



Effects of Thermal Treatments on Physico-Chemical Properties and Antinutritional Factor Reductions of Sacha Inchi (*Plukenetia volubilis* L.) Meal

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Received: 17 August 2020; Revised: 26 October 2020; Accepted: 2 November 2020; Available online: 8 February 2021

Abstract

Sacha inchi seeds are normally used for the extraction of oil due to its high content of oil. The main by-product of the oil extraction process is the seed residue or meal, which is highly nutritious. However, the presence of naturally-occurring anti-nutritional factors (ANFs) especially phytate, tannins and trypsin inhibitors could limit the utilization of sacha inchi seed meal in foods and feeds. The aim of this study is to study the effects of thermal treatments including extrusion process (barrel temperature of 80, 90, 100^o C and feed moisture of 61.8 %) and autoclaving (sterilizing temperature of 105, 110, 121^o C for 30 min) of reduction of ANFs (tannins, phytic acid contents and trypsin inhibitor) and physico-chemical properties (water absorption index (WAI), water solubility index (WSI), protein solubility (PS), foam capacity (FC), emulsifying capacity (EC), emulsion stability (ES) and oil binding capacity (OBC)), antioxidant activities (ABTS^{••}, FRAP assays and total phenolic content (TPC)) and in vitro protein digestibility of sacha inchi meal. The results show that autoclaving at 121^o C for 30 min caused a significant ($p < 0.05$) reduction in tannins (7.40 mg/g) and trypsin inhibitor (1.76 mg/g) compared to non-thermal treatment sample (control) (tannins = 94.4 mg/g and trypsin inhibitor = 8.52 mg/g). The phytic acid of non-thermal treatment sample (control) was significantly ($p < 0.05$) decreased by extrusion at high barrel temperature of 100^o C (0.43 mg/g). Furthermore, extrusion at barrel temperature of 100^o C was the most effective in improving protein digestibility (55.8%). With an increase in temperature process of extrusion cooking and autoclaving, the WSI of treatment sample increased while the WAI decreased. An increase in barrel temperature enhanced the hydrophobicity of proteins as observed from the improvement of EC, ES, PS, FC and OBC values of the extruded samples. The barrel temperature of 100^o C yielded highest EC (60.0%) ES (60.7%), PS (16.53%), FC (13.6%) and OBC (16.3%) in comparison with those of non-thermal treatment (control) (EC=46.5%, ES=37.0%, PS=5.48%, FC=4.90% and OBC=2.1%, respectively). The sample with extrusion at a lower temperature of 80^o C possessed highest antioxidant activities indicated by ABTS^{••} (2.34 mg Trolox /g powder), FRAP (1.07 mg FeSO₄ /g powder) and TPC (2.16 mg gallic acid/g powder). Overall, in vitro protein digestibility, antioxidant activities, antinutritional factor reduction and functional properties of sacha inchi meal was improved by thermal processes. The autoclaving might serve as a tool for ANFs reduction. While, extrusion cooking could improve in vitro protein digestibility, antioxidant activities and functional properties of sacha inchi meal. The sacha inchi meal through thermal processes might be helpful to produce highly nutritious foods, alternative protein related products and enhance its suitability as novel functional ingredients for the food system for industrial applications.

Keywords: sacha inchi meal, thermal treatment, antinutritional factors, physico-chemical properties, antioxidant activity

Introduction

Sacha inchi (*Plukenetia volubilis* L.) was originally cultivated in the Amazon rainforest area of Peru. Sacha inchi seeds have high oil (35–60%) and protein (25–30%) contents (including essential amino acids such as cysteine, tyrosine, threonine, and tryptophan), vitamin E, polyphenols, minerals, and others) (Wang, Zhu, & Kakuda, 2018). The oil obtained from the sacha inchi seeds is an excellent source of polyunsaturated fatty acids, such as 51% alpha-linolenic acid (C18:3, Omega-3) and 34% linoleic acid (C18:2, Omega-6) (Chirinos, Pedreschi, Dominguez, & Campos, 2015). However, after cold press extraction of sacha inchi seeds



generates by-products (seed residue or meal) are discarded as waste or used as animal feeds. Based on the nutritive values of sachu inchi meal especially proteins contents (Wang et al., 2018), it has potential to be utilized as alternative protein in food products. In addition, the by-products have been supported by numerous findings that many polyphenols and other chemically active compounds are located specifically in waste materials (Tian, 2016). As the search for effective and non-toxic natural compounds with antioxidant activity intensifies, it provides means for reusing the waste which is both highly beneficial and economically advantageous. However, plant diets are associated with poor bioavailability of major minerals such as Ca, Fe, Cu, Zn and Mn, vitamins and low protein digestibility (Nikmaram et al., 2017). Although many factors including inadequate dietary intake are responsible for the onset of Zn and Fe deficiency, the most likely cause is the poor bioavailability of dietary Zn and Fe, especially from seed-based food. The negative effects of such antinutritional factors (ANFs) could be suppressed using different processing techniques by lowering or removing of these harmful compounds prior to their human and animal consumption (Krupa, 2008). To improve the nutritional quality of edible plant seeds by thermal treatment are commonly applied (Nadeem et al., 2010). In recent years, several other techniques such as high pressure processing (HPP) and extrusion have been introduced as alternatives to reduce the ANF levels (Nikmaram et al., 2017). Moreover, through these processing technologies, some desirable modification in the functional properties of edible seeds are to be expected, and particularly the removal or reduction of the ANFs could be attained (Li, Qiu, Liu, Ren, & Li, 2014). The thermal processing can improve the functional properties of proteins (e.g., solubility, foaming, gelling, water binding and oil binding properties) (Obradovic, Babic, Subaric, & Ackar, 2014). However, processing at high temperature could cause undesirable physicochemical changes in proteins and other valuable heat sensitive constituents of edible plant seeds (Li et al., 2014).

Extrusion cooking is a multi-step, multi-functional thermal process which utilizes high heat, pressure and shear (Rafe & Sadeghian, 2017). One advantage of extrusion cooking lies in the destruction of ANFs, especially trypsin inhibitors, tannins and phytates and increase phenolic content, antioxidant activities and it has been used to modify the functional properties, such as viscosity, water solubility and water absorption of edible plant seeds. (Kaur, Sharma, Singh, & Dar, 2015; Rathod & Annapure, 2016; Nikmaram et al., 2017; Norajit, Gu, & Ryu, 2018). Other various methods and technologies as autoclaving can be used to reduce the levels of ANFs. In a study conducted by Ertop & Bektas (2018), autoclaving improves functional properties (foaming and emulsifying properties) and increase the nutritional quality of food grains due to reduction in ANFs. Therefore, this study investigated the influence of thermal parameters in extrusion and autoclaving processes at various temperatures on reduction of ANFs (phytic acid, tannins and trypsin inhibition) and enhancement of functional properties (water absorption index (WAI), water solubility index (WSI), protein solubility (PS), foam capacity (FC), emulsifying capacity (EC), emulsion stability (ES) and oil binding capacity (OBC)), antioxidant activities (ABTS^{••}, FRAP assays and total phenolic content (TPC)) as well as the bioavailability (in vitro protein digestibility) of sachu inchi meal.



Methods and Materials

Materials

Sacha inchi meal, a by-product of oil extraction was kindly provided by the Tai.C.M.S. Standard industrial Co., Ltd. (Chaing Rai Province, Thailand). All chemical reagents were of analytical grade and were used without further purification, whereas distilled water was used for the preparation of the reagents.

Extrusion process

The extrusion was carried out on a pilot-scale co-rotating twin-screw extruder (Chareon tut co., ltd, CET-D25L32, Thailand). The extruder was operated at 200 rpm screw speed 10.9 kg/h feed rate and 4 mm circular die. In-barrel moisture content in the extruder was 61.8 %. The barrel zone temperatures were set constant at 45–80 °C; temperature of the transition section between the barrel and the die was set at 80, 90, and 100 °C throughout the experiments. The extrudate was dried directly in an oven at 50 °C for 6 h. The dried extrudate was ground to powder using a stainless steel mixer (Welljun, WJ-1820) and then passed through 0.5 mm sieve (No. 35 mesh US Standard Sieve Series) and stored at 4 °C until analysis.

Autoclaving process

The sachu inchi meal was prepared according to the method described by Polesi and Sarmento (2011) with some modifications. The sachu inchi meal (500 g) was weighed into cheesecloth and the sample was then pressure-cooked in an autoclave at three different sterilizing temperatures (105, 110, 121 °C) for 30 min (vertical pressure sterilization machine model DY04-13-44-0; Shanghai Boxun Co., Ltd, Shanghai, China). The samples were dried directly in an oven at 50 °C for 6 h. The dried sample was ground to powder using a stainless steel mixer and then passed through 0.5 mm sieve and stored at 4 °C until analysis.

Antinutritional factors

Tannic Acid. The tannins in samples were determined according to the method described by Makkar, Blummel, Borowy, and Becker (1993) using Folin-Denis reagent. This method involves the preparation of a standard curve of pure tannic acid. The sample (1 g) was extracted with 40 ml of 10% methanol, then filtrated and adjusted the volume to up to 50 ml. Suitable aliquots of the extract of about 1 ml were taken in a volumetric flask, and 10 ml of 35% sodium carbonate reagent was added, and the volume adjusted to up to 100 ml. Then the absorbance was recorded at 760 nm after 45 min. The amount was calculated as tannic acid equivalent from the standard curve.

Phytic Acid. Phytic acid was determined with a slight modification to the method of Reddy, Balakrishnan, and Salunkhe (1978). About 2 g of the ground sample was taken into a beaker and was soaked in 100 ml of 2% HCl for 5 h, which was then filtered. Then 25 ml of the filtrate was taken into a conical flask and added with 5 mL of 0.3% potassium thiocyanate solution, and the mixture was titrated with a standard solution of FeCl₃. Persistence of brownish-yellow color for 5 min indicates the point. The concentration of the FeCl₃ was 1.04% w/v and the mole ratio of Fe to phytate = 1:1

Concentration of phytate phosphorous = $\text{Titre value} \times 0.064 / 100 \times \text{weight of sample}$

Phytate was calculated by supposing that it contains 20% of phosphorus by weight

Trypsin inhibitor activity. The trypsin inhibitor (TIA; mg/g) of the sample was assayed according to the method of Hamerstrand, Black, & Glover (1981). The trypsin inhibitor extract was prepared by extracting 1 g of sample with 50ml of 0.01M sodium hydroxide for 3 h by mechanical shaking at room temperature. The



suspension was filtered through Whatman paper No 1 and diluted with distilled water (1:60) so that 2 ml of the extract inhibited 40–60% of trypsin used as a standard for analysis. Trypsin activity was determined using N-Benzoyl DL arginine p-nitroanilide (BAPNA) as a substrate.

In vitro protein digestibilities

In vitro protein digestion was carried out according to Garrett, Failla, and Sarama (1999) with some modifications. Briefly, samples (5 g dry weight) were homogenized in distilled water (250 ml) using an IKA homogenizer (IKA Works Asia) for 1 min and adjusted to pH 2.0 with 5 M HCl. Pepsin was added (2.86% [w/w], based on dry substrate), and the samples were incubated in a shaking incubator at 37 °C for 1 h. The pH was adjusted to 5.3 with a 0.9 M NaHCO₃ solution and then further to pH 7.5 with 5 M NaOH. Pancreatin was then added (4.00% [w/w], based on dry substrate), and the samples were further incubated under the same conditions for 2 h. Subsequently, all digested samples were submerged in a 95 °C water bath for 10 min, cooled on ice to room temperature, and centrifuged at 10000 x g for 10 min. The peptide content of the supernatant was determined using the Lowry method with tyrosine as a standard (Wiriyaphan, Chitsomboon, Roytrakul, & Yongsawadigul, 2013). The degree of digestibility was determined based on TNBS according to Adler-Nissen (1979) using leucine as a standard.

Functional properties

Water absorption index (WAI) and water solubility index (WSI)

WAI and WSI were determined according to Lee et al. (2012). The WSI was the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample on dry basis. The WAI was calculated as the weight of sediment obtained after removal of the supernatant per unit weight of original solids as dry basis.

Protein solubility (PS)

The protein solubility was determined by Folin reaction through the method of Lowry, Rosebrough, Farr, and Randall (1951). The appropriate amount of powder was dispersed in distilled water. Then, the solution was dispersed gently at ambient temperature for 15 min. The dispersed samples were centrifuged at 1200 x g for 20 min and the supernatant was decanted and the protein was measured according to Lowry method at 750 nm (UV-visible spectrophotometer, Shimadzu, UV-160A, Japan).

Emulsifying capacity (EC) and emulsion stability (ES)

EC and ES were measured according to the method described by Wang and Kinsella (1976). Five g of sample was suspended in 10 ml of distilled water and 5 g of corn oil and then blended at high speed. After agitation, the samples were centrifuged at 10000 x g for 5 min. The EC was determined as the percentage of the emulsified layer that remained after centrifugation. A similar procedure was followed to determine the ES, but the samples were incubated at 80 °C for 30 min before centrifugation. The ES was calculated as the percentage of the emulsified layer that remained after the heat treatment.

Oil binding capacity (OBC)

The OBC to determine these binding capacities, 1 g of sample was weighed and then stirred into 10 mL of groundnut oil for one minute. These sample suspensions were then centrifuged at 2200 x g for 30 min. OBC was expressed as g of oil held per g of protein sample (Guerrero, Flores, Ancona, & Ortiz, 2002).

Foam capacity (FC)

The FC was determined according to the method described by Sze-Tao and Sathe (2000). FC was then measured as the increased (initial) volume of each sample.



Antioxidant activity

ABTS radical scavenging assay. Free radical scavenging activity of samples was determined by ABTS radical cation decolorization assay (Re et al., 1999). ABTS^{•+} cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12–16 h before use. ABTS^{•+} solution was then diluted with methanol to obtain an absorbance at 730 nm. After the addition of 5 μ l of sample extract to 3.995 ml of diluted ABTS^{•+} solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent of ABTS^{•+} scavenging inhibition was calculated.

Ferric reducing antioxidant power (FRAP) assay. The ability of the samples to reduce iron (III) was determined according to the method of Benzie & Strain (1996) with some modifications. Briefly, the FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (sodium acetate buffer, pH 3.6), 10 mM 4,6-tripyridyls-triazine (TPTZ) in 40 mM HCl and 20 mM ferric chloride in a ratio 5:1:1 (v/v) before evaluation. Two hundred mL of FRAP reagent (preheated to 37^o C) was added to 40 ml of samples (0.125, 0.25, 0.5 and 1 mg/ml) or GSH in a 96-well microplate. Absorbance at 593 nm was measured relative to a reagent blank. Ferrous sulfate (0.025–0.25 mM) was used to prepare a standard curve and the results of the samples were expressed in mM FeSO₄. Increased absorbance of the reaction mixture indicated increased reducing power.

Total phenolic content (TPC). The TPC was determined using the method described by Pinsirodom and Changnoi (2001) with some modifications. To create a standard curve, gallic acid solution was used and the absorbance was measured at 734 nm. Each result was reported as gallic acid equivalent (mg) in 1 g dry sample (mg GAE/1g DM).

Statistical Analysis

Data were collected in triplicates and subjected to one way analysis of variance using statistical analysis of variance (ANOVA) was done to determine the significant differences among means followed by Duncan's new multiple range tests were carried out to determine the significance of differences within a 95%, any significant differences were defined at p<0.05 confidence interval using SPSS 11.0 software (SPSS Inc., Chicago, Ill., U.S.A.).

Results and Discussion

Antinutritional factors (ANFs)

ANFs contents of samples were studied. ANFs have the tendency to bind to dietary proteins and digestive enzymes, which result in the formation of complexes that are not readily digestible (Norajit et al., 2018). The tannic acid content in Table. 1 showed that autoclaving at 121^o C could effectively reduce the amount of tannin in sachai meal. The results show that autoclaving at 121^o C for 30 min caused a significant (p<0.05) reduction in tannins (7.40 mg/g) compared to the non-thermal treatment sample (control) (94.4 mg/g), while extrusion at a high barrel temperature of 100^o C was found to be 65.90 mg/g. Moreover, the amount of tannin reduction in autoclaving at 105–121^o C varied from 35.14 to 7.40 mg/g compared to the extruded sample at 80–100^o C of 84.30 to 65.90 mg/g. The results indicate that an increase in sterilizing temperature of autoclaving led to significant reduction of tannins. These results agree with those of Shimelis and Rakshit (2007) found that autoclaving caused a significant reduction in tannins contents of kidney bean. Tannins are

water-soluble polyphenolic compounds and have the tendency to bind to proteins, which resulted in the formation of complexes that are not readily digestible (Raes, Knockaert, Struijs, & Camp, 2014). The structures of tannins contain several ester linkages which are easily cleaved when at higher temperatures (Mphahlele, Fawole, Makunga, & Opara, 2016). During autoclaving phenolic compounds may undergo decarboxylation due to higher melt temperature, which may promote polymerization of tannins, leading to reduced soluble (Obiang-Obounou & Ryu, 2013). The reduction of tannin may be due to the loss of compounds or interaction with other components such as proteins, to form insoluble complexes (Mphahlele et al., 2016).

The phytic acid content of sachinchi meal through thermal processes are shown in Table 1. The results were significantly ($p < 0.05$) lower for phytic acid content of extruded sample at a high barrel temperature of 100°C (0.43 mg/g). Extrusion at barrel temperatures of 80°C and 90°C has phytic acid content values of 1.53 mg/g and 1.10 mg/g , respectively. Moreover, phytic acid content of autoclaving at 110°C and 121°C was found to be 0.57 mg/g , while autoclaving at 105°C was found to be 0.74 mg/g . The non-thermal treatment (control) had the highest value of phytic acid content of 1.63 mg/g . The extrusion is a method that uses high temperature and shear forces causing a denaturation effect on molecules of phytic acid by changing their structures. Thus, extrusion have a significant impact on lowering of phytates. In addition, reduction in phytic acid content followed by extrusion might be due to the hydrolization of phytates into lower molecular weight forms like penta-, tetra and triphosphates (Kumar, Mani, Aradwad, & Samuel, 2018). Fragmentation of phytates and formation of insoluble complexes with other components could be another reason for phytic acid reduction in the extruded product (Andrews, 2015).

Trypsin inhibitors are low molecular weight proteins capable of binding to and inactivating the digestive enzyme (Yang, Hsu, & Yang, 2014). The autoclaving at 121°C reduced trypsin inhibitor to 1.76 mg/g followed by autoclaving at 110°C (2.52 mg/g), 105°C (6.66 mg/g), extrusion at barrel temperature of 100°C (7.74 mg/g), while non-thermal treatment (control) was found to be 8.52 mg/g . The trypsin inhibitors were thermosensitive, being inactivated at temperatures higher than 100°C (Aviles-Gaxiola, Chuck-Hernandez, & Saldívar, 2018). Therefore, extrusion at a barrel temperature of 80°C and 90°C has high value for trypsin inhibitors at more than 7.74 mg/g . The autoclaving promotes the breakage of intermolecular bonds responsible of holding the tertiary structure of trypsin, which consequently causes changes over the active site conformation (Yang et al., 2014). The autoclave was widely accepted as the most effective way to improve the nutritional value of legumes, because it enhances protein digestibility mainly through the inactivation of trypsin inhibition (Aviles-Gaxiola et al., 2018).

Table 1 Antinutritional properties and in vitro protein digestibility of sachinchi meal through thermal treatments

Thermal treatments	Tannin (mg/g)	Phytic acid (mg/g)	In vitro protein digestibility (%)
Extrusion (80°C)	84.30 ± 0.19^b	1.53 ± 0.05^b	38.87 ± 0.03^f
Extrusion (90°C)	71.11 ± 0.11^c	1.10 ± 0.07^c	42.44 ± 0.06^d
Extrusion (100°C)	65.90 ± 0.16^d	0.43 ± 0.04^f	55.81 ± 0.04^a
Autoclaving (105°C)	35.14 ± 0.02^e	0.74 ± 0.07^d	41.53 ± 0.07^e
Autoclaving (110°C)	20.70 ± 0.04^f	0.57 ± 0.05^e	44.17 ± 0.05^c
Autoclaving (121°C)	7.40 ± 0.07^g	0.57 ± 0.06^e	49.91 ± 0.07^b
Non-thermal treatment sample (control)	94.40 ± 0.22^a	1.63 ± 0.05^a	15.40 ± 0.06^g

Results are presented as mean \pm SD ($n = 3$). For each column, mean values that contain different letters are significantly different at $p < 0.05$



In vitro protein digestibility

The effects of thermal processes on in vitro protein digest are depicted in Table 1. A significant ($p < 0.05$) increase in vitro protein digestibility was observed by extrusion at a high barrel temperature of 100°C (55.81%), followed by autoclaving at 121°C for 30 min (49.91%). In vitro protein digestibility in the extruded sample at 80°C and 90°C was 38.87% and 42.44% compared to autoclaving at 105°C and 110°C of 41.53% and 44.17%, respectively. Non-thermal treatment (control) showed the lowest in vitro protein digestibility of 15.40%. Thermal treatment by extrusion increased shearing action and developed heat through dissipation of mechanical energy then caused the loss of structural integrity and finally increased the digestive enzyme susceptibility. Improvement of protein digestibility after thermal processes could be attributed to the reduction or elimination of antinutritional factors. Exposure to high temperatures may increase the digestibility of native proteins by unfolding the polypeptide chain and rendering the protein more susceptible to digestive enzymes (Onesmo, 2011). In addition, thermal processing promoted structural changes of protein, thereby increasing chain flexibility and accessibility to proteases (Yu-Wei & Wei-Hua, 2013).

Functional properties

The protein solubility (PS) is an important prerequisite in food protein functionality and is a good index for potential of protein applications. The PS of samples are shown in Table 2. Results indicate that extrusion at barrel temperature of 100°C was highest PS (16.53%) in comparison with autoclaving at 105 – 121°C and non-thermal treatment (control) (PS=5.48%) due to high temperature and shear lead to the protein degradation and the release of low molecular weight compounds. Furthermore, high temperature processes can change the molecular structure of proteins or breakdown of S–S bonds with the release of H_2S , the release of NH_3 from amide groups, dissociation of subunits and /or breakdown of these subunits into compounds of small molecular weights (Esmaeili, Rafe, Shahidi, & Hasan-Saraei, 2016), which may increase the PS of the sample.

The effects of thermal processes on WSI and WAI are shown in Figure 1. Increasing in barrel temperature had significant increases in WSI of extruded. The highest value of WSI (5.63%) was in extrusion in barrel temperature of 100°C . Furthermore, it seems that the PS of sachinchi can be affected by other components such as the dietary fibre, which may be attached to the protein and improve its solubility. Besides, the high mechanical shear and temperature caused breakdown of macromolecules to small molecules with higher solubility (Pardh, Singh, Nayik, & Dar, 2019). However, WSI of all samples increased while the WAI decreased as presented in Figure 1. The WAI decreased with an increase temperature process, probably attributed to the reduction of elasticity of protein and starch gelatinization and swelling of the crude fiber, which occurred during the process responsible for the decreased WAI (Esmaeili et al., 2016).

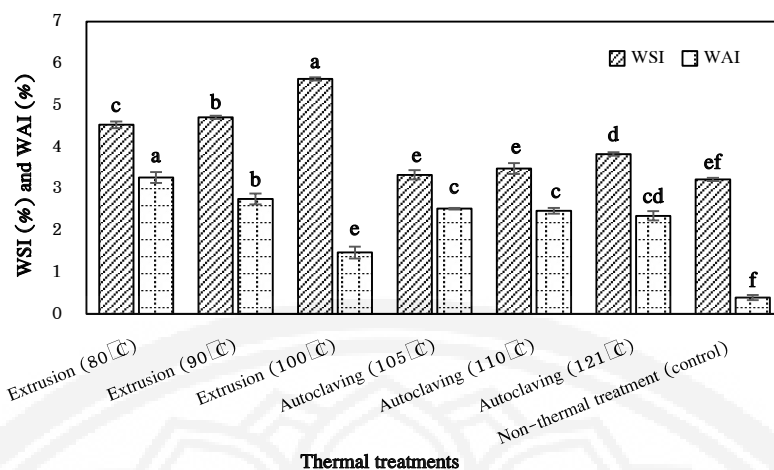


Figure 1 Effect of thermal treatments on WAI and WSI of sachu inchi meal

It has been reported that the PS has a close relationship with emulsifying and foaming properties (Gharbi, Labbafi, & Madadlou, 2017). An increase of the barrel temperature enhanced the hydrophobicity of proteins as observed from the improvement of EC, ES, FC and OBC of proteins after extrusion (Table 2). A significant ($p < 0.05$) increase in functional properties was observed by extrusion at a high barrel temperature of 100°C . Extruding at 100°C yielded the sample with the highest EC (60.0%), ES (60.7%), FC (13.6%) and OBC (16.3%), compared to those of autoclaving at a high temperature of 121°C , EC= 53.58%, ES= 52.42%, FC=8.22% and OBC=15.21%. The non-thermal treatment (control) was EC=46.5%, ES=37.0%, FC=4.9% and OBC=2.1%. It could be explained that heat caused the protein molecules to change their shapes, resulted in proteins with smaller sizes (monomers or oligomers). In addition, heat did not only make the protein molecule to be loosen but also it could break the bond between the monomer of protein molecules (Gharbi et al., 2017). As a result, an increase in the number of free sulfhydryl groups and the hydrophobic group on the surface of the protein molecules occurred during heat treatment. Therefore, denatured proteins could be easily absorbed between the air and water or oil and water. Generally, the emulsifying activities of proteins were affected by molar mass, hydrophobicity, conformation stability and charge and temperature. Solubility also plays an important role since highly soluble proteins were good emulsifiers (Peng et al., 2016). An improvement of emulsifying properties by heat treatment may be mainly attributed to the increased hydrophobic surface and flexibility, as a consequence of thermal denaturation (Pardhi et al., 2019).

Table 2 Functional properties of sachu inchi meal through thermal treatments

Thermal treatments	PS (%)	EC (%)	ES (%)	OBC (%)	FC (%)
Extrusion (80°C)	13.56 ± 0.04^c	46.51 ± 0.23^c	47.11 ± 0.32^c	15.71 ± 0.07^b	5.99 ± 0.02^d
Extrusion (90°C)	13.95 ± 0.21^c	53.33 ± 0.11^b	51.46 ± 0.08^c	15.84 ± 0.01^b	9.26 ± 0.04^b
Extrusion (100°C)	16.53 ± 0.07^a	60.00 ± 0.14^a	60.74 ± 0.07^a	16.32 ± 0.07^a	13.60 ± 0.03^a
Autoclaving (105°C)	13.20 ± 0.22^c	49.56 ± 0.01^d	50.97 ± 0.03^d	13.92 ± 0.01^d	4.86 ± 0.06^e
Autoclaving (110°C)	13.56 ± 0.04^c	52.67 ± 0.11^c	52.33 ± 0.13^b	14.55 ± 0.14^c	$5.98 \pm 0.08_d$
Autoclaving (121°C)	14.93 ± 0.11^b	53.58 ± 0.12^b	52.42 ± 0.27^b	15.21 ± 0.08^b	8.22 ± 0.05^c
Non-thermal treatment sample (control)	5.48 ± 0.03^d	46.50 ± 0.08^e	37.00 ± 0.11^f	2.18 ± 0.06^e	4.90 ± 0.02^e

Results are presented as mean \pm SD ($n = 3$). For each column, mean values that contain different letters are significantly different at $p < 0.05$



Antioxidant activity

The antioxidants play an important role in protection against damage caused by the oxidant, free radicals and provides other health benefits (Nayak et al., 2011). The antioxidant activities and total phenolic content (TPC) of all samples are presented in Table 3. Extrusion at a low barrel temperature of 80° C showed significantly ($p < 0.05$) higher ABTS^{••} (2.34 mg Trolox/g powder), FRAP (1.07 mg FeSO₄/g powder) and TPC (2.16 mg gallic acid/g powder) compared to autoclaving at a low temperature of 105° C showing ABTS^{••} (1.68 mg Trolox/g powder), FRAP (0.59 mg FeSO₄/g powder) and TPC (1.54 mg gallic acid/g powder). The non-thermal treatment sample (control) showed the lowest ABTS^{••} (0.22 mg Trolox/g powder), FRAP (0.32 mg FeSO₄/g powder) and TPC (0.60 mg gallic acid/g powder). The level of antioxidant activity in extruded products increased when temperature increased. Alterations of the structure of the existing antioxidants and the formation of novel antioxidant components may enhance the initial antioxidant status varieties (Sharma, Pasricha, Satpathy, & Gupta, 2015). The level of antioxidant activity in extruded products increased when temperature increased. Alterations of the structure of the existing antioxidants and the formation of novel antioxidant components may enhance the initial antioxidant status varieties (Sharma et al., 2015). This suggests that processing of edible seeds meal by extrusion did not cause a drastic loss in antioxidant values, which is in accordance with the previous finding (Chaaban et al., 2017) that indicates that heating enhanced antioxidant activity in plant because of the enhancement of the antioxidant properties of naturally occurring compounds or the formation of novel compounds such as Maillard reaction products that had antioxidant activity. As mechanical process under temperature and pressure leads to the breakdown of cell wall structure, which might contribute to the release of compounds and their derivatives such as phenolic acids (particularly ferulic acid) that have antiradical potential (Wani & Kumar, 2016). Repo-Carrasco-Valencia and Serna (2011) observed the similar trend and found that the free radicals scavenging activity increased during the extrusion process of quinoa. However, the antioxidants activities are less resistant to temperature above 80° C may be due to the damage or change in the nature structure (Chaaban et al., 2017). The antioxidant activity may decrease with the loss of antioxidants or with the formation of compounds having pro-oxidant action (Wani & Kumar, 2016). The decrease in total TPC may be ascribed either to the disintegration of phenolic compounds as a result of high temperature and pressure during process or to the change in molecular arrangement of phenolic compounds that can cause decline in the chemical reactivity of phenolic compounds or reduce their extractability because of certain degree of polymerization (Altan, McCarthy, & Maskan, 2009).

Table 3 Antioxidant activities and total phenolic content of sachu inchi meal through thermal treatments

Thermal treatments	ABTS	FRAP	TPC
	(mg Trolox/g powder)	(mg FeSO ₄ /g powder)	(mg gallic acid/g powder)
Extrusion (80° C)	2.34 ± 0.04 ^a	1.07 ± 0.15 ^a	2.16 ± 0.04 ^a
Extrusion (90° C)	1.95 ± 0.08 ^b	0.85 ± 0.09 ^b	1.86 ± 0.06 ^b
Extrusion (100° C)	1.82 ± 0.11 ^c	0.73 ± 0.05 ^c	1.75 ± 0.07 ^c
Autoclaving (105° C)	1.68 ± 0.09 ^d	0.59 ± 0.01 ^d	1.54 ± 0.09 ^d
Autoclaving (110° C)	1.26 ± 0.04 ^e	0.31 ± 0.02 ^e	1.35 ± 0.02 ^e
Autoclaving (121° C)	1.10 ± 0.04 ^f	0.08 ± 0.05 ^f	1.11 ± 0.05 ^f
Non-thermal treatment sample (control)	0.22 ± 0.01 ^g	0.32 ± 0.07 ^e	0.60 ± 0.09 ^e

Results are presented as mean ± SD (n = 3). For each column, mean values that contain different letters are significantly different at $p < 0.05$



Conclusion and Suggestions

From the above study, it was found that thermal treatments of sachu inchi meal by autoclaving or extrusion caused the destruction of antinutritional factor such as tannins, phytates and trypsin inhibitor. Interestingly, autoclaving at 121^o C for 30 min caused a reduction in tannins (7.40 mg/g) and trypsin inhibitor (1.76 mg/g). In addition, the phytic acid was decreased by extrusion at a barrel temperature of 100^o C (0.43 mg/g). The extruded sample at barrel temperature of 100^o C was the most effective for improving in vitro protein digestibility (55.8%) and functional properties (EC=60.0%, ES=60.7%, PS=16.5%, FC=13.6% and OBC=16.3%). Meanwhile, the extruded sample at a barrel temperature of 80^o C showed the highest antioxidant activities (ABTS^{•+}= 2.34 mg Trolox /g powder, FRAP=1.07 mg FeSO₄ /g powder and TPC=2.16 mg gallic acid/g powder). Thus, there is an opportunity to apply this versatile processing technique to produce highly nutritious foods for human consumption and develop innovative products tune to the changing needs of consumers.

Acknowledgments

This work received funding support from The Research and Researcher for Industry (RRI) (Grant#PHD59I0078), Thailand Science and Research Innovation (TSRI). We would like to thank Tai.C.M.S. Standard industrial Co., Ltd. (Chaing Rai Province, Thailand), Ometien Co. Ltd. (Chaing Rai Province, Thailand) for supplying raw materials and co-funding for conducting this study.

References

- Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry*, 27(6), 1256-1262.
- Altan, A., McCarthy, K. L., & Maskan, M. (2009). Effect of extrusion cooking on functional properties and in vitro starch digestibility of barley based extrudates from fruit and vegetable by-products. *Journal of Food Science*, 74(2), 77-86.
- Andrews, R. (2015). *Phytates and phytic acid*. Retrieved from <http://www.precisionnutrition.com/all-about-phytates-phytic-acid>
- Aviles-Gaxiola, S., Chuck-Hernandez, C., & Saldívar, S. O. S. (2018). Inactivation Methods of Trypsin Inhibitor in Legumes: A Review. *Journal of Food Science*, 83(1), 17-29.
- Benzie, I., & Strain, J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power: The FRAP Assay. *Analytical Biochemistry*, 239(1), 70-76.
- Chaaban, H., Ioannou, L., Chebil, L., Slimane, M., Gerardin, C., Paris, C., ... Ghoul, M. (2017). Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *Journal of Food Processing and Preservation*, 41(5), 13203.
- Chirinos, R., Pedreschi, R., Dominguez, G., & Campos, D. (2015). Comparison of the physico-chemical and phytochemical characteristics of the oil of two *Plukenetia* species. *Food Chemistry*, 173(15), 1203-1206.



- Ertop, M. H., & Bektas, M. (2018). Enhancement of Bioavailable Micronutrients and Reduction of Antinutrients in Foods with Some Processes. *Food and Health*, 4(3), 159–165.
- Esmaceli, M., Rafe, A., Shahidi, S. A., & Hasan-Saraei, A. G. (2016). Functional properties of rice bran protein isolate at different pH levels. *Cereal Chemistry*, 93(1), 58–63.
- Garrett, D. A., Failla, M. L., & Sarama, R. J. (1999). Development of an in vitro digestion method to assess carotenoid bioavailability from meals. *Journal of Agricultural and Food Chemistry*, 47(10), 4301–4309.
- Gharbi, N., Labbafi, M., & Madadlou, A. (2017). Effect of heat treatment on foaming properties of ostrich (*Struthio camelus*) egg white proteins. *International Journal of Food Properties*, 20(12), 3159–3169.
- Guerrero, L. C., Flores, V. P., Ancona, D. B., & Ortiz, G. D. (2002). Functional properties of flours and isolates from *Phaseolus lunatus* and *Canavalia ensiformis* seeds. *Journal of Agricultural and Food Chemistry*, 50(3), 584–591.
- Hamerstrand, G. E., Black, L. T., & Glover, J. D. (1981). Trypsin inhibitors in soy products: Modifications of the standard analytical procedure. *Cereal Chemistry*, 58(1), 42–45.
- Kaur, S., Sharma, S., Singh, B., & Dar, B. (2015). Effect of extrusion variables (temperature, moisture) on the antinutrient components of cereal brans. *Journal of Food Science and Technology*, 52, 1670–1676.
- Krupa, U. (2008). Main nutritional and antinutritional compounds of bean seeds—a review. *Polish Journal of Food and Nutrition Sciences*, 58(2), 149–155.
- Kumar, A., Mani, I., Aradwad, P., & Samuel, D. V. K. (2018). Effect of extrusion technique on antinutritional factors of sorghum–soya blends. *Indian Journal of Agricultural Sciences*, 88(3), 420–428.
- Lee, I., We, G. J., Kim, D. E., Cho, Y. S., Yoon, M. R., Shin, M., & Ko, S. (2012). Classification of rice cultivars based on cluster analysis of hydration and pasting properties of their starches. *LWT - Food Science and Technology*, 48(2), 164–168.
- Li, H., Qiu, J., Liu, C., Ren, C., & Li, Z. (2014). Milling characteristics and distribution of phytic acid, minerals, and some nutrients in oat (*Avena sativa* L.). *Journal of Cereal Science*, 60(3), 549–554.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with folin phenol reagent. *The Journal of Biological Chemistry*, 193, 265–275.
- Makkar, H. P. S., Blummel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture*, 61(2), 161–165.
- Mphahlele, R. R., Fawole, O. A., Makunga, N. P., & Opara, U. L. (2016). Effect of drying on the bioactive compounds, antioxidant, antibacterial and antityrosinase activities of pomegranate peel. *BMC Complement Altern Med*, 16, 143.
- Nadeem, M., Anjum, F. M., Amir, R. M., Khan, M. R., Hussain, S., & Javed, M. S. (2010). An overview of anti-nutritional factors in cereal grains with special reference to wheat—A review. *Pakistan Journal of Food Sciences*, 20(1–4), 54–61.
- Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., ... Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. *Food Control*, 79, 62–73.
- Norajit, K., Gu, B. J., & Ryu, G. H. (2011). Effects of the addition of hemp powder on the physicochemical properties and energy bar qualities of extruded rice. *Food Chemistry*, 129(4), 1919–1925.



- Obiang-Obounou, B. W., & Ryu, G. H. (2013). The effect of feed moisture and temperature on tannin content, antioxidant and antimicrobial activities of extruded chestnuts. *Food Chemistry*, *141*(4), 4166–4170.
- Obradovic, V., Babic, J., Subaric, D., & Ackar, D. (2014). Improvement of nutritional and functional properties of extruded food products. *Journal of Food and Nutrition Research*, *53*(3), 189–206.
- Onesmo, N. O. (2011). *Effects of Malting and Fermentation on the Composition and Functionality of Sorghum Flour*. University of Nebraska: Lincoln.
- Pardh, S. D., Singh, B., Nayik, G. A., & Dar, B. N. (2019). Evaluation of functional properties of extruded snacks developed from brown rice grits by using response surface methodology. *Journal of the Saudi Society of Agricultural Sciences*, *18*(1), 7–16.
- Peng, W., Kong, X., Chen, Y., Zhang, C., Yang, Y., & Hua, Y. (2016). Effects of heat treatment on the emulsifying properties of pea proteins. *Food Hydrocolloids*, *52*, 301–310.
- Pinsirodom, P., & Changnoi, W. (2001). Comparison of total polyphenol content and antioxidant potential of extracts obtained from seeds of different citrus fruits cultivated in Thailand. *Food*, *34*, 300–307.
- Polesi, L. F., & Sarmiento, S. B. S. (2011). Structural and physicochemical characterization of RS prepared using hydrolysis and heat treatments of chickpea starch. *Starch – Starke*, *63*(4), 226–235.
- Rafe, A., & Sadeghian, A. (2017). Stabilization of Tarom and Domesiah cultivars rice bran: Physicochemical, functional and nutritional properties. *Journal of Cereal Science*, *74*, 64–71.
- Rathod, R. P., & Annapure, U. S. (2016). Effect of extrusion process on antinutritional factors and protein and starch digestibility of lentil splits. *LWT – Food Science and Technology*, *66*, 114–123.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237.
- Reddy, N. R., Balakrishnan, C. V., & Salunkhe, D. K. (1978). Phytate phosphorus and mineral changes during germination and cooking of black gram (*Phaseolus mungo*) seeds. *Journal of Food Science*, *43*(2), 540–543.
- Repo-Carrasco-Valencia, R. A. M., & Serna, L. A. (2011). Quinoa (*Chenopodium quinoa*, Willd.) as a source of dietary fiber and other functional components. *Food Science and Technology (Campinas)*, *31*(1), 225–230.
- Shimelis, E. A., & Rakshit, S. K. (2007). Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chemistry*, *103*(1), 161–172.
- Sze-Tao, K. W. C., & Sathe, S. K. (2000). Walnuts (*Juglans regia* L.): proximate composition, protein solubility, protein amino acid composition and protein in vitro digestibility. *Journal of the Science of Food and Agriculture*, *80*(9), 1393–1401.
- Raes, K., Knockaert, D., Struijs, K., & Camp, J. V. (2014). Role of processing on bioaccessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. *Trends in Food Science & Technology*, *37*(1), 32–41.
- Sharma, K., Pasricha, V., Satpathy, G., & Gupta, R. K. (2015). Evaluation of phytochemical and antioxidant activity of raw *Pyrus communis* (1), an under exploited fruit. *Journal of Pharmacognosy and Phytochemistry*, *3*(5), 46–50.



- Tian, X. (2016). Food Processing By-Products as Natural Sources of Antioxidants: A Mini Review. *Advances in Food Technology and Nutritional Sciences*, 2, 7-17.
- Wang, J. C., & Kinsella, J. E. (1976). Functional properties of novel proteins: Alfalfa leafprotein. *Journal of Food Science*, 41(2), 286-292.
- Wang, S., Zhu, F., & Kakuda, Y. (2018). Sacha inchi (*Plukenetia volubilis* L.): Nutritional composition, biological activity, and uses. *Food Chemistry*, 265, 316-328.
- Wani, S. A., & Kumar, P. (2016). Effect of Extrusion on the Nutritional, Antioxidant and Microstructural Characteristics of Nutritionally Enriched Snacks. *Journal of Food Processing and Preservation*, 40(2), 166-173.
- Wiriaphan, C., Chitsomboon, B., Roytrakul, S., & Yongsawadigul, J. (2013). Isolation and identification of antioxidative peptides from hydrolysate of threadfin bream surimi processing byproduct. *Journal of Functional Foods*, 5(4), 1654-1664.
- Yang, H. W., Hsu, C. K., & Yang, Y. F. (2014). Effect of thermal treatments on anti-nutritional factors and antioxidant capabilities in yellow soybeans and green-cotyledon small black soybeans. *Journal of the Science of Food and Agriculture*, 94(9), 1794-1801.
- Yu-Wei, L., & Wei-Hua, X. (2013). Effect of different processing methods on certain antinutritional factors and protein digestibility in green and white faba bean (*Vicia faba* L.). *Journal of Food*, 11(1), 43-49.