Efficiency of protein extraction from palm kernel cake via different chemical extraction methods

Namfon Chaipet¹, Marisa Raita², Wanwipa Siriwatwechakul¹, Verawat Champreda²

¹Sirindhorn International Institute of Technology, Thammasat University – Rangsit Campus, 99 Moo 18, on Phahonyothin Road, Klong Luang, Pathum Thani 12120, Thailand
²National Center for Genetic Engineering and Biotechnology - Integrative Biorefinery Laboratory (IBL) 113 Thailand Science Park, Phahonyothin Road, Klong Nueng, Klong Luang, Pathum Thani 12120 Thailand

*Email: wanwipa@siit.tu.ac.th

Abstract. Protein isolation from defatted palm kernel cake (DPKC) was investigated under 3 conditions, including different chemical reagents (alkaline, saline, acidic), concentrations in the range of 0.01-0.3M and reaction time (0.5-4 h). The reaction contained 1 g biomass in 50 mL of solutions, with mixing speed 250 rpm, and the extraction occurred at 35°C for 2 hrs. Extractable protein of 35.06%, 12.40% and 4.34% based on available protein in the raw material were obtained in aqueous of 0.25M NaOH, 0.01M NaCl and 0.1M HCl. Afterward, DPKC was isolated with 0.25M NaOH for 0.5, 1, 2, 3 and 4 hrs, achieving the protein concentration of 24.16%, 28.99%, 32.72%, 34.39%, and 34.84%, respectively. The protein isolation process resulted in 48.32-60.57 %w/w and 7.27-34.60 %w/w removals of protein and ash, according to analysis of the solid residues. The oil extraction process mostly effected the composition of lignin, which can remove lignin up to 54.71% (w/w).

1. Introduction

Oil palm is a major economic crop in Malaysia, Indonesia, and Thailand and a main source for producing cooking oil and biodiesel. Generally, the palm oil industry provides in crude palm oil of 22% and palm residues of 78% based on a weight basis [1]. Several by-products including empty palm fruit bunch (EPFB), palm fiber (PF), palm kernel shell (PKS) and palm kernel cake (PKC) are main palm wastes in industrial palm oil processing. PKC is palm residue obtained from cracking and pressing of palm nuts. It can be used for producing animal feed supplement in ruminants, swine, and poultry due to its high protein and fiber content, approximated at 15-21% and 50% respectively [2, 3]. The variety of essential amino acids from PKC such as Arginine, Valine, Methionine, Cysteine, Lysine, and others have been reported in previous works [4], leading to the possibility of its use as food supplements in infants, preschool children, and adults [5]. Moreover, the hydrolyzed protein from PKC showed a high value of antioxidant activities [6].

Many researches have reported protein isolation from PKC using physical and chemical treatment such as alkaline, salt-alkaline, and enzymatic methods [6-8]. The chemical method such as alkali treatment
leads to a simple and low cost process. However, the method provides lower efficiency, generates of unwanted products, and produces wastewater treatment. Arifin et al (2009) had reported the use of alkaline and salt treatment in protein extraction of PKC [9]. It was found that the isolated protein concentration from alkaline treatment based on 0.03 M NaOH at 35°C for 2 hr with the ratio of 30:1 liquid/solid showed in 38.51% (g/g) of biomass. While, protein concentration of saline treatment was 25.81% (g/g) contained in 0.2 M NaCl at 35°C for 2 hr with the ratio of 60:1 liquid/solid [9]. The increase of reaction temperature at 80°C extracted PKC protein by 0.03 M NaOH for 4 h resulted in higher soluble protein with 56.6% as reported by Choi and Mohamad (2019). The obtained protein had potential for using in cosmetic products [10]. Besides, a study on the synergistic method of protein extraction using alkaline and enzymatic treatment found that papain gave the extractable protein with the highest antioxidant activity compared to the other protease enzymes (Alcalase, Chymotrypsin, Pepsin, Trypsin, Flavourzyme, and Bromelain). The extracted protein can be considered for its potential use in food industries [6]. In addition to alkaline, salt alkaline and enzyme methods, some studies investigated extraction of protein from plants using acidic solution. Eromosele et al (2007) studied the effects of pH in extracting protein from African yam bean. The protein was extracted with distilled water, adjusted to pH 1-12 with HCl and NaOH, and extracted at room temperature (ca. 30°C) for 2 hr [11]. The extraction resulted in protein concentration of 14.8% and 18.4% at pH 2 and pH 10, respectively [11].

This work studied the efficiency of protein extraction from PKC using different chemical reagents (saline, acidic, and alkaline solution), reagent concentration, and reaction time. The efficiency of protein extraction was evaluated based on the soluble protein by Bradford method. In addition, the effect of oil and protein extraction process was evaluated in form of % removal and % soluble liquid product recovery (by-product).

2. Materials and methods

2.1. Material

Palm kernel cake (PKC) obtained from Suksomboon Palm Oil, Co. Ltd. (Chonburi, Thailand). The biomass was physically grinded by a cutting mill (Retsch ZM2000, Haan, Germany), and sieved (mesh #14-40). The milled PKC was dried at 70°C for 3 days, and stored in plastic bag at room temperature before use. All analytical grade chemicals were provided from major chemical suppliers (Sigma-Aldrich, Fluka, and Merck).

2.2. Oil extraction

The PKC oil extraction was performed in a Soxhlet extractor using hexane as a solvent with the ratio of 1:35 (w/v) for 6 h. After that, the mixture of hexane and oil was distillated by a rotary evaporator (USA N-1200BV-W). The isolated oil was dried at 100±3°C, until the weight remained unchanged following the AOAC method (2014) [12]. The percentage of fat removal was calculated according to Equation 1. Whereas, the defatted solid sample was dried at 70°C for 3 days, and then weighed before further use.

\[
\text{Fat removal} (% \text{w/w}) = \frac{\text{Weight of extracted oil (g)}}{\text{Initial weight of oil in sample (g)}} \times 100
\]  

1

2.3. Protein extraction

The defatted PKC was extracted by different aqueous media that were alkali (NaOH), salt (NaCl pH 9), and acid (HCl) under the ratio of solid and liquid (1:50 (w/v)) at 35°C for 2 h, and stirred at 250 rpm on multi-position hot plate stirrer (CHINCAN, SP200-2T, China). The reaction was performed by different aqueous media concentrations (0.01-0.3 M) and mixing times (0.5-4 h). The slurry from protein extraction was separated by filtered through Whatman paper No.4. The solid residue after protein extraction was washed by distilled water until neutral, and then dried 70°C for 3 days before analyzing the biomass composition [13]. The concentration of soluble protein obtained from liquid phase was
analyzed by Bradford assay using bovine serum albumin as a standard [14]. The protein concentration was determined according to Equation 2. The concentration of the extracted protein was based on percentage of protein recovered in the soluble phase compared to the available protein in the raw material.

\[
\text{Protein concentration (\% \text{g/g protein})} = \left( \frac{\text{Weight of total protein in supernatant (gram)}}{\text{Weight of total protein in PKC (gram)}} \right) \times 100
\]  

(2)

2.4. Biomass composition

Chemical biomass compositions (cellulose, hemicelluloses, lignin and ash) were analysed by the National Renewable Energy Laboratory (NREL) method [8]. The biomass samples (0.3 g) was added to 3 mL of 72\% (v/v) H\textsubscript{2}SO\textsubscript{4}, and then vortexed for 2 h. Afterward, the sample mixture was added to 84 mL of distilled water, and sterilized at 121°C for 1 h. The liquid phase was adjusted to pH 7 with CaCO\textsubscript{3} powder before analysing the sugar profiles and acid soluble lignin. The sugar profile was analysed by high-performance liquid chromatography (SPD-M10A DAD, Shimadzu, Japan) equipped with a refractive index detector using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65°C with 5 mM H\textsubscript{2}SO\textsubscript{4} as the mobile phase at a flow rate of 0.5 ml/min. The acid soluble lignin was determined by UV-Visible spectrometer at 240 nm. Whereas, the solid residue was filtered through crucible por. 5, and then burnt in the furnace at 575°C for 3 h for analysing the acid insoluble lignin. The 0.3 g of biomass sample was burnt in the furnace at 575°C for 3 h for analysing ash content. The efficiency of the operated conditions was determined in term of the percentage of pulp yield, hemicellulose removal, and lignin removal according to the Equation 3-5, respectively.

\[
\text{Pulp yield (\%w/w) =} \frac{\text{Final weight after pretreated PKC (g)}}{\text{Initial weight of raw PKC (g)}} \times 100
\]  

(3)

\[
\text{Hemicellulose removal (\%w/w) =} \frac{\text{H}_{I}-(\text{H}_{F} \times \% \text{pulp yield})}{\text{H}_{I}} \times 100
\]  

(4)

Where hemicellulose removal is the percentage of hemicelluloses removed after protein extraction compared with the initial hemicellulose content; \( \text{H}_{I} \) is the percentage of initial hemicellulose content; \( \text{H}_{F} \) is the percentage of remaining hemicellulose content in the final solid recovery.

\[
\text{Lignin removal (\%w/w) =} \frac{\text{L}_{I}-(\text{L}_{F} \times \% \text{pulp yield})}{\text{L}_{I}} \times 100
\]  

(5)

Where lignin removal is the percentage of lignin removed after protein extraction compared with the initial lignin content; \( \text{L}_{I} \) is the percentage of initial lignin content; \( \text{L}_{F} \) is the percentage of remaining lignin content in the final solid recovery.

The oil content was extracted with light petroleum for 6 h in a Soxhlet extractor according to AOAC method. The extracted fat was weighed, and calculated as a percentage of the sample [12]. The protein content of PKC sample was determined according to Kjeldahl method (protein conversion factor of 6.25 following the method of AOAC (2000) [15]. The biomass sample (1.5 g) was mixed with 20 mL of 98\% v/v sulfuric acid, and added to 14 g of K\textsubscript{2}SO\textsubscript{4} and 1.6 g of CuSO\textsubscript{4} \cdot 5H\textsubscript{2}O. The sample was digested at 420°C until the color changed, and then added 100 mL of distilled water in the mixture. After that, it was distilled, and 100 mL of NaOH was added during process. The NH\textsubscript{3} gas from distillation process was trapped in boric acid solution contained in 1 mg of methyl red, 1 mg of bromocresol green, and 1 mL of ethanol. Later, the boric acid solution was titrated with 0.1 N HCl to the endpoint. The protein content was determined according to Equation 6-7.
Kjeldahl nitrogen, % = \frac{(V_s - V_b) \times M \times 14.01}{W \times 10 \times A} \quad (6)

Protein content (%w/w) = \%Kjeldahl nitrogen \times F \times P \quad (7)

Where
- \( V_s \) = volume (mL) of standardized acid used to titrate a test
- \( V_b \) = volume (mL) of standardized acid used to titrate reagent blank
- \( M \) = molarity of standard HCl
- 14.01 = atomic weight of N
- \( W \) = weight (g) of test portion or standard
- 10 = factor to convert mg/g to percent
- \( A \) = the accuracy of procedure and equipment as 0.8244
- \( F \) = factor to convert N to protein (PKC conversion factor of 6.25)
- \( P \) = Pulp yield (%)

The protein content (P_F) of biomass can be used to calculate the efficiency of protein removal of each condition. The equation was expressed as:

\[
\text{Protein removal} (\%\text{w/w}) = \left( \frac{P_I - P_F}{P_I} \right) \times 100
\]

Where protein removal is the percentage of protein removed after protein isolation process compared with the initial protein content; \( P_I \) is the percentage of initial protein content; \( P_F \) is the percentage of protein content in the final solid recovery.

3. Results and discussion

3.1 Raw PKC composition

The chemical composition of raw PKC is shown in Figure 1. The result showed that the PKC sample contained 46.43% carbohydrate by weight, which were divided into 7.04% cellulose and 39.39% hemicellulose. The other major components included 20.03% lignin, 17.37% crude protein, 12.65% of oil/fat, and 4.02% of ash on a dry weight basis. Normally, the protein content of PKC was in a range of 14.5-19.6% (w/w), carbohydrates 50-62% (w/w) and fat content depending on the extraction methods and the type of palm [4].

![Figure 1. Proximate compositions of the raw material on a dry weight basis](image-url)
3.2 Oil extraction
The PKC oil was extracted by hexane, and the result showed that the pulp yield after defatted PKC was 81.71%, leading to 90.04% (w/w) fat removal (Table 2 and 3). After oil extraction, the composition of defatted PKC composed of cellulose 6.99%, hemicellulose 38.35%, lignin 9.07%, ash 3.67%, fat 1.26% and protein 17.11% by weight (Table 2). The result showed that the process affected the composition of oil and lignin, resulting in lignin removal up to 54.71% (w/w). Chang et al. 2014 extracted oil from PKC with hexane at room temperature (24°C) for 6 h. The defatted PKC still contained oil in pulp up to 7.87% (w/w), but the research did not report in % protein yield from alkaline extraction because the process was preparing protein isolates to hydrolyse with enzymes [16]. However, Lau et al 2015 reported that extracting protein from mesocarps of palm oil was challenging because of high level of interfering compounds such as oil, polysaccharide and phenolics. They found that oil removing could improve the efficiency of protein extraction [17].

3.3 Protein extraction
3.3.1. The effect of chemical reagent types and concentrations. The effect of chemical reagent types (NaOH, NaCl, and HCl) and concentrations (0.01-0.3 M) on protein extraction was studied. The investigation included the ratio of solid and liquid (1:50, w:v) at 35°C for 2 h with mixing speed 250 rpm, as shown in Figure 2. An increase in NaOH concentration from 0.01 to 0.3 M led to an increase in the amount of the extracted protein from 5.88% to 35.06%. Particularly, the extraction with 0.25 M NaOH achieved the highest extraction with 35.06% of protein extracted compared to the other chemical reagents. Increasing NaCl concentration from 0.01 to 0.3 M with pH adjusted to 9 resulted in the decline in the protein extraction (6.01-12.40%). The isolated protein extracted by HCl solution showed the lowest protein content with 3.05-4.34%.

The highest protein isolated by saline and acid treatment was 12.40 and 4.34% with 0.01 M of NaCl and 0.1 M of HCl, respectively. The use of NaOH in protein extraction provided disruption of the biomass structure and increased the protein solubility. The high concentration of NaOH helps to break down hydrogen bond of polypeptide. Thereby, the surface change of protein would increase, and then the protein would have more ability to dissolve in water. The result of protein extracted from PKC by NaOH treatment is comparable to that of previous works [5]. The addition of 1.5% sodium hexametaphosphate–assisted (SHMP) in alkaline treatment adjusted to pH 10 led to protein yield at 28.37% contained at 50°C for 1 h [5]. Besides, the protein concentration extracted from different feedstocks such as red pepper seed [18], watermelon seed [19], quinoa seed [20], Africa yam bean [11], cottonseed [21] and rice bran [22] using alkaline process was the range of 12-84%.

![Figure 2. Effect of chemical reagents and concentrations on the protein concentration](image-url)

The analysis of Pearson Product Movement Correlation was calculated by using 2-tailed (significant at the 0.05 level) to compare the effects of chemical reagents and concentrations on protein concentration (Table 1). It found that increase of soluble protein concentration extracted by NaOH concentration
significantly (P<0.05), and showed a positive correlation to 86.2%. While, rising of NaCl concentration is significant (P<0.05), and showed a negative correlation to 80.5%. Increasing of HCl concentration is no significant (P>0.05) corresponded to the lowest efficiency for extracting protein from PKC.

**Table 1.** Analysis of Pearson Product Movement Correlation in different chemical reagents and concentrations on protein content.

| Correlations | NaOH | NaCl | HCl |
|--------------|------|------|-----|
| Pearson Correlation | 862’ | -805’ | .137 |
| Sig. (2-tailed) | 0.013 | 0.029 | 0.770 |
| N | 21 | 21 | 21 |

3.3.2 *The effect of reaction time.* The protein extraction from defatted PKC was investigated in using 0.25 M NaOH with the ratio of solid to liquid of 1:50 (w/v) at 35°C for different reaction times (0.5-4 h) with mixing speed 250 rpm. The increase in reaction time from 0.5 to 4 h led to an increase of protein concentration in the range of 24.16-34.84%. However, the extracted protein after 2 h increased slightly (Figure 3). Therefore, the reaction time at 2 h was optimum for protein isolation from PKC. An increase of protein yield was consistent with rising extraction time, which is similar to the previous works. Shen et al 2008 reported that extractable protein from tea using alkaline method was performed in the range of 1-6 h, leading to increase the protein yield from 12 to 38%; however, after 4 h, the protein yield showed marginal increase [23].

![](image)

**Figure 3.** Effect of reaction time on protein concentration

3.3.3 *Biomass compositions and soluble liquid products.* The biomass composition from PKC after oil and protein extraction was analyzed by NREL and Kjeldahl method as listed in **Table 2**. In oil extraction, hexane was showed to efficiently reduce the fat and lignin content from 12.65% to 1.26% and 20.03% to 9.07%, respectively. These were equivalent to the fat and lignin removal of 90.04% and 54.71%, respectively (Table 3). The protein, ash, cellulose, and hemicellulose were not significantly removed from the defatting process. Later, the extractable protein with 0.25 M NaOH at 0.5-4 h presented the reduction of protein content in the solid residues to the range of 6.49-8.59% compared to the raw PKC (17.37%) corresponding to the efficiency of protein removal with 48.32-60.57%. While, the composition of cellulose, hemicellulose, lignin, and fat composition had slightly affected in this stage of protein extraction process at different reaction times, except the efficiency of ash removal showed in the range of 7.27-34.60%.

In the liquid phase, it found that the recovery of cellulose, hemicellulose and soluble protein achieved in the range of 1.63-5.38% (g/g cellulose), 0.88-4.12% (g/g hemicellulose), and 24.16-34.84% (g/g...
protein), respectively after protein extraction process in different reaction times at 0.5, 1, 2, 3 and 4 h (Table 4). This result showed the efficiency of product recovery. However, the loss of cellulose, hemicellulose, and protein content was still observed. The loss of cellulose and hemicellulose under the protein extraction condition using 0.25 M NaOH at 35 °C might be due to their degraded to by-products e.g. hydroxymethylfurfural (HMF), furfural, acetic acid, and formic acid according to previous works [24].

| Process                  | Time (h) | Pulp yield (%) | Composition in the solid phase (%/w/w) | %Efficiency in the solid phase |
|-------------------------|----------|----------------|----------------------------------------|-------------------------------|
| Raw material            | -        | -              | 7.04 39.39 20.03 4.02 12.65 17.37 -  |
| Oil extraction          | 6        | 81.71 699      | 38.35 9.07 3.67 12.6 17.11 5.26       |
| Protein extraction      | 0.5      | 67.91 673      | 37.89 8.44 3.38 Nf 8.59 2.89          |
|                         | 1        | 67.24 640      | 37.62 8.86 3.22 Nf 8.08 3.07          |
|                         | 2        | 65.41 629      | 37.35 8.79 2.84 Nf 7.91 2.22          |
|                         | 3        | 64.00 639      | 36.53 8.63 2.64 Nf 7.69 2.12          |
|                         | 4        | 61.70 626      | 36.02 8.74 2.28 Nf 6.49 1.91          |

Nf; no information, *other: oil, wax, starch and other

| Process                  | Time (h) | Composition in the liquid phase (g/100g of initial composition) |
|-------------------------|----------|---------------------------------------------------------------|
| Oil extraction          | 6        | Cellulose Hemicellulose Protein content | 0.5 1.63 0.88 24.16 |
|                         | 1        | 2.27 1.67 28.99                                              |
|                         | 2        | 3.47 1.84 32.72                                              |
|                         | 3        | 4.56 3.86 34.39                                              |
|                         | 4        | 5.38 4.12 34.84                                              |

4. Conclusion
Alkaline (NaOH) was the best solution for isolating protein from palm kernel cake (PKC). Increasing solution concentration influenced the concentration of soluble protein except when acidic solution (HCl) used. This research suggested the time for protein extraction from PKC as 2 h, resulted in a high yield of protein and had lower by-products. From the study suggested extracting protein from PKC with 0.25 M NaOH, but the concentration was higher than other research, which usually perform at 0.03-0.15 M of NaOH concentration. Thereby, my research had studied the effect of high concentration in form of by-products and composition of biomass after treatment. For functional properties of protein isolates such as nutrition value and purity will be further analyzed.

Acknowledgements
This research obtained financial supports from Thailand Advanced Institute of Science and Technology-Tokyo Institute of Technology (TAIST-TokyoTech) and Sirindhorn International Institute of Technology, Thammasat University. In addition, we had received facilities of laboratory equipment, chemical, location and assistance from Integrative Biorefinery Laboratory (IBL), BIOTEC (NSTDA). The authors thank everyone involved on this occasion.

References
[1] Abdullah, N. and F. Sulaiman, *The oil palm wastes in Malaysia. Biomass now-sustainable growth and use*, 2013. 1(3): p. 75-93.
[2] Aspar, H.M., *Malaysian palm kernel cake as animal feed*. Notes, 2001. 1(366): p. 360.
[3] Saeed, M., et al., *Use of mannann-oligosaccharides (MOS) as a feed additive in poultry nutrition*. J. World Poult. Res, 2017. 7: p. 94-103.
[4] Alimon, A., *The nutritive value of palm kernel cake for animal feed*. Palm Oil Dev, 2004. 40(1): p. 12-14.
[5] Chee, K.-L. and M.-K. Ayob, *Optimization of hexametaphosphate-assisted extraction and functional characterization of palm kernel cake protein*. Food science and technology international, 2013. 19(2): p. 109-122.
[6] Zarei, M., et al., *Production of defatted palm kernel cake protein hydrolysate as a valuable source of natural antioxidants*. International Journal of Molecular Sciences, 2012. 13(7): p. 8097-8111.
[7] Mi, S., et al., *The synergism of hot water pretreatment and enzymatic hydrolysis in depolymerization of lignocellulosic content of palm kernel cake*. Journal of Molecular Catalysis B: Enzymatic, 2016. 134: p. 37-42.
[8] Klein-Marcuschamer, D., et al., *The challenge of enzyme cost in the production of lignocellulosic biofuels*. Biotechnology and bioengineering, 2012. 109(4): p. 1083-1087.
[9] Arifin, B., et al., *Protein extraction from palm kernel meal*. Journal of Applied Sciences, 2009. 9(17): p. 2996-3004.
[10] Choi, W., et al., *Antioxidant properties of crude and cold ethanol precipitated protein from palm kernel cake (PKC) as potential cosmeceutical agent*. Journal of oil palm research, 2019. 31(1): p. 159-164.
[11] Eromosele, C., et al., *Extractability of African yam bean (Sphenostylis stenocarpa) protein in acid, salt and alkaline aqueous media*. Food Hydrocolloids, 2008. 22(8): p. 1622-1628.
[12] Sanderson, P., *A new method of analysis of feeding stuffs for the determination of crude oils and fats*, in Recent advances in animal nutrition. 1986, Butterworths, London, UK. p. 77-81.
[13] Sluiter, A., et al., *Determination of structural carbohydrates and lignin in biomass*. Laboratory analytical procedure, 2008. 1617: p. 1-16.
[14] Kruger, N.J., *The Bradford method for protein quantitation*, in The protein protocols handbook. 2009, Springer. p. 17-24.
[15] Thiex, N.J., et al., Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. Journal of AOAC International, 2002. 85(2): p. 309-317.

[16] Chang, S.K., et al., Biochemical characterisation of the soluble proteins, protein isolates and hydrolysates from oil palm (Elaeis guineensis) kernel. Food Bioscience, 2014. 7: p. 1-10.

[17] Lau, B.Y.C., et al., Method developments to extract proteins from oil palm chromoplast for proteomic analysis. SpringerPlus, 2015. 4(1): p. 1-13.

[18] Firatligil-Durmus, E. and O. Evranuz, Response surface methodology for protein extraction optimization of red pepper seed (Capsicum frutescens). LWT-Food Science and Technology, 2010. 43(2): p. 226-231.

[19] Wani, A.A., et al., Effect of temperature, alkali concentration, mixing time and meal/solvent ratio on the extraction of watermelon seed proteins—a response surface approach. Biosystems engineering, 2006. 94(1): p. 67-73.

[20] Aluko, R. and E. Monu, Functional and bioactive properties of quinoa seed protein hydrolysates. Journal of Food Science, 2003. 68(4): p. 1254-1258.

[21] Zhang, B., et al., Alkaline extraction method of cottonseed protein isolate. Mod Appl Sci, 2009. 3(3): p. 77-82.

[22] Gadalkar, S.M., P.R. Gogate, and V.K. Rathod, Recovery of Proteins from Rice Mill Industry Waste (Rice Bran) Using Alkaline or NaCl-Assisted Alkaline Extraction Processes. Journal of Food Process Engineering, 2017. 40(3): p. e12430.

[23] Shen, L., et al., Studies on tea protein extraction using alkaline and enzyme methods. Food Chemistry, 2008. 107(2): p. 929-938.

[24] Jönsson, L.J., B. Alriksson, and N.-O. Nilvebrant, Bioconversion of lignocellulose: inhibitors and detoxification. Biotechnology for biofuels, 2013. 6(1): p. 16.