high-shear processing without sufficient thermal pretreatment have been reported to yield only moderate improvements in gastric digestibility, highlighting the importance of combined processing strategies (Loveday, 2020).

4.7.3 Intestinal digestion stage

Following intestinal digestion (I2), protein solubility further increased to 11.02 ± 0.12 mg/g for CL, compared with 9.24 ± 0.12 mg/g for AC. Although the relative

difference between samples narrowed at this stage, CL still exhibited significantly higher overall gastrointestinal digestibility.

The reduced magnitude of difference during the intestinal phase suggests that pancreatin-mediated proteolysis partially compensates for structural limitations present in AC. However, the consistently higher solubilization observed for CL indicates that prior processing history strongly influences digestion kinetics. Similar trends have been reported for hydrothermally treated legume and oilseed proteins, where early-stage structural disruption accelerates protein hydrolysis throughout gastrointestinal digestion (Minekus et al., 2014; Vogelsang-O'Dwyer et al., 2020).

4.7.4 Structural and molecular basis for enhanced digestibility

The superior digestibility of CL is strongly supported by microstructural (Section 4.4) and molecular evidence (Section 4.5). SEM images revealed fragmented, porous particles with reduced agglomeration after colloid milling, while FTIR and DSC analyses confirmed protein unfolding and reduced structural order. These changes facilitate enzyme diffusion and increase the availability of cleavage sites for proteolytic enzymes.

Moreover, reductions in antinutritional factors such as phytic acid, trypsin inhibitors, and saponins observed after processing (Table 4.2) likely contributed to improved digestibility by reducing enzyme inhibition and protein–mineral complexation. Similar reductions in antinutritional factors following hydrothermal treatment have been shown to enhance protein digestibility in oilseed meals and plant-based by-products (Etzbach et al., 2024; Ketnawa et al., 2022).

4.7.5 Comparison with dry fractionation and implications

Comparison with Part 1 highlights that cyclone-based dry fractionation improved protein concentration but did not substantially enhance digestibility due to preservation of matrix integrity. In contrast, Part 2 processing fundamentally altered matrix architecture, enabling substantial improvements in gastrointestinal protein solubilization.

These findings align with recent literature emphasizing that protein digestibility is governed more by structural accessibility than by protein content alone (Loveday, 2020; Njoroge et al., 2025). The present study therefore demonstrates that integrating

autoclaving with colloid milling is an effective strategy to enhance nutritional quality of Sacha inchi press cake by improving protein bioaccessibility during digestion.

Table 4.12 Protein quality indices of autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) compared with FAO/WHO reference values a -nd soybean protein.

Parameters	**CL**	**FAO/WHO reference pattern**
		Soybean
E/T (%)	34.47	39.20
EAA (%)	34.47	103.23
EAAI	108.00	81.15
AAS	57.17	0.74
PER1	1.85	32.21
PER2	1.58	31.56
PER3	0.59	40.05
p-BV	106.00	76.75
NI	69.14	32.46

Note CL: autoclaved press-cake meal followed by colloid-milled press-cake meal (CL)
Protein quality indices were calculated based on amino acid composition.
E/T: ratio of essential amino acids to total amino acids;
EAA: essential amino acid content;
EAAI: essential amino acid index;
AAS: amino acid score;
PER: predicted protein efficiency ratio;
p-BV: predicted biological value;
NI: nutritional index.
FAO/WHO reference values were obtained from soybean protein.

Table 4.13 Protein solubility during *in vitro* digestion of autoclaved press-cake meal (-AC), and autoclaved press-cake meal followed by colloid-milled press cake meal (CL).

Digestion stage	**AC-SP**	**CL-SP**
Before digestion (BF)	0.23 ± 0.01^{b}	0.40 ± 0.01^{a}
Gastric digestion stage for 2 h (G2)	5.62 ± 0.11^{b}	9.34 ± 0.13^{a}
Intestinal digestion stage for 2 h (I2)	9.24 ± 0.12^{b}	11.02 ± 0.12^{a}

Note Values are expressed as mean ± standard deviation (n = 3).

Mean values with different superscript letters within the same row are significantly different ($p < 0.05$).

BF, G2, and I2 refer to the samples before digestion, during the gastric phase, and during the intestinal phase, respectively.

Protein solubility was determined using the Bradford assay, with bovine serum albumin (BSA) as the standard.

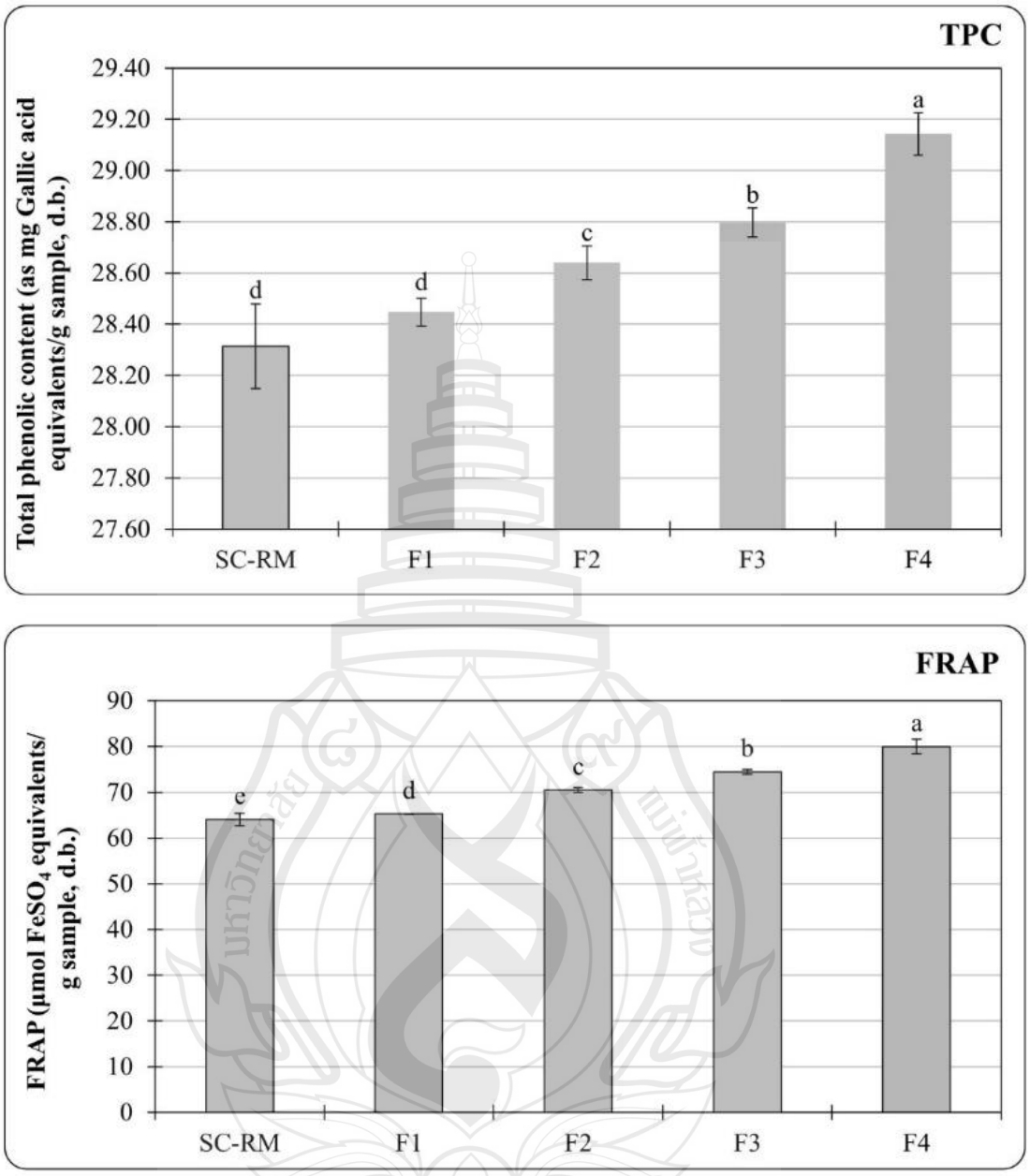


Figure 4.8 Total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) of the fractionated Sacha inchi press cake obtained from the multi-stage cyclone separator.

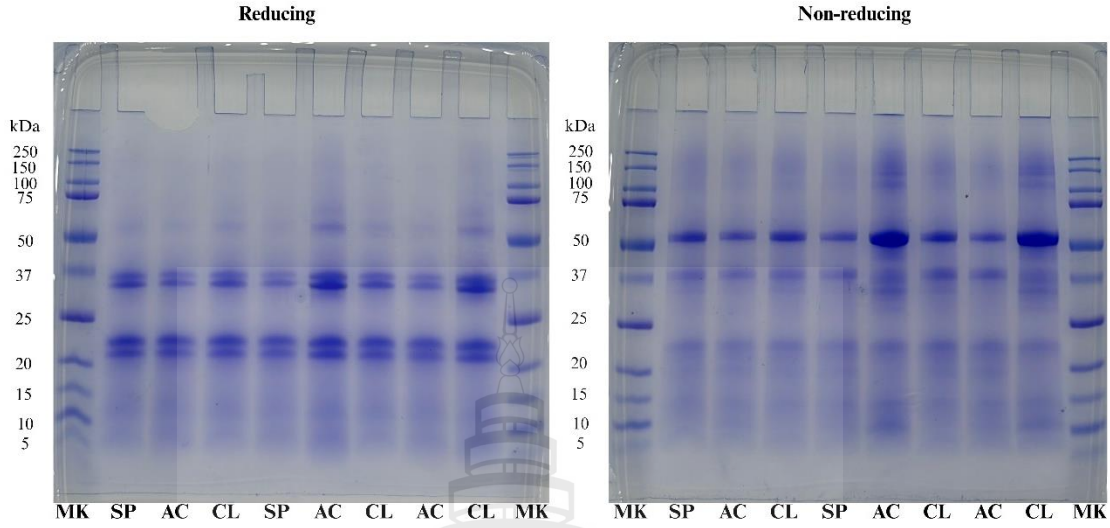


Figure 4.9 SDS–PAGE profiles of proteins extracted from raw Sacha Inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) under reducing and non-reducing conditions.

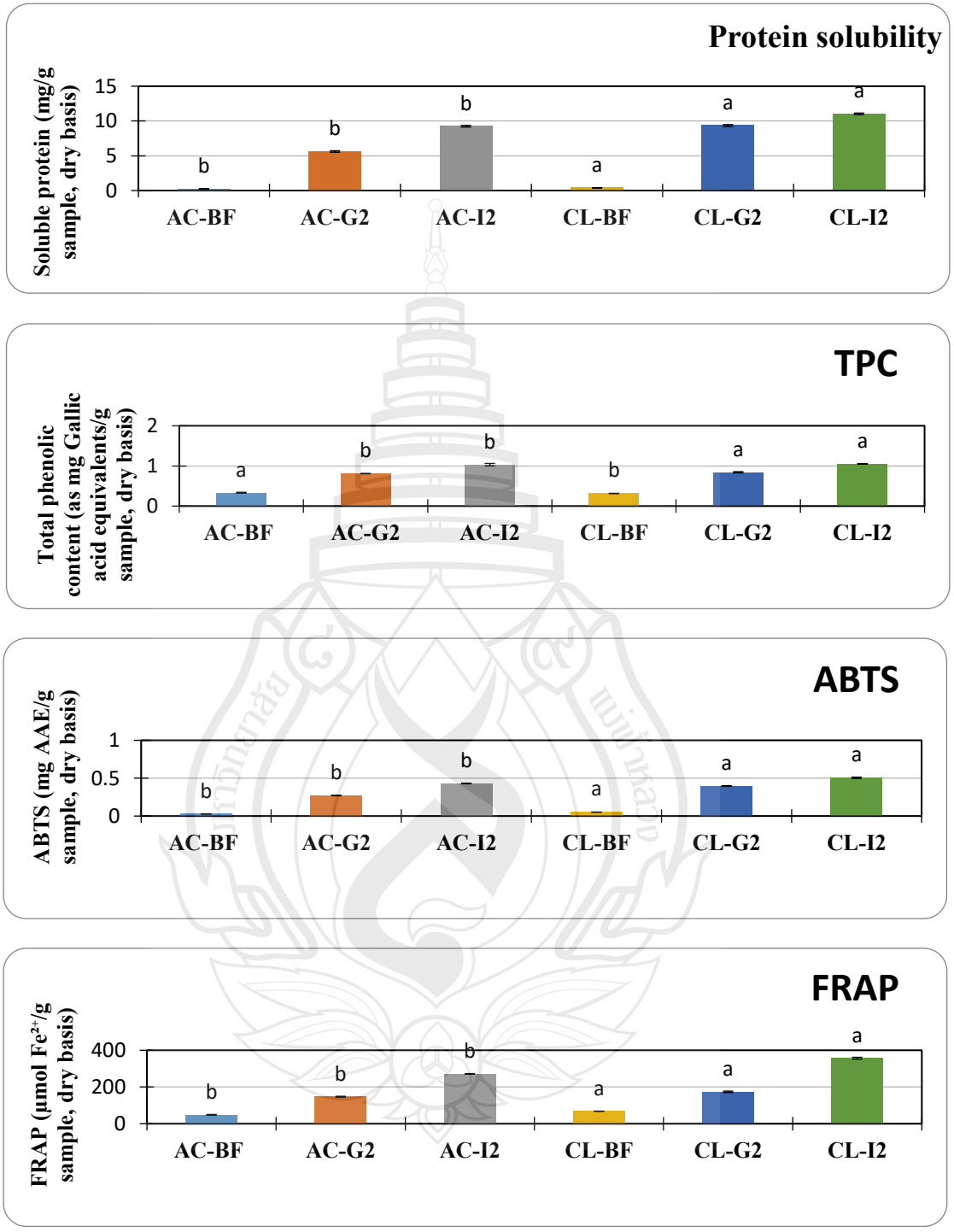


Figure 4.10 Changes in protein solubility, total phenolic content (TPC), and antioxidant activities (ABTS and FRAP) of autoclaved press-cake meal (AC) and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) before and during *in vitro* gastrointestinal digestion.

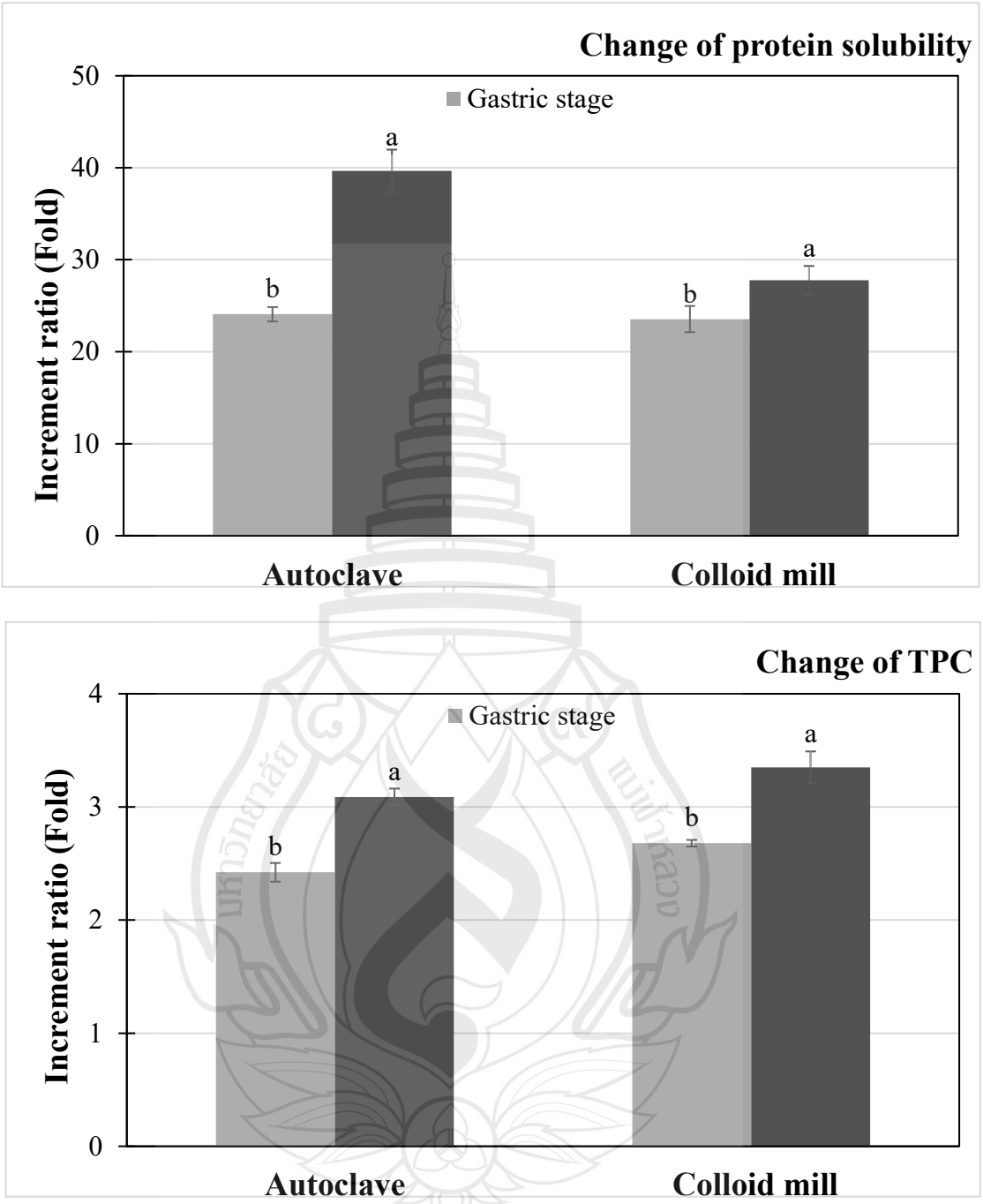


Figure 4.10 Changes in protein solubility, total phenolic content (TPC), and antioxidant activities (ABTS and FRAP) of autoclaved press-cake meal (AC) and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) before and during *in vitro* gastrointestinal digestion.

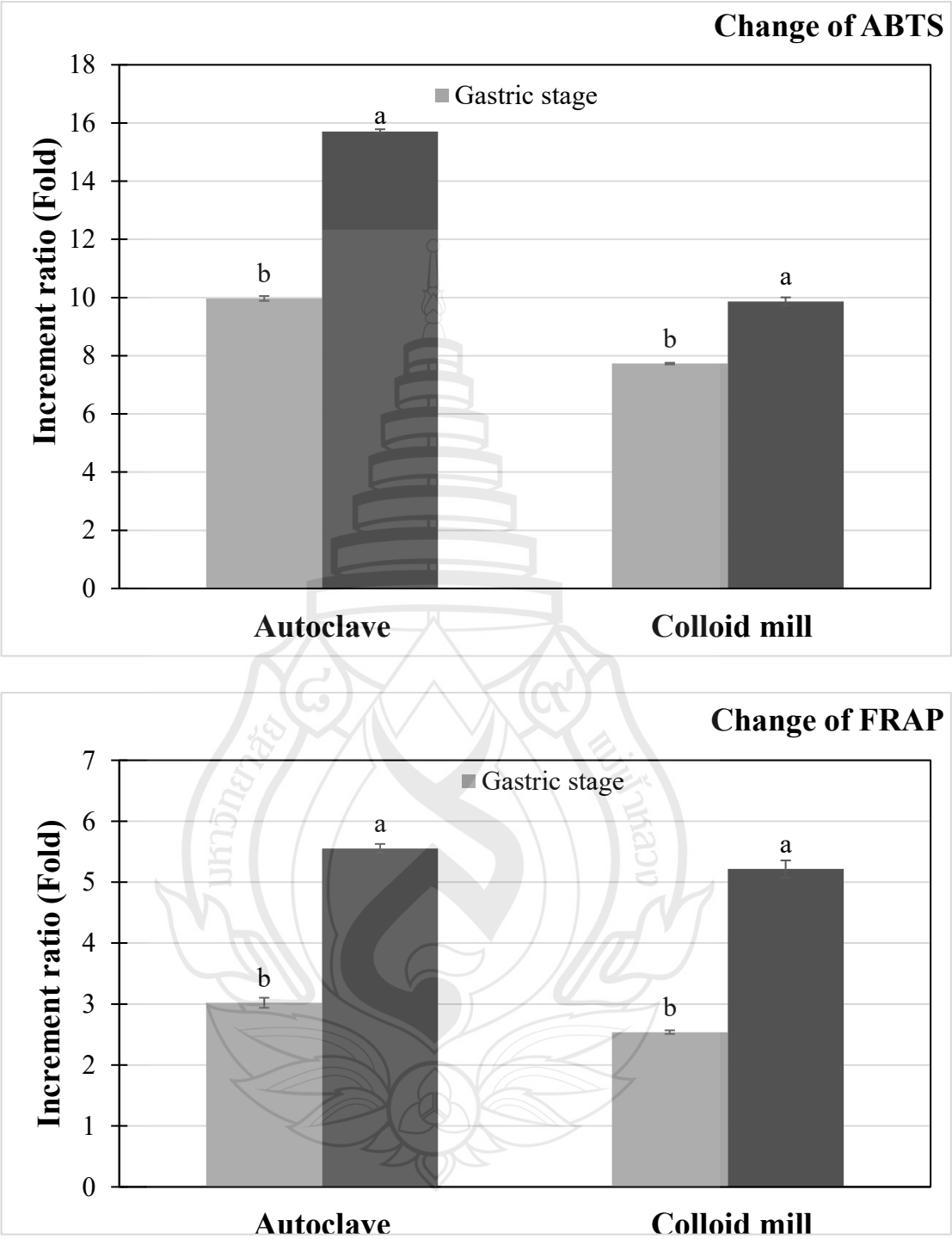


Figure 4.10 Changes in protein solubility, total phenolic content (TPC), and antioxidant activities (ABTS and FRAP) of autoclaved press-cake meal (AC) and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) before and during *in vitro* gastrointestinal digestion.

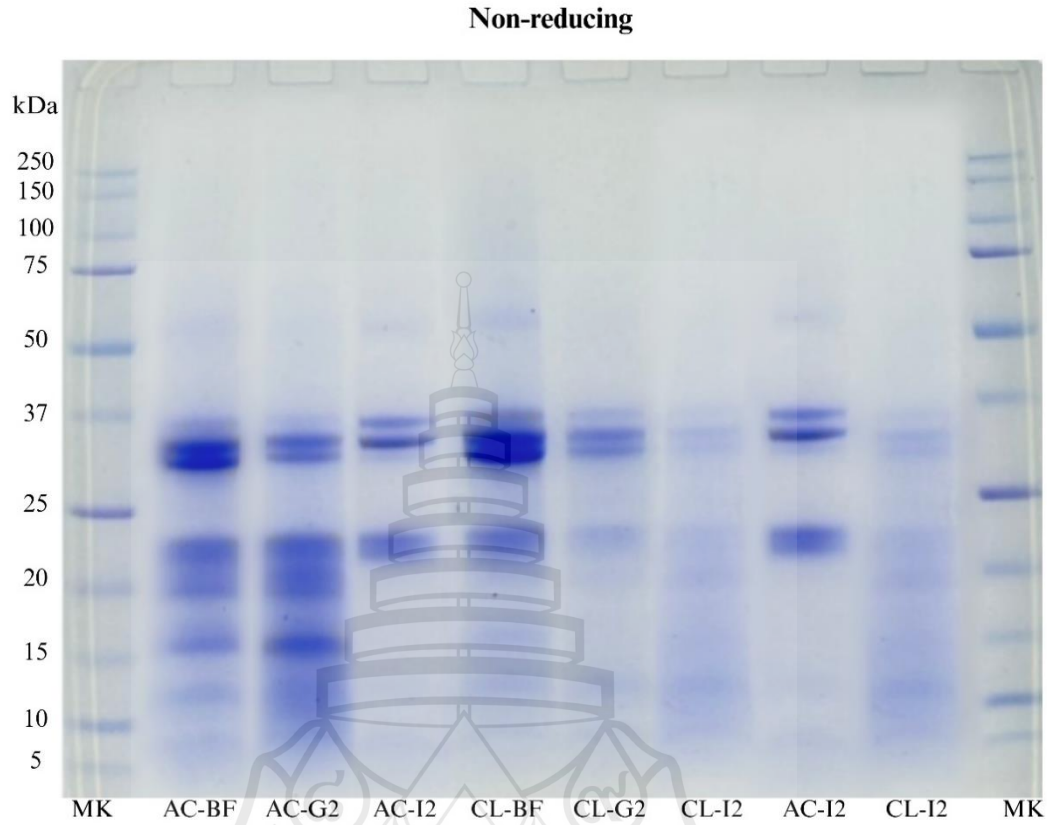


Figure 4.11 SDS–PAGE protein profiles under non-reducing conditions of autoclaved press-cake meal (AC) and autoclaved press-cake meal followed by colloid milled press-cake meal (CL) before and during *in vitro* gastrointestial digestion.

4.8 Effect of Thermomechanical Treatments on Protein Structure

4.8.1 SDS–PAGE profiles under reducing and non-reducing conditions

Figure 4.9 illustrates the SDS–PAGE protein profiles of raw Sacha inchi press-cake powder (SP), autoclaved press-cake meal (AC), and autoclaved press-cake meal followed by colloid milling (CL) under reducing and non-reducing conditions. SDS–PAGE is a widely used technique for characterizing protein molecular weight distribution and aggregation behavior in food systems (Laemmli, 1970).

Under reducing conditions, all samples exhibited major protein bands primarily in the molecular weight range of approximately 15–40 kDa. The similarity of band

patterns among SP, AC, and CL indicates that thermomechanical treatments did not markedly alter the primary structure of the proteins, as reducing agents cleave disulfide bonds without affecting the peptide backbone (Damodaran & Parkin, 2017). This suggests that the applied treatments mainly influenced higher-order protein structures rather than covalent amino acid sequences.

In contrast, under non-reducing conditions, notable differences were observed between the raw and processed samples. Both AC and CL samples displayed increased band intensity and apparent shifts toward higher molecular weight regions compared with SP, indicating the formation of protein aggregates during autoclaving. Heat treatment is known to induce protein unfolding, which promotes intermolecular interactions and aggregation through disulfide bond formation (Damodaran & Parkin, 2017). The presence of similar aggregated bands in the CL sample suggests that colloid milling did not disrupt disulfide-linked aggregates but primarily improved the physical dispersion of proteins within the matrix. Overall, these results demonstrate that autoclaving played a dominant role in modifying protein secondary and tertiary structures via aggregation, while colloid milling acted mainly as a physical treatment affecting protein dispersion rather than covalent structure.

4.8.2 SDS–PAGE profiles during *in vitro* gastrointestinal digestion

Color Figure 4.11 presents the SDS–PAGE protein profiles of AC and CL samples under non-reducing conditions before and during simulated *in vitro* gastrointestinal digestion. The digestion procedure was conducted following a standardized static *in vitro* digestion model, which is commonly applied to evaluate protein breakdown under gastrointestinal conditions (Minekus et al., 2014).

Before digestion, both AC and CL samples exhibited dominant protein bands around 30–40 kDa, corresponding to aggregated protein structures formed during thermal processing. These aggregates are typical of heat-treated plant proteins and can influence subsequent enzymatic hydrolysis (Damodaran & Parkin, 2017).

During the gastric phase, major protein bands remained visible in both samples, although a reduction in band intensity was observed, indicating partial proteolysis by pepsin. The persistence of aggregated protein bands suggests that compact protein structures formed during autoclaving exhibit relative resistance to gastric digestion, as limited enzyme accessibility can slow protein hydrolysis (Kaushik, 2015).

After the intestinal phase, a substantial decrease in high-molecular-weight protein bands was observed, accompanied by the appearance of low-molecular-weight fragments, indicating extensive proteolysis by pancreatic enzymes. The CL sample showed a greater reduction in intact protein bands compared with the AC sample, suggesting enhanced enzymatic hydrolysis. This improvement can be attributed to increased protein dispersion and surface area resulting from colloid milling, which enhances enzyme accessibility during intestinal digestion (Kaushik, 2015).

Taken together, these findings indicate that although autoclaving induced protein aggregation through structural modifications, subsequent colloid milling partially mitigated the negative impact of aggregation on digestibility by improving protein dispersion and susceptibility to gastrointestinal enzymes.

4.9 Food Application and Sensory Evaluation

Evaluation of food application and sensory acceptance provides a critical link between laboratory-scale functional characterization and practical utilization of modified protein ingredients. In this study, autoclaved press-cake meal followed by colloid milling (CL) was incorporated into cassava starch–based crackers at different substitution levels to assess its effects on physical quality attributes and consumer acceptance. Results are discussed in relation to the techno-functional properties (Section 4.6) and protein digestibility outcomes (Section 4.7). This assessment bridges laboratory-scale functional characterization with product-level performance and consumer acceptance.

4.9.1 Effects of CL incorporation on physical and textural properties of crackers

Color attributes and texture profile parameters of crackers formulated with varying CL substitution levels are presented in Table 4.14. Incorporation of CL significantly influenced cracker appearance and mechanical properties, with effects becoming more pronounced as substitution level increased.

Crackers formulated with a 90:10 cassava starch–CL ratio exhibited the highest lightness (L* = 56.51) and whiteness index (53.19), exceeding even the control formulation. This enhancement can be attributed to the improved dispersion and hydration behavior of CL, which promotes more uniform bubble formation and expansion during

frying. In contrast, higher substitution levels (80:20 and 70:30) resulted in darker products with lower L* values and higher redness (a*), reflecting increased protein content and intensified Maillard reactions during frying.

Texture profile analysis revealed that moderate CL incorporation improved cracker texture, whereas excessive substitution adversely affected mechanical properties. The 90:10 formulation exhibited significantly lower hardness (5533 g) and chewiness (174) compared with the control, indicating a lighter and crispier texture. These improvements are consistent with the enhanced water-holding capacity and emulsifying properties of CL (Section 4.6), which facilitate uniform dough hydration and expansion.

In contrast, crackers containing 20% and 30% CL exhibited markedly higher hardness and chewiness, particularly at the 70:30 ratio (hardness >50,000 g). Excessive protein incorporation likely interfered with starch gelatinization and expansion, leading to dense, rigid structures. Similar texture deterioration at high protein substitution levels has been reported for starch-based snacks fortified with plant proteins and is commonly attributed to protein–starch competition for water and restricted bubble growth.

4.9.2 Sensory acceptance of CL-fortified crackers

Sensory evaluation results are summarized in Table 4.14, with overall sensory profiles illustrated in Figures 18 and 19. The 90:10 cassava starch–CL formulation achieved the highest overall acceptability score (8.62), significantly outperforming the control and higher substitution levels.

Panelists rated the 90:10 crackers highest for appearance, color, texture, and crispness, which aligns with the favorable physical properties observed in Table 4.14. The improved texture and crispness can be directly linked to the enhanced techno-functional properties of CL, particularly its water-holding and emulsifying capacities, which contribute to uniform expansion and desirable mouthfeel.

At higher substitution levels (80:20 and 70:30), sensory scores for texture, crispness, and overall acceptance decreased significantly. Increased hardness and chewiness negatively affected eating quality, confirming that excessive CL incorporation compromises product structure. These findings underscore the importance of balancing protein enrichment with textural integrity in starch-based snack formulations.

Notably, bitterness and aftertaste scores did not differ significantly among formulations, indicating that CL incorporation did not introduce undesirable off-flavors.

However, the perceived “Sacha inchi note” increased slightly at the 70:30 level, suggesting that higher inclusion rates may accentuate characteristic seed flavors, which could limit consumer acceptance if not masked or formulated appropriately.

4.9.3 Relationship between functional properties, digestibility, and sensory performance

The superior sensory performance of crackers containing 10% CL reflects a favorable balance between functional enhancement and structural compatibility. At this substitution level, CL contributed improved hydration, emulsification, and dispersion without overwhelming the starch matrix. This observation aligns with the lower least gelation concentration and enhanced solubility of CL reported in Section 4.6, as well as its improved protein digestibility discussed in Section 4.7.

Importantly, the results demonstrate that improvements in protein functionality and digestibility do not automatically translate to improved product quality at all inclusion levels. Instead, an optimal incorporation range exists where functional benefits are maximized without compromising texture and consumer acceptance. Similar conclusions have been reported in studies on protein-enriched snack products, emphasizing that moderate protein substitution is generally preferable for maintaining desirable sensory attributes.

4.9.4 Implications for food product development

From an application perspective, the present findings demonstrate that CL is a promising protein-enriched ingredient for starch-based snack products when incorporated at appropriate levels. The 90:10 cassava starch–CL formulation achieved the best balance between nutritional enhancement, functional performance, and sensory acceptance, highlighting its potential for commercial development.

These results further confirm that the combined autoclaving and colloid milling strategy not only improves protein accessibility and digestibility but also enables successful integration of Sacha inchi press cake into real food systems. This outcome supports the broader objective of valorizing oilseed by-products into functional, consumer-acceptable food ingredients and provides a practical foundation for future product optimization and scale-up. Overall, these results demonstrate the feasibility of incorporating colloid-milled Sacha inchi press cake into starch-based snack products without

compromising consumer acceptance, supporting its potential for commercial utilization.

Table 4.14 Color attributes and texture properties of crackers formulated from cassava starch and autoclaved press-cake meal followed by colloid-milled press cake meal (CL) at varying ratios.

Properties	**Control***	**90:10**	**80:20**	**70:30**
Color attributes				
L* (Lightness)	50.53±0.43^{b}	56.51±0.42^{a}	44.13±0.43^{c}	40.58±0.37^{d}
a* (Redness/ Greenness)	-0.34±0.12^{d}	5.03±0.46^{c}	6.44±0.27^{b}	8.43±0.15^{a}
b*(Yellowness/Blueness)	3.22±0.44^{d}	16.55±0.47^{a}	10.34±0.42^{c}	11.45±0.27^{b}
ΔE	0.52±0.21^{d}	15.58±0.30^{ab}	11.73±0.49^{c}	15.61±0.37^{a}
Whiteness	50.42±0.40^{b}	53.19±0.36^{a}	42.81±0.44^{c}	38.90±0.37^{d}
Texture profile				
Hardness (g)	6685.54±148.53^{c}	5533.31±249.84^{d}	18137.00±988.77^{b}	50832.00±2326.95^{a}
Chewiness	675.45±241.62^{c}	173.63±76.74^{d}	4138.38±1824.46^{b}	23850.49±3027.48^{a}
Springiness	0.41±0.15^{bc}	0.22±0.09^{d}	0.41±0.11^{b}	0.58±0.04^{a}
Cohesiveness	0.25±0.08^{c}	0.17±0.08^{d}	0.58±0.24^{b}	0.81±0.02^{a}

Note Values are expressed as mean ± standard deviation (n = 3).

Mean values with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Formulation ratios represent the weight ratio of cassava starch to autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) (w/w).

*The control sample was prepared using 100% cassava starch.

Figure 4.12 Representative images of crackers formulated with cassava starch and colloid-milled press-cake meal (CL) at different ratios.

Table 4.15 Sensory characteristics of crackers formulated from cassava starch and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) at varying ratios.

Attributes	**Control**	**90:10**	**80:20**	**70:30**
Overall	7.17±0.81^{b}	8.62±0.49^{a}	6.02±0.90^{c}	5.92±0.68^{c}
Appearance	7.02±0.98^{b}	8.60±0.50^{a}	6.46±0.67^{c}	6.23±0.73^{c}
Color	7.04±0.84b	8.48±0.50a	6.19±0.86c	6.00±0.66c
Aroma	6.15±0.78^{c}	7.48±0.92^{a}	6.75±0.81^{b}	6.13±0.74^{c}
Flavor	6.90±0.89^{b}	8.56±0.50^{c}	6.13±0.79^{c}	6.19±0.77^{c}
Texture	7.02±0.92^{b}	8.92±0.27^{a}	6.37±0.89^{c}	6.15±0.92^{c}
Crispness	8.19±0.63^{b}	8.90±0.30^{a}	6.75±0.97^{c}	6.21±0.89^{c}
Aftertaste	5.02±0.70^{a}	5.12±0.32^{a}	5.23±0.51^{a}	5.21±0.67^{a}
Sacha inchi note	5.10±0.30^{b}	5.02±0.14^{b}	5.21±0.54^{b}	5.58±0.82^{a}
Bitterness	5.04±0.19^{a}	5.06±0.31^{a}	5.12±0.32^{a}	5.17±0.38^{a}

Note Values are expressed as mean ± standard deviation.

Mean values with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Formulation ratios represent the weight ratio of cassava starch to autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) (w/w).
The control sample was prepared using 100% cassava starch.
Sensory evaluation was performed using a 9-point hedonic scale (1 = "dislike extremely," 9 = "like extremely") with 52 untrained panelists.

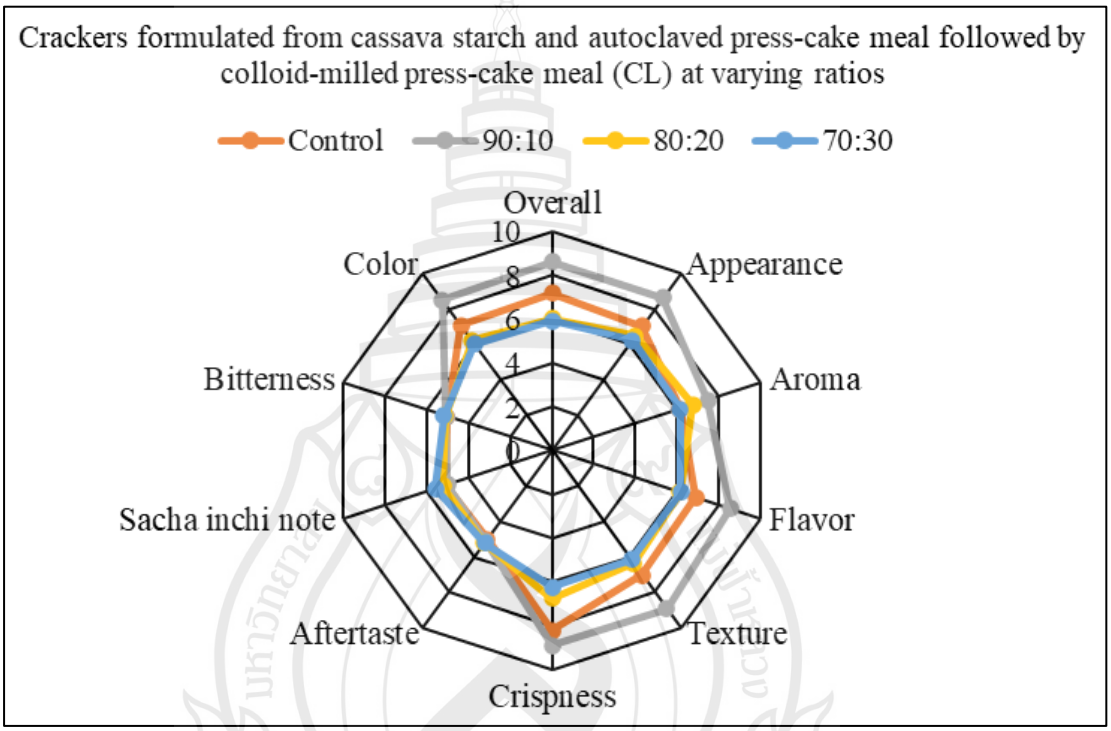


Figure 4.13 Sensory profile of crackers formulated from cassava starch and autoclaved press-cake meal followed by colloid-milled press-cake meal (CL) at varying ratios.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study provides novel mechanistic insight into the valorization of Sacha inchi press cake by demonstrating that protein accessibility, rather than protein enrichment alone, is the primary determinant governing functional performance and digestibility in oilseed press cakes. In contrast to previous studies that typically focus on single processing strategies, this work systematically disentangled the respective roles of physical redistribution and structural modification through a two-part experimental design.

The first key contribution of this research is the direct comparison between cyclone-based dry fractionation and integrated thermal–mechanical processing applied to the same raw material. Cyclone-based fractionation increased protein content by approximately 8%; however, only marginal improvements were observed in protein solubility, techno-functional properties, and digestibility. These limited gains were attributed to the preservation of the native press-cake matrix, which restricted protein accessibility. This finding provides quantitative evidence that protein enrichment without effective matrix disruption yields limited functional benefits, a distinction that has not previously been explicitly demonstrated for Sacha inchi press cake.

The second major contribution is the demonstration that autoclaving followed by colloid milling produces synergistic, non-additive effects on protein accessibility and functionality. Autoclaving partially disrupted protein–polysaccharide interactions and softened the matrix, while subsequent colloid milling further enhanced these effects by reducing particle size, increasing surface area, and promoting protein unfolding. This synergistic interaction was reflected in protein recovery values exceeding 112%, a substantial increase in acidic protein solubility (~65%), enhanced water- and oil-holding capacities, and a reduced least gelation concentration. Importantly, protein recovery

values above 100% were rigorously interpreted as increased analytical nitrogen accessibility rather than true mass gain, providing a critical methodological clarification that is often overlooked in protein recovery studies.

A further novel aspect of this work is the integration of molecular, structural, functional, and digestive evidence into a unified structure–function–digestibility framework. Spectroscopic and thermal analyses (FTIR, DSC, and TGA) confirmed that processing-induced molecular rearrangements and reduced thermal stability translated directly into improved hydration behavior, interfacial properties, and gastrointestinal protein solubilization. This multi-scale approach moves beyond descriptive characterization and establishes causal links between processing history, protein structure, and nutritional functionality.

From an application perspective, this study demonstrates for the first time that colloid-milled Sacha inchi press cake can be incorporated into starch-based snack products at substitution levels of up to 10% while maintaining or improving sensory acceptability. This outcome bridges laboratory-scale protein modification with real food system performance and highlights the feasibility of transforming Sacha inchi press cake from a low-value by-product into a functional ingredient aligned with sustainable protein development and circular bioeconomy objectives.

In summary, the novelty of this study lies not only in the processing techniques employed, but also in the conceptual advancement that overcoming matrix-imposed constraints through controlled structural disruption is essential for unlocking the functional and nutritional potential of oilseed press cakes. The integrated processing framework presented here offers a transferable and scalable strategy for the valorization of other underutilized plant protein by-products beyond Sacha inchi.

5.2 Scientific and Practical Implications

This study provides quantitative evidence linking processing-induced structural modifications to functional and nutritional outcomes in oilseed press cakes. By systematically comparing cyclone-based dry fractionation with integrated thermal–mechanical processing, the findings demonstrate that protein enrichment alone, without matrix disruption, yields limited improvements in solubility, techno-functional properties, and

digestibility. In contrast, combined autoclaving and colloid milling substantially enhance protein accessibility, hydration behavior, interfacial functionality, and in vitro digestibility, highlighting the critical role of controlled structural disruption in unlocking the potential of oilseed proteins.

Industrial and practical implications

From an industrial perspective, the proposed processing strategy achieves substantial functional enhancement while maintaining high production yield (>80%). The ability to incorporate colloid-milled Sacha inchi press cake at up to 10% substitution in starch-based snack formulations without compromising sensory acceptance demonstrates its potential as a sustainable and functional plant protein ingredient. These results suggest that the sequential processing approach can be applied in the development of protein-enriched foods, supporting circular bioeconomy principles and the valorization of oilseed by-products.

5.3 Limitations of the Study

Several limitations should be considered when interpreting the findings. Protein digestibility was assessed indirectly through protein solubilization during *in vitro* gastrointestinal digestion; direct measures such as degree of hydrolysis, free amino nitrogen, or peptide profiling were not performed. Thermal analyses (DSC and TGA) were primarily qualitative and did not provide detailed quantitative thermodynamic parameters. In addition, food application testing was restricted to a single model product (starch-based crackers), limiting generalizability to other food systems.

5.4 Recommendations for Future Research

Future research should address the identified limitations and expand the applicability of the findings. Suggested directions include:

1. Incorporating advanced digestion metrics (e.g., degree of hydrolysis, pep tide profiling) to provide a more comprehensive assessment of protein bioavailability.

2. Optimizing autoclaving and colloid milling conditions to reduce energy consumption while maintaining or enhancing functional properties.

3. Evaluating the applicability of processed Sacha inchi press cake in a wider range of food systems, such as baked goods, emulsified products, and meat analogues.

4. Conducting scale-up studies, including techno-economic analysis and life cycle assessment, to support industrial feasibility and sustainable production.

Collectively, these efforts will strengthen the practical and industrial relevance of Sacha inchi press cake as a functional plant protein ingredient and guide its adoption in sustainable food systems.

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APPENDIX A

ADDITIONAL EXPERIMENTAL DATA

This appendix provides additional experimental details, representative calculations, and definitions used in data analysis to support interpretation of results presented in Chapter 4.

B1. Calculation of production yield

Production yield (%) = (Weight of recovered product / Weight of raw material input) × 100

B2. Calculation of protein recovery and protein recovery yield

Protein recovery (%) = (Protein content in processed sample / Protein content in raw sample) × 100

Protein recovery yield (%) = (Recovered protein mass / Initial protein mass) × 100

B3. Example calculation of antioxidant activity (FRAP assay)

Absorbance value, dilution factor, standard curve equation, and final results expressed as μmol FeSO4 equivalents/g sample (dry basis).

APPENDIX B

SENSORY EVALUATION QUESTIONNAIRE

This appendix presents the sensory evaluation questionnaire used for consumer acceptance testing of cassava starch–based cracker formulations enriched with autoclaved and colloid-milled Sacha inchi press-cake meal.

C1. Participant information

Age: _____ Gender: _____

C2. Sensory evaluation form (9-point hedonic scale)

Participants were asked to evaluate appearance, color, texture, flavor, and overall acceptability using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely).

C3. Ethical statement

This sensory evaluation study was conducted in accordance with institutional ethical guidelines, and informed consent was obtained from all participants prior to participation.

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RESEARCH INTERESTS

Sustainable plant protein ingredients

Valorization of food and agricultural by-products

Dry fractionation and physical processing of plant proteins

Structure–function–digestibility relationships of food proteins

Functional and nutritional food product development

RESEARCH PROJECTS AND PRODUCT DEVELOPMENT

Development of high-protein mushroom-based minced meat products

Development of low-calorie cinnamon–ginger pudding with aloe vera jelly

Development of black garlic jelly products (R2M Thailand, 8th)

Development of plant-based bacon products (FoSTAT Food Innovation Concept Contest, Finalist 2022)

AWARDS AND ACADEMIC ACTIVITIES

Young Rising Stars of Science, 2024

ASEAN International Mobility for Students (AIMS), 2023

FoSTAT Food Innovation Concept Contest, Finalist (2022)