การแยกโพลีแซคคาไรด์จากผงเนื้อในเมล็ดมะขามด้วยเอนไซม์โปรติเอสและคลื่นเหนือเสียงความเข้มสูง

นายสุขุม ภุมรินทร์วรากุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรดุษฎีบัณฑิต
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TAMARIND SEED POLYSACCHARIDE ISOLATION FROM TAMARIND KERNEL POWDER BY PROTEASE ENZYME AND HIGH-INTENSITY ULTRASOUND

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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สุขุม ภุมรินทร์วรากุล : การแยกโพลีแซคคาไรด์จากผงเนื้อในเมล็ดมะขามด้วยเอนไซม์ โปรติเอสและคลื่นเหนือเสียงความเข้มสูง. (TAMARIND SEED POLYSACCHARIDE ISOLATION FROM TAMARIND KERNEL POWDER AND HIGH-INTENSITY ULTRASOUND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ .ดร. จิรกานต์ เมืองนาโพธิ์, อ:ที่ปรึกษาวิทยานิพนธ์ร่วม. ผศ.ดร.จิรารัตน์ ทัตติยกุล 125 หน้า

โพลีแซคคาไรด์จากผงเนื้อในเมล็ดมะขามหรือไซโลกลูแคน สามารถนำไปใช้ในอุตสาหกรรมอาหารแทนสารให้ความหนืด สารให้ความคงตัว หรือใช้แทนแป้งดัดแปรเพื่อปรับปรุงสมบัติการไหลและสมบัติทางความร้อนของผลิตภัณฑ์ ผงเนื้อในเมล็ดมะขามประกอบด้วย โพลีแซคคาไรด์ โปรตีน น้ำมัน และอื่น ๆ ร้อยละ 69.84 18.82 8.00 **และ** 3.34 **ตามลำดับ การศึกษาการแยกโพลีแซคคาไรด์ออกจากผงเนื้อในเมล็ดมะขามด้วยเอนไซม์โปรติเอสและคลื่นเหนือเสียงความเข้มสูง แบ่งการทดลองออกเป็น** 3 **ส่วน ได้แก่ การกำจัดน้ำมัน การแยกโพลีแซคคาไรด์ และการเพิ่มความบริสุทธิ์ให้โพลีแซคคาไรด์ด้วยไดอะลัยเซอร์**

ในขั้นตอนการกำจัดน้ำมัน ด้วยตัวทำละลายไอโซโพรพานอล และ เฮกเซน พบว่ามีประสิทธิภาพของการกำจัดน้ำมันเหมือนกัน ทั้งในด้านของเวลาในการสกัด อุณหภูมิในการสกัด และอัตราส่วนระหว่างตัวทำละลายต่อของแข็ง ปริมาณน้ำในเอทานอลร้อยละ 95 **ทำให้ประสิทธิภาพในการกำจัดน้ำมันลดลง การใช้คลื่นเหนือเสียงความเข้มสูงช่วยเพิ่มประสิทธิภาพในการกำจัดน้ำมันที่สกัดด้วยเอทานอลร้อยละ** 95 **แต่จะทำให้ผงเนื้อในเมล็ดมะขามมีขนาดเล็กลงและ ค่าเฉลี่ยมวลโมเลกุลของโพลีแซคคาไรด์มีขนาดเล็กลงด้วย การลดลงของน้ำมันในผงเนื้อในเมล็ดมะขามที่สกัดน้ำมันแล้วทำให้ผงมีกระจายตัวได้ดีในน้ำและมีสมบัติทางความร้อนที่ดีขึ้น ภาวะที่เหมาะสมในการกำจัดน้ำมันคือการสกัดด้วยไอโซโพรพานอลที่อัตราส่วนระหว่างตัวทำละลายต่อของแข็งที่** 3 **ต่อ** 1 **มิลลิลิตรต่อกรัม เวลาในการสกัด** 10 **นาที และอุณหภูมิ** 30 **องสาเซลเซียส จะได้ผลิตภัณฑ์ที่มีองค์ประกอบของโพลีแซคคาไรด์ โปรตีน น้ำมัน และอื่น ๆ เท่ากับ ร้อยละ** 75.40, 20.32, 0.65 **และ** 3.63 **ตามลำดับ**

การทดลองแยกโพลีแซคคาไรด์จากเมล็ดมะขาม แบ่งการทดลองออกเป็น 3 **ส่วน คือการกำจัดโปรตีนด้วยเอนไซม์โปรติเอส การกำจัดโปรตีนด้วยคลื่นเหนือเสียงความเข้มสูง และการใช้เอนไซม์โปรติเอสร่วมกับคลื่นเหนือเสียงความเข้มสูง การใช้เอนไซม์โปรติเอสร้อยละ** 0.3 **และ** 0.5 **ที่เวลาในการย่อย** 3 **และ** 5 **ชั่วโมง ได้ผลิตภัณฑ์ที่มีการปนเปื้อนของโปรตีนต่ำและมีสมบัติทางความร้อนที่ดี จลนพลศาสตร์ของการย่อยโปรตีนสามารถเขียนได้ด้วยสมการของ** Michaelis-Menten **การใช้คลื่นเหนือเสียงความเข้มสูงเพียงอย่างเดียวไม่สามารถลดปริมาณโปรตีนปนเปื้อนในผลิตภัณฑ์ การเพิ่มระดับแอมพลิจูดและเวลาในการสั่น ทำให้ค่าเฉลี่ยมวลโมเลกุลโพลีแซคคาไรด์ลดลงและสามารถประมาณได้จากแบบจำลองของ** Schmid **การทำงานร่วมกันของโปรติเอสและคลื่นเหนือเสียงความเข้มสูงมีแนวโน้มเพิ่มผลได้สูงและปริมาณโปรตีนปนเปื้อนต่ำในเวลาที่สั้น ภาวะที่เหมาะสมคือการใช้ปริมาณเอนไซม์ร้อยละ** 0.3 **และแอมพลิจูดร้อยละ** 50 **ที่เวลาในการทำงาน** 30 **นาทีให้ผลิตภัณฑ์ที่กำจัดโปรตีนออกไปร้อยละ** 93.49 **และมีผลได้ร้อยละ** 60.12 **และมีองค์ประกอบของโพลีแซคคาไรด์ โปรตีน และอื่น ๆ เท่ากับ ร้อยละ** 95.86, 2.06 **และ** 2.08 **ตามลำดับ**

ในการทดลองสุดท้าย เพื่อผลิตโพลีแซคคาไรด์ดัดแปรจากเมล็ดมะขามด้วยการเพิ่มความบริสุทธิ์ด้วยไดอะลัยเซอร์ พบว่า การเพิ่มอัตราเร็วของสายป้อนช่วยเพิ่มประสิทธิภาพการทำงานของไดอะลัยเซอร์รวมถึงสัมประสิทธิ์การส่งผ่านมวลรวม (k_{ov}) และช่วยลดค่าสัมประสิทธิ์รีเจคชันของโปรตีน ภาวะที่เหมาะสมในการทำงานคืออัตราเร็วของสารป้อน 250 **และ สายไดอะลัยเซต** 1,500 **มิลลิลิตรต่อนาทีทำให้ได้ผลิตภัณฑ์ที่มีความบริสุทธิ์ของโพลีแซคคาไรด์ร้อยละ** 99.13 **และมีผลได้ร้อยละ** 63.12 **โพลีแซคคาไรด์ดัดแปรที่ได้ประกอบด้วยโพลีแซคคาไรด์และโปรตีนร้อยละ** 99.13 **และ** 0.86 **สามารถละลายได้อย่างรวดเร็วในน้ำที่อุณหภูมิต่ำและให้ความหนืดเริ่มต้นสูงกว่าผลิตภัณฑ์อื่น ๆ การเพิ่มความบริสุทธิ์ของโพลีแซคคาไรด์ทำให้ผลิตภัณฑ์มีความหนืดมากขึ้น และมีสมบัติทางความหนืดดีขึ้น นอกจากนี้ยังพบว่าผงผลิตภัณฑ์สามารถละลายน้ำที่อุณหภูมิต่ำได้อย่างรวดเร็ว**

ภาควิชา วิศวกรรมเคมี ชื่อนิสิต .....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก. .....

ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม. .....

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SUKHUM POOMMARINVARAKUL: TAMARIND SEED POLYSACCHARIDE ISOLATION FROM TAMARIND KERNEL POWDER BY PROTEASE ENZYME AND HIGH-INTENSITY ULTRASOUND. ADVISOR: ASSOC.PROF.CHIRAKARN MUANGNAPOH, Dr.Ing., CO-ADVISOR: ASST. PROF. JIRARAT TATTIYAKUL, Ph.D., 125 pp.

Tamarind seed polysaccharide, xyloglucan (XG), is obtained from Tamarind Kernel Powder (TKP). In food industry, XG is widely used as a thickener, stabilizer, fat replacer, or starch modifier to improve rheological and thermal properties of many products. The TKP composes of polysaccharide, protein, oil and others 69.84, 18.82, 8.00 and 3.34%, respectively. Isolation of tamarind seed polysaccharide from TKP by using protease enzyme and high-intensity ultrasound in this research was divided into three parts; the de-oiled processing, the isolation of tamarind seed polysaccharide processing and the purification of isolated tamarind seed polysaccharide using dialyzer.

For the de-oiled processing, isopropanol and hexane have similar extraction efficiency on the basis of extraction time, extraction temperature and solvent to solid ratio. The water content in 95% ethanol decreased the effective of oil removal. The use of high-intensity ultrasound in the 95% ethanol extraction increased the percentage of oil removal but reduced the size of TKP and the average molecular weight of polysaccharide. The decreasing of oil content in DTKP (de-oiled TKP) made the good dispersions in water. The oil extraction using isopropanol at the solvent to solid ratio of 3:1 ml g^{-1}, extraction time 10 min and the temperature of 30°C was a suitable condition for DTKP production. The obtainable product compositions are 75.40, 20.32, 0.65 and 3.63% for polysaccharide, protein, oil and others, respectively.

For tamarind seed polysaccharide isolation process, the experiment was divided into three parts. The use of protease, high-intensity ultrasound and combination of protease and high-intensity ultrasound were studied. The solely utilization of protease digestion for 3 to 5 hours and 0.3 to 0.5% enzyme concentrations produced low protein ITSP (isolated tamarind seed polysaccharide) with good thermal properties. The kinetics of protein digestion could be fit with Michaelis-Menten equation. The high-intensity ultrasound could not decrease the protein contamination in ITSP. The increasing of amplitude level and sonication time was declining the average molecular weight of polysaccharide. The Schmid polymer degradation model was used to fit the kinetics of molecular weight of polysaccharide decay as a function of time. A combination of protease and high-intensity ultrasound treatment has a potential to produce ITSP in high yield and low protein contaminated in short time. A 0.3% protease and 50% amplitude level at 30 min sonication time gave the 93.49% protein eliminated and 60.12 % yield for ITSP. ITSP composes of polysaccharide, protein and others at 95.86, 2.06 and 2.08 respectively.

The last experiment, MTSP (modified tamarind seed polysaccharide) was the purification of ITSP by using dialyzer. The increase of feed flow rate increased the dialysance (D, the performance of dialyzer) and the overall mass transfer coefficient (k_{OV}) and also decreased the rejection coefficient (R) of protein. The highest purity of polysaccharide, 99.13%, at 63.12% yield was achieved at 250 ml min^{-1} feed flow rate and 1500 ml min^{-1} dialysate flow rate. The MTSP could rapidly dissolve in water at low temperature. So, the initial viscosity was higher than that of other polysaccharides. The increase of polysaccharide purity could increase the viscosity and the pasting properties of product. It was also found that the powder could be rapidly dissolved in water at low temperature.

Department : Chemical Engineering	Student's Signature .....
Field of Study : Chemical Engineering	Advisor's Signature .....
Academic Year : 2008	Co-Advisor's Signature .....

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NOMENCLATURE

TKP = Tamarind kernel powder

DTKP = De-oiled tamarind kernel powder

ITSP = Isolated tamarind seed polysaccharide

MTSP = Modified tamarind seed polysaccharide

k_E = Protein digestion rate constant (h^{-1})

k_U = Depolymerization rate constant (mol min^{-1})

k_{OV} = Over all mass transfer coefficient (cm s^{-1})

UF = Ultrafiltration (ml min^{-1})

Back-UF = Back ultrafiltration (ml min^{-1})

D = Dialysance (ml min^{-1})

E = Fraction extraction (-)

R = Rejection coefficient (-)

CHAPTER I

INTRODUCTION

1.1 Motivation

Tamarind (*Tamarindus indica* L.) is the most important common tree that grows abundantly in the dry tracts of South Asian and South East Asian countries such as India and Thailand (Department of Agricultural Extension, Thailand, 2005). The pulpy portion of fruit is used in foods and fruit juice (Shankarachaya, 1998). The by-product from tamarind pulp industry is tamarind seed which is the source of tamarind kernel powder (TKP). After removing protein and fat from TKP, tamarind seed polysaccharide (TSP) which is an expensive natural gum is obtained (Mathur and Mathur, 2004). In Thailand, about 570,000 Rais are used to grow Tamarind trees and these will give about 280,000 tons/year of total tamarind products (Department of Agricultural Extension, Thailand, 2005). From these figures, it has been estimated that the potential availability of tamarind seed is about 80,000 tons/year and about 50,000 tons/year of TKP can be produced in Thailand.

In Thailand, G.M. Ichihara Company Limited is the TKP production factory and they sell their product to Japanese company in a trade name of MAKAM200 (G.M. Ichihara (THAILAND) Co., Ltd). As mentioned above, TSP is an important, expensive product. Therefore, it is interesting to produce TSP from local TKP instead of sending abroad. There are many applications in use of TSP, for example, as sizing, finishing, and printing cotton and artificial silk in the textile industry, as a thickening, stabilizing, and jellying agent in the food industry, as a base in the cosmetics and pharmaceutical industries (for binding material in tablet and thickening solution in artificial tear), as a creamer for latex, in explosives, in borax printing, paste, and paper manufacturing (Parija et al., 2001). High purity tamarind polysaccharide, in a trade name of Glyloid produced in Japan is internationally well-known (Dainippon Pharmaceutical Co., Ltd).

The composition of tamarind kernel powder (TKP) usually composes of 60-70% of non-fiber carbohydrate (polysaccharide), 15-20% of protein and 4-8% of oil, fiber and moisture (Jones et al., 1978, Sila et al., 1994). The polysaccharide in tamarind kernel is xyloglucan which has β-(1→4) linked D-glucan backbone variously major substituted with xylose and minor substituted with arabinose and galactose (Parija et al., 2001). Xyloglucan from TKP is totally dissolved in water at 80°C or higher temperature and highly viscous solution is obtained (Mathur and Mathur, 2004). The advantage of this solution is that no gelation takes place unless another substance is added (Mathur and Mathur, 2004).).

Non-polysaccharide fraction chiefly protein, oil and fiber is undesirable because it contributes little or nothing to intend in any applications. Moreover, oil or fat in TKP make the product tacky, rancidity, and non-free flowing. As a result, such TKP is difficult to convey and disperse. The water-insoluble fiber in TKP can build up in the processing equipment which makes it necessary to periodically shut-down for removal. Protein is the major composition to separate because it may produce foam when dispersed in aqueous solution and its denaturation will pose the insoluble precipitation problems.

At present, tamarind kernel oil is extracted from TKP by solvent extraction. The solvent such as hexane is widely used in oil extraction industry. Hexane is a byproduct of petroleum refining and is a good extraction solvent. Andriamanantena et al. (1983) used hexane and a mixture of chloroform and methanol to extract oil from tamarind seed. However, the disadvantages of hexane and other organic solvents that are used in industry are flammability, toxicity and restrictive to governmental regulations. Thus, the recent interest in alternative solvents has shifted towards finding a solvent that is less flammable and produces less noxious emissions.

Ethanol has widespread use as a solvent of substances intended for human contact or consumption, including scents, flavorings, colorings, and medicines. Absolute ethanol is obtained from petrochemical, through the hydration of ethylene or benzene. Because a small amount of the ethylene or benzene used remains in the solution, absolute alcohol produced by this method is not suitable for consumption, as benzene is carcinogenic. Ethanol for use in alcoholic beverages (food grade) is

produced by fermentation. But, the highest fractional distillation can concentrate ethanol to 95.6% by volume (89.5% mole). The water content in alcohol solution decreased the efficiency of extraction (Zhang et al., 2005).

From literature reviews, high-intensity ultrasound is a potential new technology to improve extraction efficiency of lipophilic compounds from plants. High intensity ultrasound can accelerate heat and mass transport in a variety of food process operation and has been successfully used to improve drying, mixing, homogenization and extraction. Li et al, 2004 and Zhang et al, 2008 used ultrasonic to improve oil extraction from soybeans and flaxseed. They found that the oil yield increased in a shorter time.

From the reasons mentioned above, the methods of protein removal are the objective of this research. From our literature review, there are many attempts to remove protein from the crude TKP. Deguchi et al., (1966) reported that polysaccharide dissolved in hot water was co-precipitated with different kinds of salt solution. However, this method also gave rise to another problem of protein precipitation at the same time. Natural protein in TKP composes of 38.7% insoluble protein and 61.3% soluble protein (21.7% salt-soluble protein, 19.6 water-soluble protein, 16.7% alkali-soluble protein and 3.9% alcohol-soluble protein) (Bhattacharya et al., 1994). For completely separation of soluble protein, it requires successive chemical treatments which create great loss of polysaccharide.

In case of dry powder, Jone et al., (1978) used air classification to separate protein particles from polysaccharide particles by applying different density phenomenon. The result has showed that its yield is significantly low, due to the fact that some fibers and protein are still a lot in the product. Hydrocyclone is another simple way to remove protein particles from polysaccharide particles (Teraoka et al., 1990). This related patent illustrated that 36 hydrocyclone units in series should be used. However, this system did not give only low purity product but also difficulty to handle.

Rattanaporn, (2001) and Poommarinvarakul, (2004) used rotating filtration to pre-separate polysaccharide from protein particles in aqueous system (TKP suspension, TKP dispersed in water). They reported that filtration method can be used

to decrease protein particle in TKP suspension. However, the loss of polysaccharide in suspension was increased when protein separation proceeded for getting high purity polysaccharide.

Enzyme hydrolysis is an interesting method because it is widely used to separate protein in rice starch (Lumdubwong and Seib, 2000). Protease is a famous enzyme that catabolise protein by hydrolysis the peptide bonds that link amino acids in the polypeptide chain. After the addition of protease (0.01% neutral protease solution) to TKP suspension, polysaccharide and protein particles were distributed and size of particles was reduced because protein bound on the surface of TKP particle is cracked by enzyme reaction (Sangyon, 2002). Lumdubwong and Seib, (2000) used alkaline protease to digest protein in rice starch. They also found that, by the effect of enzyme, the recovery of starch was higher with the protease method than with the NaOH method.

The size of TKP from the factory is very large, about 200 meshes (74 micrometer). This will cause the difficulty of enzyme to enter inside TKP particles. The aid of high intensity ultrasound may be applicable for enhancing particle breaking (Jaruwattanayon, 2000). High intensity ultrasound is also widely used in many food applications, such as emulsification, sterilization, extraction, filtration, drying and enhancing oxidation (Mason, 1998). By combining neutral protease with high-intensity ultrasound, the efficacy of starch isolation was greatly improved, and sonication following protease digestion facilitated the separation process (Wang and Wang, 2003).

From our reviews, most of the researchers pay attention to the removal of protein from rice (Lumdubwong and Seib, 2000, Wang and Wang, 2004) and wheat (free gluten) (Kong et al, 2007) using high-intensity ultrasound and protease enzyme. Therefore, we are interested in use of these methods for protein removal from TKP in pre-separation and then dialysis technique will be investigated for higher purity grade polysaccharide (TSP).

1.2 Objectives

1. To investigate protein removal from TKP using ultrasound and protease enzyme in pre-separation step.

2. To investigate protein removal using dialysis technique for high purity TSP.

1.3 Working scopes

1.3.1 Part 1: De-oiled TKP processing

(i) The parameters and conditions of oil extraction were shown in Table 1.1.

(ii) The parameters and conditions of high-intensity ultrasound assisted extraction were shown in Table 1.2.

(iii) Evaluation of the suitable condition for oil removal from TKP by considering the percentage of oil removal.

Table 1.1 Oil extraction process parameters

Parameters	Conditions
Solvent type	95% ethanol, isopropanol or hexane
TKP to solvent ratio	1:3 – 1:10 g ml^{-1}
Extraction time	3 – 60 min
Extraction temperature	30 - 60°C

Table 1.2 High-intensity ultrasound assisted extraction process parameters

Parameters	Conditions
Solvent type	95% ethanol
TKP to solvent ratio	1:3
Amplitude level	0-60%
Extraction time	3 - 60 min
Extraction temperature	30 - 60°C

1.3.2 Part 2: Isolated tamarind seed polysaccharide processing

The raw material (de-oiled TKP, DTKP) in this part was chosen from the suitable condition of de-oiled processing.

(i) The parameters and conditions of protease digestion were shown in Table 1.3.

(ii) The parameters and conditions of high-intensity ultrasound sonication were shown in Table 1.4.

(iii) The combination condition was selected by the suitable condition from protease digestion and high-intensity ultrasound sonication. It cover the following scope:
   (a) Enzymatic digestion and followed by sonication.
   (b) Dispersion with sonication and followed by enzymatic digestion.
   (c) Enzymatic digestion and dispersion with sonication in the same time.

(iv) Evaluation of the suitable condition for isolation tamarind seed polysaccharide from DTKP by considering the percentage of protein eliminated and yield.

Table 1.3 Protease digestion parameters

Parameters	Conditions
Protease type	Esperase 8.0 L
Enzyme level	0.1 – 0.5% (based on powder)
Digestion time	1 – 5 h
Digestion temperature	37°C

Table 1.4 High-intensity ultrasound sonication parameters

Parameters	Conditions
High-intensity ultrasound	500W, ½ in probe.
Amplitude level	0-100% (0-2 W cm^{-3})
Sonication time	15 – 60 min
Sonication temperature	37°C

1.3.3 Part 3: Purification of isolated tamarind seed polysaccharide (ITSP) using dialyzer

The raw material (ITSP) in this part was chosen from the suitable condition of isolated tamarind seed polysaccharide processing. The powder will be dissolved in hot water (85°C) and the residual protein in this solution will be removed by dialyzer. The process conditions were shown in Table 1.5.

Table 1.5 Dialysis process parameters

Name	Hemoflow F70S
Feed flow rate (ml min^{-1})	150, 200 or 250
Dialysate flow rate (ml min^{-1})	900, 1,200 or 1,500
Flow direction	Counter current

1.4 Expected benefits

1.4.1. Suitable condition for polysaccharide production from TKP will be obtained.

1.4.2. This study will provide fundamental information and be useful for production scale.

1.4.3. This process will illustrate more value added product from agricultural waste.

CHAPTER II

BACKGROUND & LITERATURE REVIEWS

2.1 Tamarind (Kaur et al., 2006)

Tamarind is the ripe fruit (pod) of the tree (Tamarindus indica) (Figure 2.1). Every part of the tamarind tree is useful, especially the fruit. The sweetish acidic pulp of the fruit is a product of commercial importance. It's used in culinary preparations, beverages, like wine. The tender leaves and flowers are used as vegetables. Tamarind is thought to have originated in Africa and later India. It was called meaning the 'Date of India'. India is one of the major producers of tamarind in the world. Many states of the country export tamarind to west Asia, Europe and America where it is used in Worcestershire sauces because of its special flavor.

Figure 2.1 Tamarind (Tamarindus indica).

Tamarind is a good source of carbohydrates and protein. It also has small amounts of vitamins, carotene, Vitamin B and nicotinic acid. Tamarind is indigenous

to tropical Africa and probably also to India. It is found throughout the tropics and subtropics and has been naturalized at many places. Tamarind is now cultivated in India, the Middle East, Africa, South-East Asia and the Caribbean. India and Thailand are country which is producer of tamarind on a commercial scale.). In Thailand, about 570,000 Rais are used to grow Tamarind trees and these will give about 280,000 tons/year of total tamarind products (Department of Agricultural Extension, Thailand, 2005) (Figure 2.2)

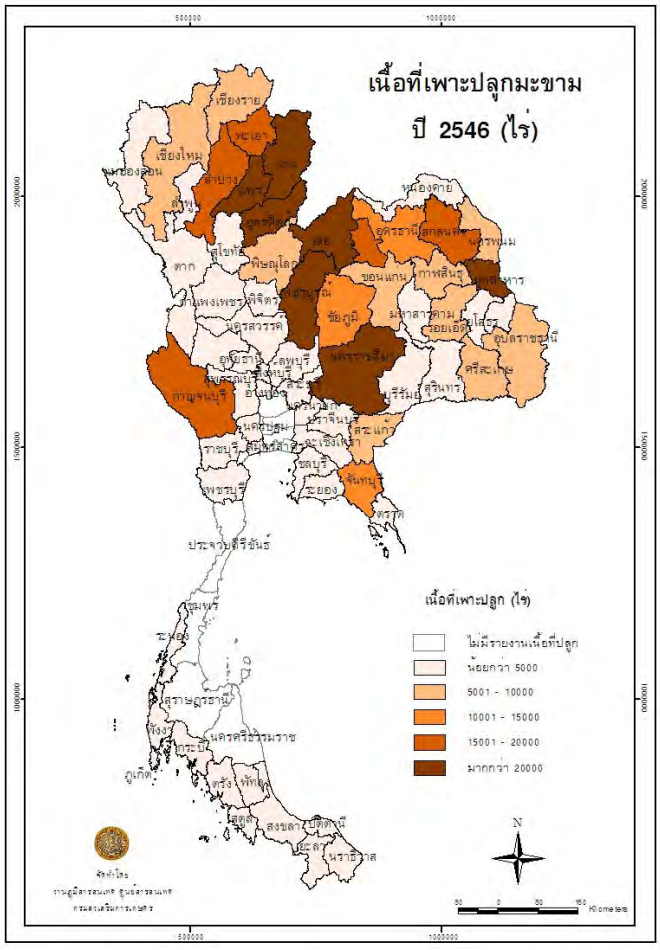


Figure 2.2 Tamarind plant areas in Thailand.

The fruit starts bearing 13-14 years after transplanting. The pods which contain about 10 brown seeds surrounded by an abundant pulp are left in the tree, until the outer shell is dry. Trees can produce up to 15 tons of fruits on annual basis. They are harvested by merely shaking the tree branches, to avoid damage to the new

buds. A full grown tree yield about 180-225 kg of fruits per season. Tamarind is harvested in second week of February to end of the June. The pulp color varied from reddish brown to different shade of black. The pod length ranged from 7.62-20.68 cm while the pod girth varied from 4.56-10 cm. The mean pod weight varied significantly from 7-46.8 g (Figure 2.3).

Figure 2.3 Tamarind pod and pulp.

Tamarinds are slow growing, long lived, evergreen trees that under optimum conditions can grow 80 feet high with a spread of 20 to 35 ft. in its native eastern Africa and Asia. However, in Southern California it seldom reaches more than 15 to 25 ft. in height.

Foliage: The bright green, pinnate foliage is dense and feathery in appearance, making an attractive shade tree with an open branch structure. The leaves are normally evergreen but may be shed briefly in very dry areas during the hot season. There are usually as many as 10 to 20 nearly sessile ½-1 inch, pale green leaflets per leaf. The leaflets close up at night.

Flowers: The inconspicuous, inch wide, five petal flowers are borne in small racemes and are yellow with orange or red steaks. The flower buds are pink due to the outer color of the 4 sepals which are shed when the flower opens.

Fruit: The 3-8 inch long, brown, irregularly curved pods are borne in abundance along the new branches. As the pods mature, they fill out somewhat and the juicy, acidulous pulp turns brown or reddish brown. When fully ripe, the shells are brittle and easily broken. The pulp dehydrates to a sticky paste enclosed by a few coarse stands of fiber.

The pods may contain from 1 to 12 large, flat, glossy brown, obviate seeds embedded in the brown, edible pulp. The pulp has a pleasing sweet/ sour flavor and is high in both acid and sugar. It is also rich in Vitamin B and high in calcium. There are wide differences in fruit size and flavor in seedling trees. Indian types have longer pods with 6-12 seeds, while the West Indian types have shorter pods containing only

2.2 Tamarind kernel powder (Mathur et al, 2004)

Tamarind Kernel Powder (Tamarind seed Polysaccharide or Tamarind Gum) obtained from the endosperm of seeds of the tamarind tree (*Tamarindus indica* Linn.), is a seed gum with potential industrial application. The seeds are by-products from the production of tamarind pulp, which is used as a food flavor. It is a creamy white to light tan and is a mixture of substances. The presence of a small amount of fat causes a tendency for the gum to form lumps. The composition of tamarind kernel on dry basis shows in Table 2.1. The molecular weight of the polysaccharide is above 52,000. It disperses in water, giving a smooth, continuous, and elastic film. It forms a gel over a wide range of pH in the presence of a high sugar concentration.

Table 2.1 Compositions of Tamarind kernel Powder

Composition	%age
Moisture	8.1%
Protein	17%
Fat	7%
Non Fibre Carbohydrates	65%
Crude Fibre	5%
Mineral Matter	2.8%

2.2.1 Manufacture of tamarind kernel powder (TKP)

For the manufacture of TKP, firstly adhering pulp and hollowed seeds are removed by floatation in the process of TKP seed washing with water. The seed kernel is tenaciously held by the seed coat. To remove seed coat, the seeds are perched in an oven or with hot sand at 150°C for 10-25 min. It caused that the seed coat becomes loose and brittle. Care is taken that the kernel is not charred during heating. When partially charred, the TKP produced is darker in color and has lower viscosity. The testae are then removed by light pounding or winnowing. This operation is generally carried out manually, although mechanical disintegrators have also been devised. Broken kernel pieces are sometimes bleached using sulfur dioxide or sodium bisulfite, to improve the whiteness of the final powder. This treatment is also a safeguard against bacterial infection during processing. Finally the broken kernel is powdered using impact-cum-attrition type pulverizer or hammer mill.

Most of the TKP is processed in small or medium scale industries and the product coming in the market is not of uniform or reproducible quality. Since the product is basically meant for non-edible purpose, microbial control is not carried out. Generally the TKP is of such fineness, that > 90% of it pass through 250 standard US-mesh i.e. of 60 microns fineness. The powder is generally mixed with appropriate preservatives i.e. chlorinated phenols, benzoate and bi-sulfite. The product is packaged into 25-50 Kg. bags, and it is recommended for storage in dry place. According to one's estimation, 60- 80 thousand tons of TKP is processed in India.

2.2.2 Properties of TKP

Good quality of de-oiled TKP is a colorless or off-white, free flowing powder, having a faint smell. It is dispersible in cold water, but hydrates only on heating to > 60°C for 25- 30min. A l% solution of TKP, when completely hydrated by heating at 60- 75°C will produce a viscosity of 50-80 cp, when measured by Brookfield Viscometer. The increase in viscosity with concentration is logarithmic and the viscosity of 8 % solution can be 30,000-45,000cp. When measured by a Brabender Amylograph, the viscosity change pattern is somewhat similar to that of starch. A 5%

solution of TKP shows a viscosity of -l600 Brabender Units. For use as a size, this viscosity is very high compared to that of 5% cornstarch, which is about 300 only. Hence depolymerized TKP is preferred for sizing. Solutions of TKP have a non-Newtonian (pseudo- plastic or shear-thinning) rheology and the viscosity is reduced with rise in temperature. The structural organization of tamarind seed granule appears to be similar to that of starch in being subjected to disruption by heat and alkali.

Compared to many plant polysaccharides, TKP pastes are more stable to heat and shearing. In preparation of TKP printing pastes for polyester, with dispersed dyes, cooking of the paste for a rather long period and often over-night is done. TKP pastes are also more resistant to acid and alkaline depolymerization, compared to other gums. Chemical Structure of Tamarind Polysaccharide

Tamarind polysaccharide has a cellulosic, b, 1 --> 4-linked glucan backbone. Nearly three-fourth of the glucose units in the backbone are substituted by a-linked xylopyranose group at C-6 of glucose in the main chain, and nearly one out of every three-xylose units, is linked to a (3-galactose unit at C-2 of xylose. The ratio, glu: xyl: gal is 4:3: 1- 1.5 which is somewhat like a cell- wall polysaccharide, though it is a reserve polysaccharide (Figure 2.4). Thus tamarind polysaccharide is regarded as a galactoxyloglucan. High degree of substitution of glucan chain, produce a stiff, extended conformation for tamarind polysaccharide molecule, with large volume occupancy in solution.

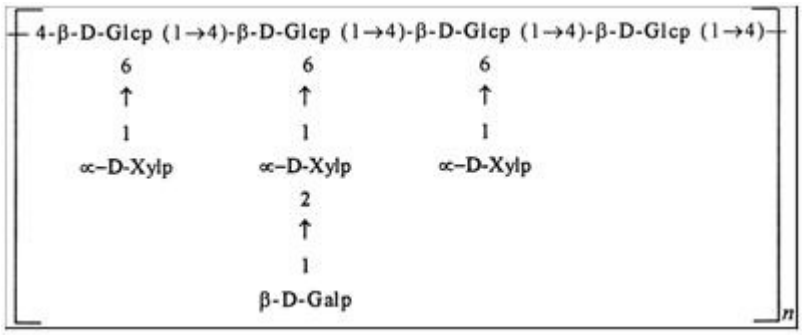


Figure 2.4 Repeating unit of Tamarind Polysaccharide. (Parija et al, 2001)

Stability of tamarind polysaccharide to heat and mild acids and bases, can be attributed to the high degree of substitution of the backbone, which, to some extent, shields it from hydrolyzing agents. High substitution of glucan backbone produces a ribbon like conformation for the polysaccharide. Cellulase enzymes (from aspergillus oryzaen-iger) hydrolyze the glucan backbone and reduce the viscosity, ultimately producing mainly 7-9 oligomers. Hydrolyzed tamarind oligo-saccharides, along with non-sugar sweeteners e.g. aspartame, have been recommended as non-calorie sugar mimics, which can replace major part of sugar in food products, without a significant change in the original texture of a food.

2.2.3 Modified and derivative tamarind polysaccharides

TKP is the powdered kernel of tamarind seed, form which purified polysaccharide call Tamarind Seed Polysaccharide (TSP or Jellose), free from protein, fibre and oil, can be isolated. Both wet and dry methods, have been used for isolating commercially purified (> 95%) polysaccharide, in 50-60%yield. The protein has been separated by precipitation, by heat coagulation or decomposed by concentrated alkali or enzymes. Polysaccharide is then obtained by alcohol or salt precipitation, protein and polysaccharide particles in TKP and these appear to be more attractive. Currently only Japan (Dainippon Pharmaceutical Co.) is making purified tamarind polysaccharide (trade name Glyloid), which is claimed to be a substitute for pectin, for making jams and jellies. This is an important field of research for India, looking to the underdeveloped pectin industry and availability of raw material. As a gelling agent, tamarind polysaccharide can function even better compared to pectin, as is evident from the gel strength data. TKP and tamarind polysaccharide have been derivative into anionic (carboxymethyl) and nonionic (hydroxyethyl) derivatives, which show improved solubility and wash ability in textile printing. A cold water-soluble TKP is also known.

2.2.4 Major applications of TKP and TSP

Textile Applications: Being one of the cheapest gums, TKP has been used as a substitute for other hydrocolloids, wherever it can serve reasonably well and is cost

effective. Currently its largest application is in textile, where it is being used as a sizing material for cotton and jute. Lowest grade TKP goes in textile sizing. It is also used; sometimes blended with other polysaccharides, in printing of polyester, with disperse dyes. The results are reasonably good and it works well. However, it is unsuitable in printing cotton with vat dyes, due to the formation of insoluble complex with dyes. It is also unsuitable in- cotton printing with fiber reactive dyes. The presence of large amounts of proteins in TKP based thickener, which also reacts (via amino and hydroxyl groups) with reactive dyes in preference to cotton cloth, reduce the color yield. Purified tamarind polysaccharide and its anionic derivatives, are likely to act as suitable thickener for reactive printing where these may prove comparable to sodium alginate. No work has so far been carried out on these lines. For making TKP print paste, heating is required, but cold water hydrating TKP has also been produced. Anionic derivatives have been shown to offer some advantage over regular TKP in print paste thickener.

Food Applications: Purified tamarind polysaccharide can be used as a general, low viscosity thickener and a non-calorie bulking agent in food. Tamarind polysaccharide based thickeners produce better freeze-thaw stable products and: emulsion stabilization is better. At low water activity i.e. at sugar concentration > 60%, it forms gel just like HM-Pectin, and its use in making jams and jells is done. It has been used in confectioneries, and as a binder in pharmaceutical tablets. Being non-metabolized, it acts as a soluble food fiber. Enzymatically depolymerised tamarind oligosaccharides have been used to replace sugar in low calorie food products. Overall there can be good value addition in making purified, food grade tamarind polysaccharide as has been done in Japan. The starting material is cheap and in substituting high priced pectin it can give good returns to a processor.

Adhesive paints and binder: TKP can substitute starch in many adhesive applications, and as a binder in particleboard, corrugated board and books. It has been used in briquetting of coal powder and palletizing of minerals. It has also been used for stabilizing soil. In paints, TKP can act as a rheology control additive and it can be used in water-based distempers, rubber and synthetic emulsions.

Other Applications: Because of its better thermal stability, TKP is being evaluated as thickener in drilling fluids for petroleum oil, including by O.N.G.C. in India. Use of purified tamarind polysaccharide can be done in cosmetics. Scientists at the C.D.R.I, have developed absorbable dressing for wounds based on purified and sterilized tamarind polysaccharide. It has been used as a creaming agent in latex.

2.2.5 Tamarind kernel protein (Bhattacharya and Mukherjee, 1994)

Tamarind kernel proteins are divided into four groups according to the solubility-fractionation. The proteins are fractionated into 19.6% albumin, 21.7% globulin, 3.9% prolamine, and 16.1% glutelins according to their solubility in distilled water (pH 7.0), 5% NaCl solution (pH 7.0), 70% ethanol, and 0.25% NaOH (pH 10.0) solution, respectively. A total of 61.3% protein can be extracted by these solvents, so that the sum of non-protein nitrogen and insoluble proteins accounted for the remaining 38.7%. The amino-acid composition of the tamarind seed protein is showed in Table 2.2. The proportions of hydrophobic amino acids (alanine, valine, leucine, isoleucine, and phenylalanine) and hydrophilic amino acids (lysine, histidine, aspartic acid, glutamic acid, and arginine) are 28.2% and 42.3%, respectively.

2.2.6 Tamarind kernel oil

The tamarind kernel oil is amber in color, free of smell, and sweet to taste. It can be used for making varnishes, paints, and burning in oil lamps. Tamarind kernel oil contained a relatively large proportion of unsaturated fatty acids (75%) with linoleic acid (56.10%) as the predominant fatty acid. The fatty acids composition and physico-chemical properties of Tamarind kernel oil are tabulated in Table 2.3 and 2.4 (Shankaracharya, 1998).

Table 2.2 Amino-acid composition of the tamarind seed protein

Amino acid	mg/16g N
Lysine	5.96
Histidine	2.01
Arginine	4.20
Aspartic acid	11.59
Threonine	3.75
Serine	7.71
Glutamic acid	18.53
Proline	6.19
Glycine	9.12
Alanine	6.96
Cysteine	0.30
Valine	4.60
Methionine	0.33
Isoleucine	4.12
Leucine	8.21
Tyrosine	1.99
Phenylalanine	4.33

Table 2.3 Composition of fatty acids of tamarind kernel oil

Fatty acid	(%)
Lauric (12:0)	trace-28.2
Myristic (14:0)	trace-0.4
Palmitic (16:0)	8.7-20.0
Stearic (18:0)	Trace-7.0
Oleic (18:1)	15.0-27.0
Linoleic (18:2)	7.5-56.1
Linolenic (18:3)	1.1-5.6
Arachidic (20:0)	1.9-12.2
Behenic (22:0)	3.0-12.2
Decosenoic (22:1)	0.1
Decosa-tetraenoic (22:4)	5.6
Lignoceric (24:0)	3.0-22.3

Table 2.4 Characteristics of tamarind seed kernel fatty acids

Characteristics	Value
Saponification value	183.4-192.0
Iodine value	99.1-140.0
Titre	45.8 ^{0}C
Bellier's turbidity temp.	52 ^{0}C
Specific gravity	0.9194-0.9324
Refractive Index	1.4510-1.4770
Acid value	0.10-12.70
Unsaponifiable matter, %	1.60-3.70
Ester value	164
Sterols	
β-Sitosterol	66-72% of total sterols
Campesterol	16-19% of total sterols
Stigmasterol	11-14% of total sterols

2.3 Protease

Proteolytic enzymes are involved in a great variety of physiological processes and their action can be divided into two different categories limited proteolysis and unlimited proteolysis.

Limited proteolysis, in which a protease cleaves only one or a limited number of peptide bonds of a target protein leading to the activation or maturation of the formely inactive protein e.g conversion of prohormones to hormones.

Unlimited proteolysis, in which proteins are degraded into their amino acid constituents. The proteins to be degraded are usually first conjugated to multiple molecule of the polypeptide ubiquitin. This modification marks them for rapid hydrolysis by the proteasome in the presence of ATP. Another pathway consists in the compartmentation of proteases e.g in lysosomes.

The International Union of Biochemistry and Molecular Biology (1984) had recommended using the term peptidase for the subset of peptide bond hydrolases (Subclass E.C 3.4.). The widely used term protease is synonymous with peptidase. Peptidases comprise two groups of enzymes: the endopeptidases and the exopeptidases, which cleave peptide bonds at points within the protein and remove amino acids sequentially from either N or C-terminus respectively. The term proteinase is also used as a synonym word for endopeptidase and four mechanistic classes of proteinases are recognized by the IUBMB as detailed below.

Proteinases are classified according to their catalytic mechanisms. Four mechanistic classes have been recognized by the International Union of Biochemistry and Molecular Biology:

1. The serine proteinases
2. The cysteine proteinases
3. The aspartic proteinases
4. The metallo proteinases

The Cysteine proteinases are a neutral protinases group that has optimum pH activity at 6.5-7.5. This family includes the plant proteases such as papain, actinidin or bromelain, several mammalian lysosomal cathepsins, the cytosolic calpains (calcium-activated) as well as several parasitic proteases (e.g Trypanosoma, Schistosoma). Papain is the archetype and the best studied member of the family. Recent elucidation of the X-ray structure of the Interleukin-1-beta Converting Enzyme has revealed a novel type of fold for cysteine proteinases. Like the serine proteinases, catalysis proceeds through the formation of a covalent intermediate and involves a cysteine and a histidine residue. The essential Cys25 and His159 (papain numbering) play the same role as Ser195 and His57 respectively. The nucleophile is a thiolate ion rather than a hydroxyl group. The thiolate ion is stabilized through the formation of an ion pair with neighbouring imidazolium group of His159. The attacking nucleophile is the thiolate-imidazolium ion pair in both steps and then a water molecule is not required.

The metallo proteinases may be one of the older classes of neutral proteinases and are found in bacteria, fungi as well as in higher organisms. They differ widely in their sequences and their structures but the great majority of enzymes contain a zinc atom which is catalytically active. In some cases, zinc may be replaced by another metal such as cobalt or nickel without loss of the activity. Bacterial thermolysin has been well characterized and its crystallographic structure indicates that zinc is bound by two histidines and one glutamic acid. Many enzymes contain the sequence HEXXH, which provides two histidine ligands for the zinc whereas the third ligand is either a glutamic acid (thermolysin, neprilysin, alanyl aminopeptidase) or a histidine (astacin). Other families exhibit a distinct mode of binding of the Zn atom. The catalytic mechanism leads to the formation of a non covalent tetrahedral intermediate after the attack of a zinc-bound water molecule on the carbonyl group of the scissile bond. This intermediate is further decomposed by transfer of the glutamic acid proton to the leaving group.

2.4 Literature reviews

2.4.1 Purification of tamarind seed polysaccharide

Deguchi et al. (1966) showed the art of purifying tamarind kernel powder (TKP) to obtain tamarind seed jellose (TSJ), polysaccharide constituent. Firstly, tamarind seeds were ground into about 3-16 mesh particles and then bleached by a bleaching agent (such as sulfurous acid solution or sodium hydrosulfite). Then water is added into the divided seeds (about 18-30 fold, but preferably 35 fold). The mixture was subsequently blown with steam for 2-3 hours and was acceded hot filtering treatment. After the addition of sulfates (sodium sulfate, ammonium sulfate, magnesium sulfate and aluminum sulfate), such coagulates rised to the surface of the extract as fluffy flakes. After that, the jellose coagulates were separated by a sieve and washed with alcohol in order to remove sulfates. Finally, the washed jellose could then be dried and ground. The obtained tamarind seed jellose was either white or grayish-white, tasteless, odorless and not toxic. The result of this study showed in Table 2.5.

Table 2.5 Result of Jellose extraction with salt precipitation (Deguchi et al. 1966)

Salt (kg)	Conc., percent by wt.	Ethanol (l)	Jellose (kg)
$(NH_4)_2SO_4$	50	10	1.7
$Al_2(SO_4)_3$ $18H_2O$	50	20	1.6
Na_2SO_4	25	28	1.7
$MgSO_4$ $7H_2O$	60	20	1.7

Gordon et al. (1968) taught the art of making a cold water-soluble product from TKP. They started by extracting polysaccharide from tamarind seed kernels with isopropyl alcohol at 80 C. The polysaccharide was then dispersed in 25-to 35-fold amount of water. After that, the solution was treated with steam injection and subsequently proceeded through filtration, Finally, a cold water-soluble product could then be recovered from the solution by either roll drying or precipitation with an organic solvent.

Sandford (1984) showed the clarification method of TKP by treatment with a strong base, followed by neutralization and isolation. First, TKP was mixed for about 2 hours in an alkali aqueous solution (50% NaOH) to form aqueous slurry. The slurry was diluted with five volumes of water and stirred for another 2 hours. The diluted slurry was then neutralized by concentrated acid (HCl). As a result, the polysaccharide was precipitated, as with 3 volumes of 99% IPA. The precipitate was further dried at 60 C for about 3 hours. It was found that the treatment of TKP with an aqueous alkali solution of pH 10-14 could produce a product of greatly improved clarity. The dried (less than 20% of H2O) and milled product also exhibited enhanced solubility in cold water when compared to TKP. The clarified tamarind kernel powder contained about 2-10% protein. In addition, the transmittance of a 0.25% solution in deionized water was about 70-80% at 485 nm.

Teraoka et al. (1990) used small hydro-cyclones in series to separate tamarind seed polysaccharide from TKP. In the process, the aqueous medium was added into the fine particle of TKP to form a dispersion. Then the dispersion was thoroughly stirred at 10-35 C for 30-60 minutes by a high-speed stirrer. After that, the suspension was introduced into a series of hydro-cyclones. By varying certain parameters, as shown in Table 2.6, such remarkable results could be achieved.

Table 2.6 Comparison of tamarind seed polysaccharides obtained by the application of small hydro-cyclones in series in certain aqueous media (Teraoka et al., 1990)

Aqueous medium	Solvent %	Yield %	Polysaccharide content %	Protein content %	Fat content%	Whiteness %	No. hydrocyclone
Raw Material particles	-	-	55.0	18.0	6.5	58	-
Ethanol	35	53.7	94.8	2.94	0.6	74	18
Isopropanol	40	53.7	94.4	2.99	0.5	75	18
Ethanol	20	52.1	94.8	2.79	0.6	74	36
Methyethyl-ketone	10	51.2	94.9	2.68	0.8	73	36
Acetone	40	52.8	94.7	2.98	0.4	76	18
Ethanol	35	50.2	95.4	2.38	0.4	76	18

Saettone et al. (2000) used tamarind seed polysaccharide for the production of a thickened ophthalmic solution. According to the invention, partially-purified tamarind gum product (Glyloid® 3S, Dainippon Pharmaceutical Co.), which is commercially available, was dispersed in cold deionized water and then stirred for 12 hours to obtain a homogeneous dispersion. The dispersion was further heated at 80 C for 30 minutes. After being cooled, it was subjected to centrifugation at 5000 rpm for another 30 minutes. The supernatant solution was then dialysed by using 12,000-14,000 cut-off membranes against water at 4 C for at least 48 hours. The obtained solution was finally lyophilized, giving a translucid, white, and totally water-soluble product.

Jaruwattanayon (2000) presented the method for production of tamarind seed polysaccharide from TKP by using filtration technique. Specifically, she used ultrasonic wave for protein separation from polysaccharide in ethanol solution. At the beginning, it appeared that protein particle was adhered to polysaccharide particle and the average size of TKP was 39 micrometers (in 95% v/v ethanol solution). After pretreated with ultrasonic, protein and polysaccharide particles were then thoroughly dispersed. The average particle size of powder at this stage was decreased. Filtration through a cloth filter could remove protein as much as 81% with polysaccharide loss of 71%. The polysaccharide, protein, and fat constituents of the obtained tamarind seed polysaccharide were 93.62%, 4.84% and 1.54% wt/wt, respectively.

Rattanaporn (2001) studied the protein separation from tamarind kernel powder (TKP) using the rotating filter. Various factors affecting protein separation from TKP by using the rotating filter were investigated. Those factors were the concentration of the suspension, the pressure across the filter, rotating speed, and the distance between the filter surface and the internal surface of the outer cylinder. It was found that rotating speed and pressure across the filter had a great influence on the separation at the low concentration of the suspension. With the increasing of the rotating speed, the optimal value of pressure across the filter was increasing likewise. In response to the optimal value, the filtrate flux, percentage protein removal, and percentage polysaccharide loss were higher. On the other hand, at the high

concentration of the suspension, the rotating speed was the only one that had an effect on the separation. As for the effects of the distance between the filter surface and the internal surface of the outer cylinder, it was found that the shear force occurring from Taylor's vortice was decreased with the higher and lower distance than 0.008 meters. That made the filtrate flux, percentage protein removal, and percentage polysaccharide loss decrease. In addition, the rotating filter can separate small particles by considering the particle size distributions of TKP in raw material, feed stream, and filtrate stream. At the optimal conditions for the filtration, the particle size distribution curve of TKP in raw material and that of TKP in feed stream separated, but shared the same average particle size of 22-23 micrometers. While the narrow curve particle size distribution of TKP in filtrate stream could be observed with the average particle size of 7-9 micrometers. The optimal conditions for the filtration of the TKP suspension by using the rotating filter were as follows; the suspension concentration of 20 gram per litre, the filter rotating speed of 1700 round per minute, the pressure across the filter of 0.16 bar, and the distance between the filter surface and the internal surface of the outer cylinder of 0.008 meters. Consequently, the following results were obtained; the filtrate flux of 9354.41 litre per square meter per hour, protein removal of 74.36%, and polysaccharide loss of 40.31%, protein, polysaccharide and fat content in the product of 9.70%, 66.41% and 5.10% by weight, respectively and product yield of 29.03% by weight.

Sangyon (2002) revealed of the protein separation from tamarind kernel powder using protease (neutrase) and ethanol. At the suitable condition for enzyme activity, which was at the working temperature of 45 ^{o}C, TKP concentration of 40 g/l and the viscosity of the kernel powder not exceeding than 50 cp, the enzyme was most active at the pH value of 8 and its activity was not affected by the agitation speed (200, 400, and 600 rpm). On the contrary, its activity was substantially denatured more than 10% when it was exposed to 5% v/v up of ethanol solution. The experiments were carried out at pH value of 6.58 8 and 10 in cooperation with and without enzyme condition. It was found that at pH value of 8 and the presence of enzyme could give the highest percentage protein removal, which was 6.37% and 15.89% higher than the case of no enzyme and no pH regulation respectively. The

protein separation using enzyme and ethanol at stated suitable condition was investigated by varying the ethanol concentration from 1 3 to 5 %v/v. The experimental results show that without the use of ethanol the lipid could be removed by 93%, remaining only the trace amount of lipid (0.42–0.48 % wt/wt) in the product. Even with the presence of ethanol the lipid was also removed (1, 3, and 5 % v/v), it was confirmed that the lipid removal could not be enhanced. Protein separation, comparing with the other research, the use of enzyme could also reduce the polysaccharide loss in the ranks of 40-60 % due to unchanging in size of the most polysaccharide particle.

Poommarinvarakul (2004) studied the application of diafiltration technique for the separation of protein from TKP by using a rotating filter. Before applying the diafiltration technique, the raw material TKP consisted of polysaccharide 63.32 – 73.48 %, protein 14.71 – 16.09% and the other ingredients 6.35 – 14.14% (wt.). When dispersed in water, the mean particle size and the viscosity of the suspension were 25.23 μm and 2.24 centipoises, respectively. In response to the increasing diafiltration volume, the mean particle size and the purity of tamarind seed polysaccharide could be increased. In particular, with the treatment of 5 diafiltration volumes, the purity of produced polysaccharide was 85.98% while the percentage polysaccharide loss and the percentage protein removal were 36.85% and 61.60%, respectively. With the addition of neutrase enzyme, the mean particle size could be further decreased. As a result, the polysaccharide purity, the polysaccharide loss, and the protein removal were raised to 93.35%, 47.86% and 88.11% by weight, respectively. It was concluded that neutrase enzyme utilization could not only turn protein separation much more effective but also improve quality of the product. The summary of polysaccharide separation methods from TKP were compared in Table 2.7.

Table 2.7 Research of polysaccharide separation from TKP

Researcher	Year	Method	Separation method
Deguchi	1966	Salting out	Centrifugal
Gordon	1968	Iso-propanol and water	Centrifugal
Jones et al	1978	Heptane, toluene	Air classification
Nitayanon	1978	Enzyme proteolytic	Filtration
Stanford	1984	Strong base	
Teraoka et al	1990	Methanol, ethanol, iso-propanol, acetone, and methyl-ethyl-ketone	Hydrocyclone
Jaruwattanayon	2000	Ultrasonic	Filtration
Rattanaporn	2001	Dissolve in water	Rotating filtration
Sangyon	2002	Enzyme protease and ethanol	Filtration
Poommarinvarakul	2004	Diafiltration	Rotating filtration

2.4.2. Oil extraction

Andriamanantena et al. (1983) revealed the oil content from six samples of tamarind kernel. The seed kernel was extracted with hexane and a mixture of chloroform and methanol. The yields of the oil were 6.0-6.4% and 7.4-9.0%, respectively. Investigation by gas liquid chromatography revealed 15 fatty acids, mainly palmitic (14-20%), stearic (6-7%), oleic (15-27%), linoleic (36-49%), arachidic (2-4%), behedic (3-5%), and lignoceric (3-8%) acids. Seven sterols were separated and quantitatively analyzed by gas liquid chromatography. The main sterols were β-sitosterol (66-72%), campesterol (16-19%), and stigmasterol (11-14%).

Proctor et al. (1996) reported on extraction of rice bran oil with hexane and IPA at ambient temperature. Oil yield obtained by 10 min hexane extraction was 15.21% and 16.28% for IPA. Free fatty acid levels were 2-3% in both solvents. The oil extracted with IPA was significantly more stable to heat-induced than hexane-extracted oil. Antioxidants that were more easily extracted by IPA than hexane may

be responsible for the increased stability. Thus, IPA was effective solvent, comparing with hexane, for rice bran oil extraction.

Zhang et al. (2002) showed extraction of cottonseed collets with IPA. Extractability of collets with 95% IPA was better than that of flakes. The residual oil content and solvent hold-up of the collets were 1.6 and 33.0% compared to 4.5 and 53.2% for the flakes, respectively. The aqueous IPA extraction proceeded more slowly and showed a lower oil carrying capacity than that of hexane. The residual oil content of the cottonseed collets extracted with hexane was 1.2% while the solvents containing 97, 93, and 88% IPA resulted in 1.5, 1.9, and 2.4%, respectively. Reduction in water content improved the extraction efficiency of IPA.

Kwiatkowski et al. (2002) extracted corn oil from whole ground corn using ethanol as the solvent. The yield of oil was measured as a function of temperature, extraction time, solvent-to-solids ratio, and ethanol concentration. Optimal conditions were a solvent-to-solids ratio of 4 ml/g corn, an absolute ETOH, an extraction time of 30 min, and a temperature of 50ºC. The extraction efficiency under these conditions was 70%, equivalent to 3.3 g oil/100 g corn. A three-stage extraction, where the same corn was extracted with fresh ETOH, resulted in oil recovery of 93%, equivalent to 4.5 g/100 g corn. When anhydrous ETOH was used to repeatedly extract fresh corn, moisture was absorbed linearly by ETOH from the corn in successive stages, which, in turn, decreased oil yield and increased nonoil components in the extract.

Gandhi et al. (2003) reported on alternative solvents for the extraction of soybean oil. It was found that *n*-heptane, *n*-propanol, IPA, and ETOH were equally effective in the extraction of soybean oil when compared with n-hexane. The aqueous solvent mixtures were also efficient in extracting the oil. The advantage with the higher aqueous content solvent was the lower amount of organic solvent required, however, more energy would be required to remove the water from solvent. At 8 hours of extraction, the aqueous solvent systems containing either 90:10 and 80:20 *n*-propanol, IPA or ETOH gave oil extraction percentage in range of 92.0-97.8% and 98.0-99.5% for pure solvents.

Franco et al. (2007) studied extraction of oil from rosehip seed (*Rosa rubiginosa*) with ETOH as solvent. The influence of temperature and water content on the oil solubility was also analyzed. Oil solubility dramatically decreased with the water present in ETOH (from 92% to 99.9%). The solubility of absolute ETOH (99.9%) was higher than 96% ETOH at the same range of temperatures. The values varied between 40 g/l at 20ºC and 140 g/l at 60ºC for absolute ETOH (99.9%) and decreased to 15 g/l at 20ºC and 45 g/l at 60ºC for 96% ETOH. Although solubility of ETOH highly increases with temperature, however, oil solubility in hexane is more effective. Therefore, to achieve the same performance, it is necessary to operate at high liquid to solid ratio in ETOH extraction. The advantage in use of ETOH as an extractant is also recognized to the environment.

Liauw et al. (2008) investigated neem oil extraction from Neem seed (*Azadirachta indica A. Juss*) with *n*-hexane and ETOH. Effects of particle size, temperature, and type of solvent on oil yield and oil quality were studied. The maximum yields obtained from neem oil extraction were 44.29% for *n*-hexane and 41.11% for ethanol at 50°C and 0.425-0.71 mm particle size. Based on psycho-chemical characteristics analysis, an increasing in temperature will increase saponification, acid, and peroxide value which means increasing neem oil yield. In contrary, an increasing in temperature will decrease iodine value which means decreasing neem oil quality.

2.4.3 Other researches

Lumdubwong and Seib (1998) studied the rice starch isolation by alkaline protease digestion of wet-milled rice flour (WMRF). In a 3x3 factorial modeling experiment, recoveries of starch and levels of protein contamination were determined at pH 8.5-10, protease levels of 0.5-1.5% (based on WMRF), and digestion times of 5 and 30 hour. The following digestion conditions were kept constant at 55°C with mild agitation, 34-37% (w/v) flour solids, and alkalinity within $\pm$ 0.2 pH units. Regression equations of the variances in starch recovery and protein contamination with the three variables showed 92 and 98%, respectively,. Upon digestion with 1.1% protease at pH

10 and 18 hour, starch recovery was 95% and protein contamination 0.5%. Most hydrolysis of rice protein occurred in the first 3-4 hour of digestion as determined by the consumption of sodium hydroxide. Rice starch also was isolated by extraction of WMRF with 2.5 part 0.05 M NaOH at pH 12. The recovery of starch with the protease method was 10% higher than with the NaOH method. Moreover, the effluents contained mostly amino acid salts which opposed to protein digestion with alkali.

Wang and Wang (2004) investigated the efficacy of a neutral protease and combinations of neutral protease and high-intensity ultrasound for isolation of rice starch. Rice flour slurry (33%) was treated with a neutral protease at 0.01, 0.03, or 0.05% (on flour basis) or combinations of 0.03% neutral protease and high-intensity ultrasound at 25, 50, or 75% amplitude for 15, 30, or 60 min concurrently or sequentially. The starch yield using neutral protease alone ranged from 62.5 to 71.8% with 0.53–0.88% residual protein content and 0.99–1.81% damaged starch content. The starch yield greatly improved to 79.8–86.7% with 0.50–0.96% residual protein content and 0.98–1.87% damaged starch content when neutral protease treatment was combined with high-intensity ultrasound. Increasing sonication time at 50% amplitude during protease digestion improved the starch yield from 83.9 to 86.1%, but did not improve those treated at 75% amplitude. The preferred combination was neutral protease digestion for 2 h followed by sonication at 75 or 50% amplitude for 15 or 30 min. The starch structure analyzed by a high performance size-exclusion chromatography and scanning electron microscopy revealed no damage to the molecular structure or to the starch granule surface, however, the peak viscosities of rice starch increased after sonication. The combination of neutral protease and high-intensity ultrasound was an effective technique for isolation of rice starch without generating high salt wastes.

Li et al (2004) studied the application of 20 kHz high-intensity ultrasound during extraction of oil from two varieties of soybeans (TN 96-58 and N98-4573) using hexane, isopropanol and a 3:2 hexane–isopropanol mixture. In a simplified extraction procedure, ground soybeans were added to solvents and ultrasonicated

between 0 and 3 h at ultrasonic intensity levels ranging from 16.4 to 47.6 Wcm^{-2}. Oil yield and composition were determined after oil was recovered by distillation. Using hexane as a solvent, yield generally increased as both application time and intensity of ultrasound increased. Solvent type influenced the efficiency of the extraction, i.e., the highest yield was obtained using ultrasound in combination with the mixed solvent. Gas chromatography analysis of ultrasonicated soybean oil did not show significant changes in fatty acid composition. Results were attributed to mechanical effects due to ultrasonically induced cavitation increasing permeability of plant tissues. A comparison of scanning electron microscopy images of raw and ultrasonicated soybeans indicated development of microfractures and disruption of cell walls in ground soybean flakes. Their study suggests that high-intensity ultrasound may reduce time required to extract edible oils from plant sources and hence improve throughput in commercial oil production processes.

Imai et al (2004) investigated the effective approaches to a practical high-performance enzymatic hydrolysis of cellulose using a ultrasonic irradiation as a pretreatment and a combination of cellulases from *Trichoderma viride* and *Aspergillus niger*. Their efficacy on enhancement of reactivity was discussed based on the kinetic parameters, i.e. Michaelis constant *K*m, maximum reaction rate *V*max and initial reaction rate, correlating with ultrasonic conditions and enzyme combination, respectively. A relatively high-weight fraction of *A. niger* cellulase in the mixed-enzyme system afforded rapid initiation of the hydrolysis reaction, while a kinetic analysis revealed that a *T. viride* cellulase weight fraction of ca. 0.3 was optimal in enhancing *V*max without increasing *K*m. Pretreatment of the cellulose fibers with ultrasonic irradiation prior to initiating the enzyme reaction further improved the reaction rate. Enhancement of *V*max by ultrasonication was effective in greater weight fraction of *T. viride* used system. The correlation between the ultrasonic irradiation time required to obtain a desired initial reaction rate and the irradiation power employed was examined. It was found that the time needed for effective degradation could be markedly reduced by increasing the irradiation power. This finding is considered to support the possibility of applying ultrasonication pretreatment in a practical cellulose saccharification process.

Baxter et al (2005) investigated influence of high-intensity ultrasonication on the molecular weight and degree of acetylation of chitosan. High molecular weight shrimp chitosan was purified by alkaline precipitation and dialysis from aqueous solution. A 1% (w/v) chitosan in 1% (v/v) aqueous acetic acid was sonicated for 0, 0.5, 1, 5, 15, and 30 min at 25 °C at power levels of 16.5, 28.0 and 35.2 W cm^2. Degree of acetylation determined by high-pressure liquid chromatography with photodiode array at 210 nm, monitored acetyl groups was released after complete hydrolysis and deacetylation of samples. Average molecular weight of ultrasonicated chitosan was determined by measurements of intrinsic viscosity of samples. The degree of acetylation of purified chitosan was 21.5%. Results indicated that neither power level nor sonication time altered the degree of deacetylation of chitosan molecules. Intrinsic viscosity of samples decreased exponentially with increasing sonication time. Rates of intrinsic viscosity decreased whereas ultrasonic intensity was linearly increased. Results were analyzed in terms of molecular weight decreases. The Schmid polymer degradation model was used to analyze the molecular weight decay as a function of sonication time. A first order relationship for the dependence of the reaction rate on the ultrasonic intensity was suggested. Results of this study indicate that high-intensity ultrasonication can be utilized to reduce molecular weight of chitosan with maintaining the degree of acetylation.

Zhang et al (2008) reported the ultrasound-assisted extraction of oil from flaxseed. The effects of some operating parameters such as ultrasonic power, extraction time, extraction temperature and solvent to solid ratio on the yield of flaxseed oil have been investigated and some of the results have been compared with that of conventional method. It has been found that ultrasound-assisted extraction requires a shorter extraction time and a reduction of solvent consumption. The yield of flaxseed oil has been found to increase with the increase of the ultrasonic power and to decrease as the temperature is increased. Scanning electronic microscopy analysis was carried out on the flaxseed powder after the extraction. The images are powerful evidences to show the effect of ultrasound. The fatty acid compositions of the oils extracted by the ultrasound-assisted method and the conventional method have been analyzed using gas chromatography. It has been shown that the

compositions of the flaxseed oils were not affected significantly by the application of ultrasound ($p > 0.05$). The ultrasound-assisted extraction may be an effective method for lipid production

CHAPTER III

THEORY

3.1 Liquid-solid leaching (Geankoplis, 1995)

Many biological, inorganic, and organic substances occur in a mixture of different components in a solid. In order to separate the desired solute constituent or remove an undesirable solute component from the solid phase, the solid is contacted with a liquid phase. The two phases are intimate contact and the solutes can diffuse from the solid to the liquid phase, which causes a separation of the components originally in the solid. This process is called “liquid-solid leaching or leaching”. It is an extensively used to recover many important food and biological components such as sucrose in cane or beets, lipids from oilseeds, proteins in oilseed meals, phytochemicals from plants, and functional hydrocolloids from algae, among others. Leaching may also be used to remove undesirable contaminants and toxins presented in foods and feeds.

3.1.1 Preparation of solids for leaching

Biological materials are cellular in structure and the soluble constituents are generally found inside the cells. The rate of leaching may be comparatively slow because the cell walls provide another resistance to diffusion. However, to grind the biological material sufficiently small to expose the contents of individual cells is impractical. Sugar beets are cut into thin wedge-shaped slices for leaching so that the distance required for the water solvent to diffuse to reach individual cells is reduced. The cell walls of soybeans and many vegetable seeds are largely ruptured when the original materials are reduced in size to about 0.1 mm to 0.5 mm by rolling or flaking. Cells are smaller in size, but the walls are ruptured and the vegetable oil is easily accessible to the solvent. In Figure 3.1 (Aguilera, 2003) shows the architectural arrangements of cells associated with four pretreatments. The basic structure considers that all cells are intact and completely surrounded by a thick cell wall (A). Enzymatic treatment with a cocktail of cell wall–degrading enzymes may pierce the

wall of outer cells at different places, leaving openings that connect the cytoplasm directly to the solvent (B). A third situation may be that induced by blanching, resulting in degradation of the cell wall membrane (and possibly the cell wall itself), diminishing the overall internal resistance for transport of solute as most cells become interconnected (C). Another conceivable architecture is that of a totally connected cytoplasm and broken outer cells exposed to the solvent due to extensive physical destruction of the tissue (D). In all cases, the proportion of cell wall material to cytoplasm is the same.

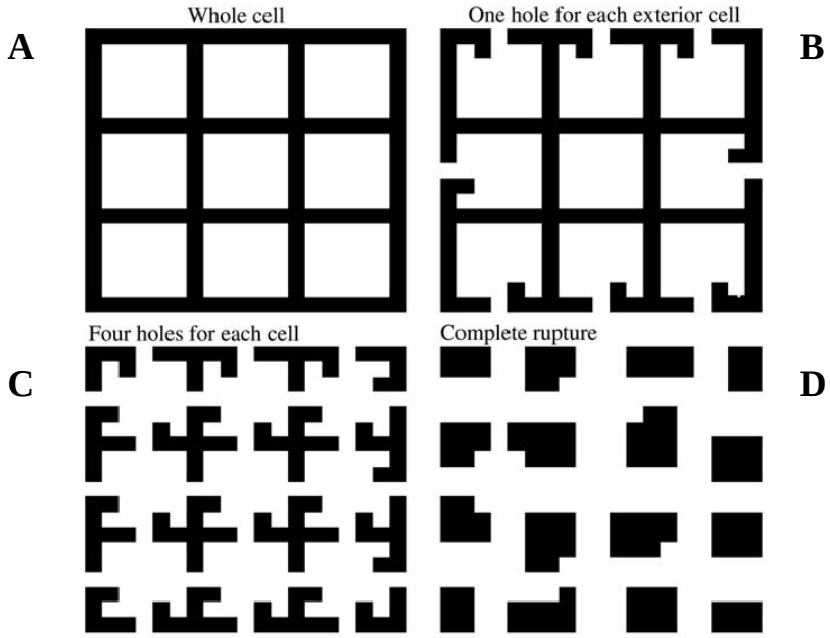


Figure 3.1 Architectural arrangements of cells of pickled cucumbers subject to different treatments (Aguilera, 2003)

3.1.2 The main steps in leaching

In the leaching of soluble materials from inside a particle by a solvent, a series of phenomenological steps have to occur during the period of interaction between solute-containing particles and solvent affecting the separation as represented schematically in Figure 3.2. These include:

1. The solvent transfers from the bulk solvent solution to the surface of the solid.

2. The solvent penetrates or diffuses into the solid.
3. The solute dissolves into the solvent.
4. The solute then diffuses through the solid solvent mixture to the surface of the particle.
5. The solute is transferred to the bulk solution.

In general, the transfer rate of the solvent from the bulk solution to the solid surface is quite rapid, and the transfer rate of the solvent into the solid can be somewhat rapid or slow.

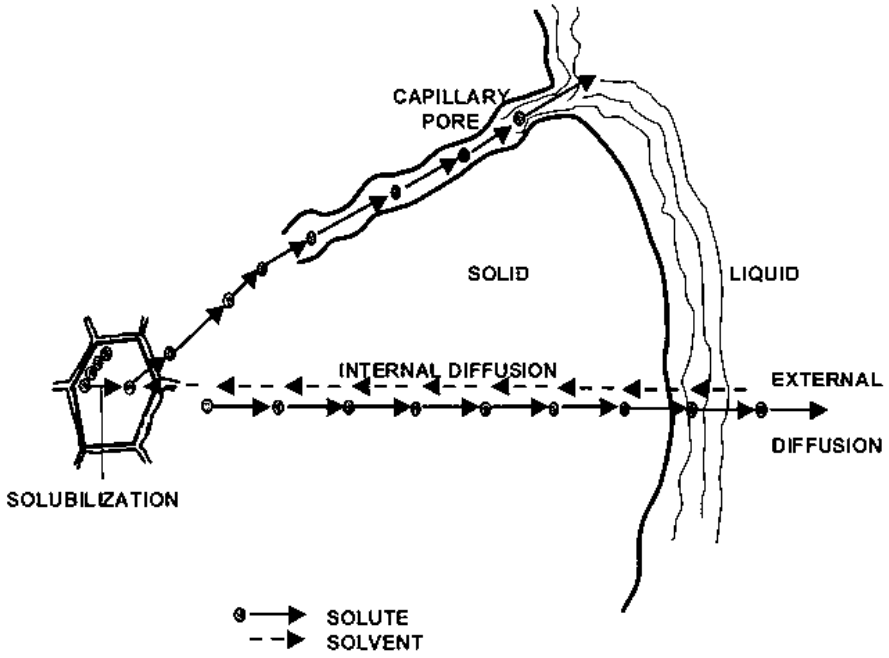


Figure 3.2 Scheme of the main steps in solvent extraction of solid food particles (Aguilera, 2003)

The diffusion rate of the solute through the solid and solvent to the solid surface is often the controlling resistance in an entire leaching process and can depend on a number of different factors. If the solid is made up of an inert porous solid structure with the solute and solvent in pores in the solid, the diffusion through the porous solid can be described by an effective diffusivity. The void fraction and tortuosity are needed. The resistance to mass transfer of the solute from the solid

surface to the bulk solvent is in general quite small compared to the resistance to diffusion within the solid itself.

3.1.3 Solvent selection

Solvent selection is based on several properties:

1. Solubility of the specific compound (or compounds) in the solvent.
2. Recovery, since the solvent will be reused in subsequent extractions. If distillation or evaporation is used, the solvent should not form azeotropes and the latent heat of vaporization should be small. Removal of the solvent from the miscella (and from the spent solids) can pose serious problems so the residual level of the solvent must be minimized.
3. Interfacial tension and viscosity. The solvent should be capable of wetting the solid matrix and its viscosity should be sufficiently low so it can flow easily. Wettability is also important if the solvent must penetrate through pores and capillaries in the solid matrix.
4. Ideally, the solvent should be nontoxic, stable, nonreactive, nonflammable, harmless to the environment, and cheap.

3.2 Enzyme kinetics

Enzymes are biological catalysts that are protein molecules in nature. They are produced by living cells and are absolutely essential as catalysts in biochemical reactions. Almost every reaction in a cell requires the presence of a specific enzyme. A major function of enzyme in a living system is to catalyze the making and breaking of chemical bonds. Therefore, like any other catalysts, they increase the rate of reaction without themselves undergoing permanent chemical changes.

Enzyme reactions are different from chemical reactions, as follows:

1. An enzyme catalyst is highly specific, and catalyzes only one or a small number of chemical reactions.

2. The rate of an enzyme-catalyzed reaction is usually much faster than that of the same reaction when directed by nonbiological catalysts.
3. The reaction conditions (temperature, pressure) for the enzyme reactions are very mild.

Enzyme mechanisms can be divided into single-substrate and multiple-substrate mechanisms. Kinetic studies on enzymes that only bind one substrate, such as triosephosphate isomerase, aim to measure the affinity with which the enzyme binds this substrate and the turnover rate. When enzymes bind multiple substrates, enzyme kinetics can also show the sequence in which these substrates bind and the sequence in which products are released. The reaction catalysed by an enzyme uses exactly the same reactants and produces exactly the same products as the uncatalysed reaction. Like other catalysts, enzymes do not alter the position of equilibrium between substrates and products. However, unlike normal chemical reactions, enzymes are saturable. This means as more substrate is added, the reaction rate will increase, because more active sites become occupied. This can continue until all the enzyme becomes saturated with substrate and the rate reaches a maximum.

There are three major types of enzyme reactions:

1. Soluble enzyme-insoluble substrate
2. Insoluble enzyme-soluble substrate
3. Soluble enzyme-soluble substrate

An example of a type 1 reaction is the use of enzymes such as protease or amylase in laundry detergents; however, this enzyme reaction has caused some controversy in relation to water pollution. Once in solution, the soluble enzyme may digest an insoluble substrate such as a blood strain. A major research effort is currently being directed at type 2 reactions. Many of these reactions are homogeneous in the liquid phase; that is, they are type 3 reaction. In the following brief presentation we limit our discussion to type 3 reactions, although the resulting equations have been found to be applicable to type 1 and type 2 reactions in certain instances. (Fogler, 1999)

3.2.1 Michaelis-Menten Kinetics (M-M Kinetics) (Levenspiel, 1999)

In a sympathetic environment, with just the right enzyme for catalyst, organic A will react to produce R. Observations show the behavior of Figure. 3.3. A simple expression which accounts for this behavior is

$$-r_A = r_R = k_E \frac{C_{E0} C_A}{C_M + C_A} \tag{3.1}$$

where C_{E0} is total enzyme. C_M is a constant, called the Michaelis constant.

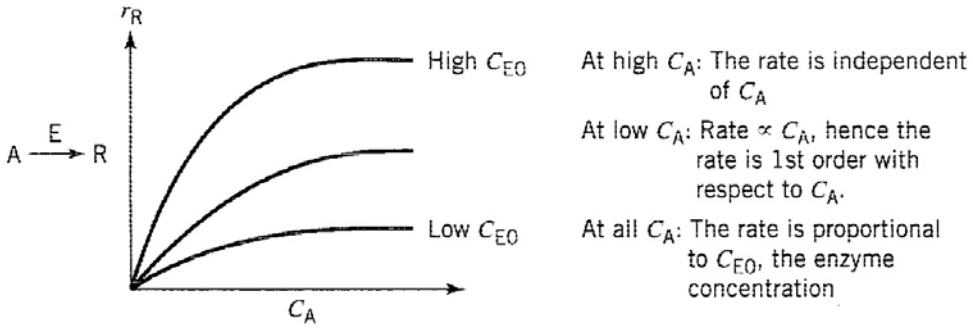


Figure 3.3 Typical rate-concentration curves or enzyme catalyzed reactions. (Levenspiel, 1999)

In searching for the simplest mechanism to explain these observations and this rate form, Michaelis and Menten (1913) came up with the two-step elementary reaction mechanism

$$A + E \underset{2}{\overset{1}{\rightleftarrows}} X \overset{3}{\rightarrow} + E \tag{3.2}$$

And

$$C_{E0} = C_E + C_X \tag{3.3}$$

where X is a intermediate. C_X is a enzyme attached to reactant.

- when $C_A = C_M$ half the enzyme is in free from, the other half combine.
- when $C_A > C_M$ most of enzyme is tied up as complex X.
- when $C_A < C_M$ most of enzyme is in free form.

Graphically show this equation in Figure 3.4

3.2.2 Batch or plug flow fermentor

For this system, integration of the M-M equation gives

$$\underbrace{C_M ln\frac{C_{A0}}{C_A}}_{\text{first order}} + \underbrace{(C_{A0} - C_A)}_{\text{zero-order}} = k_3 C_{E0} t \qquad (3.4)$$

This concentration-time behavior is shown in Figure 3.5

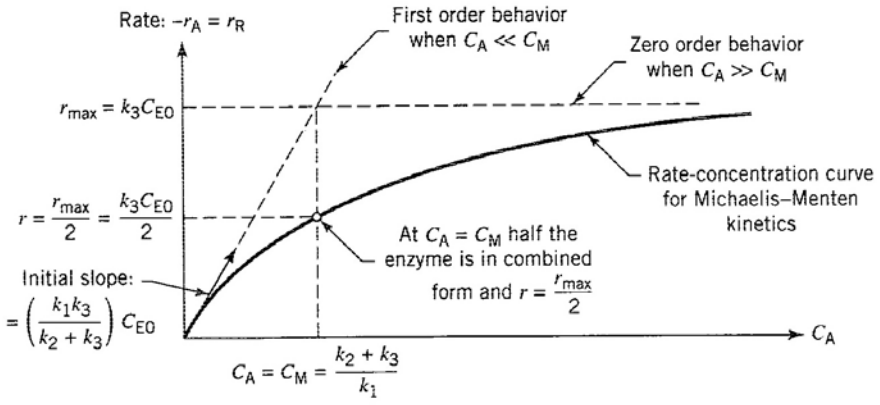


Figure 3.4 Special features of the M-M equation, Eq 3.1 (Levenspiel, 1999)

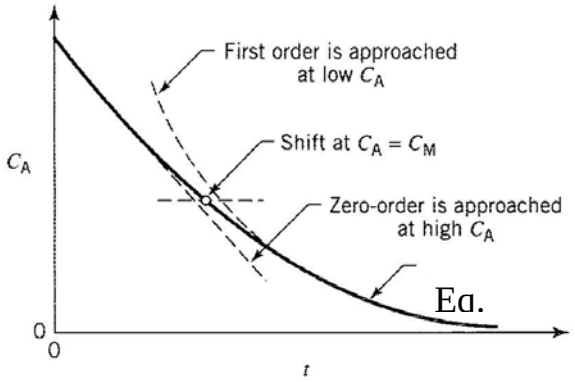


Figure 3.5 Concentration-time behavior of the M-M equation. (Levenspiel, 1999)

Unfortunately this equation cannot be plotted directly to find the values of the constants k_3 and C_M. However, by manipulation we find the following form which can be plotted, as shown in Figure 3.6 to give the rate constants

$$\frac{C_{A0}-C_A}{ln(C_{A0}/C_A)} = -C_M + k_3 C_{EO} \frac{t}{ln(C_{A0}/C_A)} \qquad (3.5)$$

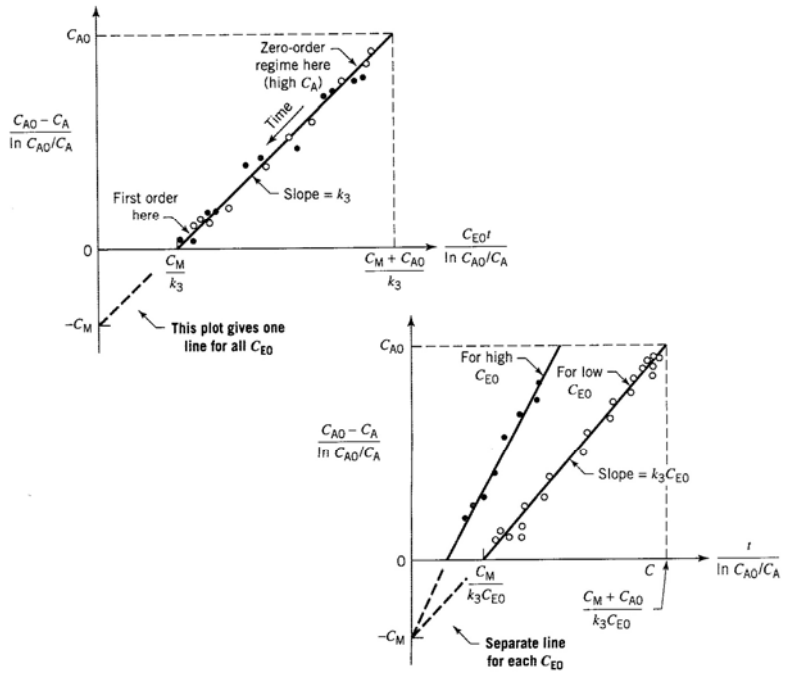


Figure 3.6 Either plot can be used to test and fit the M-M equation (3.5) from batch reactor data. (Levenspiel, 1999)

3.3 Ultrasonic (John, 1995)

Ultrasonic is the science of sound wave above the limits of human audibility. The frequency of a sound wave determines its tone or pitch. Low frequencies produce low or bass tones. High frequencies produce high or treble tones. Ultrasound is a sound with a pitch so high that it cannot be heard by the human ear. Frequencies above 18 kHz are usually considered to be ultrasonic. Exposure to ultrasound can be divided into distinct categories: airborne and liquid-borne. Exposure to airborne ultrasound occurs in many industrial applications such as cleaning, emulsifying, welding, and flaw detection and through the use of consumer devices such as dog whistles, bird and rodent controller, and camera rangefinders, and commercial device such as intrusion alarms. Liquid-borne exposure occurs predominantly through medical exposure in diagnosis, therapy, and surgery.

Acoustic energy may be transformed into several other forms of energy, which may exist at the same time within any given medium. The mechanisms of transformation into these other forms of energy are conventionally subdivided into three major categories comprising a thermal mechanism, a cavitational mechanism, and other mechanism including streaming motions.

Figure 3.7 includes examples of ultrasound devices used in medicine, industry, consumer products, and signal processing and testing, in relation to ultrasound frequency.

3.3.1 Sound Wave Generation

A sound wave is produced when a solitary or repeating displacement is generated in a sound conducting medium, such as by a “shock” event or “oscillatory” movement. The displacement of air by the cone of a radio speaker is a good example of “oscillatory” sound waves generated by mechanical movement. As the speaker cone moves back and forth, the air in front of the cone is alternately compressed and rarefied to produce sound waves, which travel through the air until they are finally dissipated. We are probably most familiar with sound waves generated by alternating

mechanical motion. There are also sound waves which are created by a single “shock” event. An example is thunder which is generated as air instantaneously changes volume as a result of an electrical discharge (lightning). Another example of a shock event might be the sound created as a wooden board falls with its face against a cement floor. Shock events are sources of a single compression wave which radiates from the source.

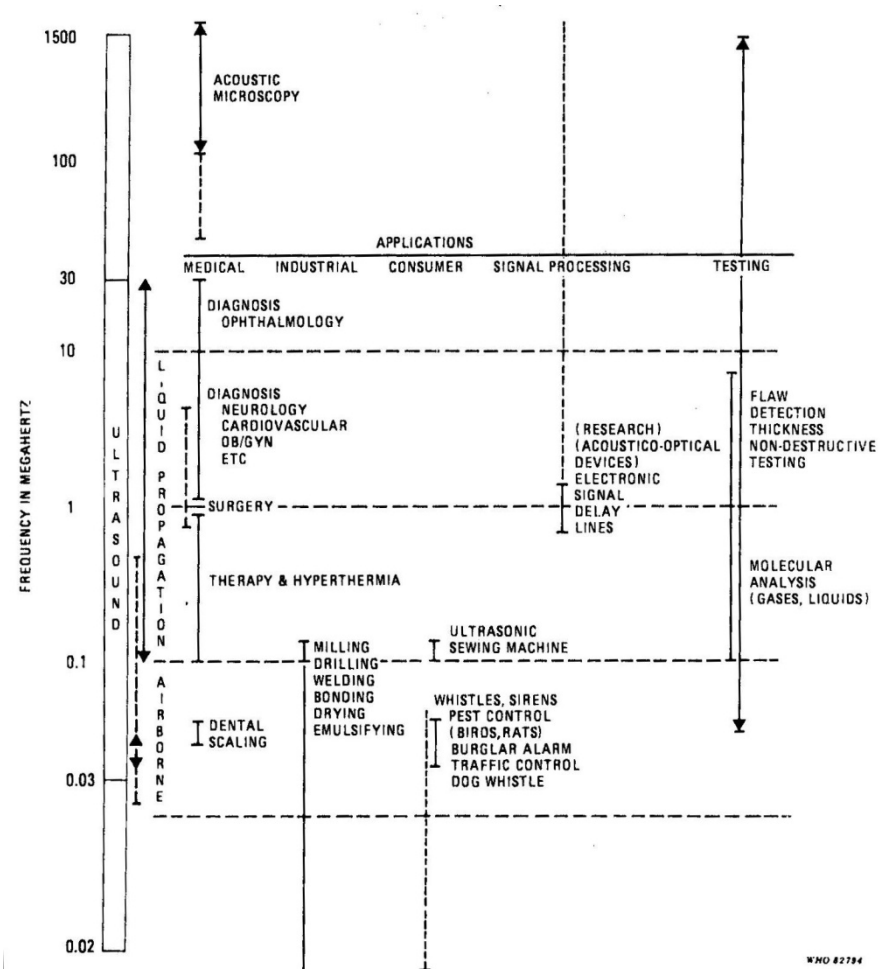


Figure 3.7 Ultrasound spectrum (John, 1995)

3.3.2 The Nature of Sound Waves

The diagram above uses the coils of a spring similar to a Slinky@ toy (Figure 3.8) to represent individual molecules of a sound conducting medium. The molecules in the medium are influenced by adjacent molecules in much the same way that the

coils of the spring influence one another. The source of the sound in the model is at the left. The compression generated by the sound source as it moves propagates down the length of the spring as each adjacent coil of the spring pushes against its neighbor. It is important to note that, although the wave travels from one end of the spring to the other, the individual coils remain in their same relative positions, being displaced first one way and then the other as the sound wave passes. As a result, each coil is first part of a compression as it is pushed toward the next coil and then part of a rarefaction as it recedes from the adjacent coil. In much the same way, any point in a sound conducting medium is alternately subjected to compression and then rarefaction. At a point in the area of a compression, the pressure in the medium is positive. At a point in the area of a rarefaction, the pressure in the medium is negative.

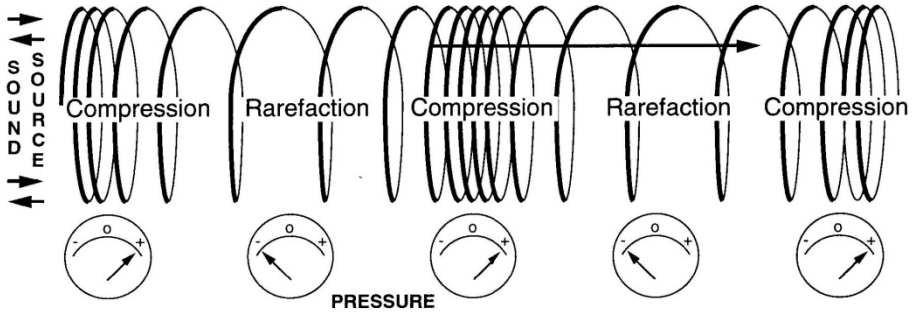


Figure 3.8 Characteristic of ultrasound (John, 1995)

3.3.3 Cavitation

In elastic media such as air and most solids, there is a continuous transition as a sound wave is transmitted. In non-elastic media such as water and most liquids, there is continuous transition as long as the amplitude or “loudness” of the sound is relatively low. As amplitude is increased, however, the magnitude of the negative pressure in the areas of rarefaction eventually becomes sufficient to cause the liquid to fracture because of the negative pressure, causing a phenomenon known as cavitation.

Cavitation “bubbles” are created at sites of rarefaction as the liquid fractures or tears because of the negative pressure of the sound wave in the liquid. As the wave fronts pass, the cavitation “bubbles” oscillate under the influence of positive pressure,

eventually growing to an unstable size. Finally, the violent collapse of the cavitation bubbles results in implosions, which cause shock waves to be radiated from the sites of the collapse (Figure 3.9).

The collapse and implosion of myriad cavitation “bubbles” throughout an ultrasonically activated liquid result in the effect commonly associated with ultrasonics. It has been calculated that temperatures in excess of 10,0000F and pressures in excess of 10,000 PSI are generated at the implosion sites of cavitation bubbles.

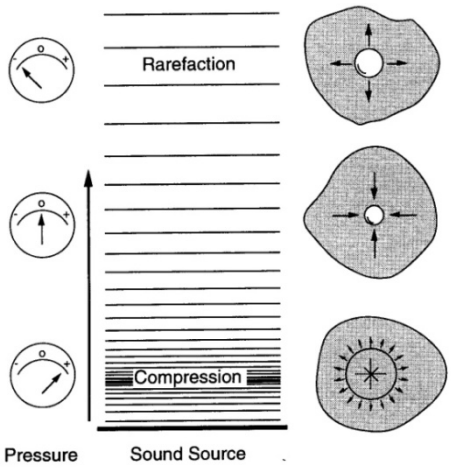


Figure 3.9 Cavitation bubble (John, 1995)

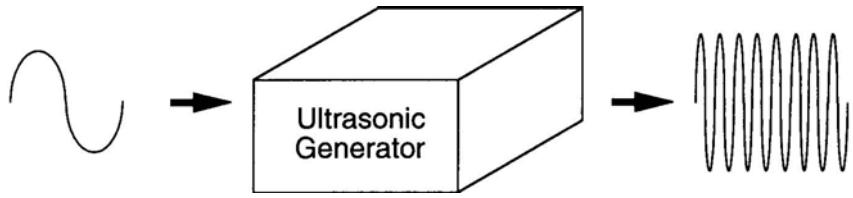
3.3.4 Ultrasonic Equipment

To introduce ultrasonic energy into a cleaning system requires an ultrasonic transducer and an ultrasonic power supply or “generator.” The generator supplies electrical energy at the desired ultrasonic frequency. The ultrasonic transducer converts the electrical energy from the ultrasonic generator into mechanical vibrations.

3.3.5 Ultrasonic Generator

The ultrasonic generator converts electrical energy from the line which is typically alternating current at 50 or 60Hz to electrical energy at the ultrasonic

frequency (Figure 3.10). This is accomplished in a number of ways by various equipment manufacturers. Current ultrasonic generators nearly all use solid state technology.



60 Cycles 20,000+ Cycles

Figure 3.10 Ultrasonic generators (John, 1995)

There have been several relatively recent innovations in ultrasonic generator technology which may enhance the effectiveness of ultrasonic cleaning equipment. These include square wave outputs, slowly or rapidly pulsing the ultrasonic energy on and off and modulating or “sweeping” the frequency of the generator output around the central operating frequency. The most advanced ultrasonic generators have provisions for adjusting a variety of output parameters to customize the ultrasonic energy output for the task.

3.3.6 Pulse

In pulse operation, the ultrasonic energy is turned on and off at a rate which may vary from once every several seconds to several hundred times per second (Figure 3.11).

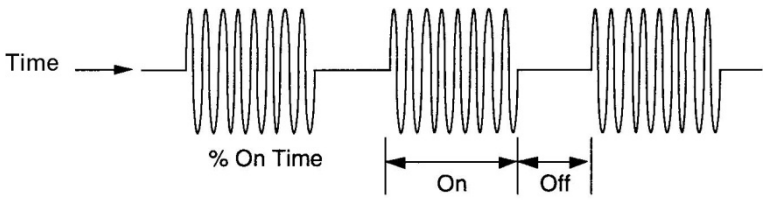


Figure 3.11 Ultrasonic pulsed (John, 1995)

The percentage of time that the ultrasonic energy is on may also be changed to produce varied results. At slower pulse rates, more rapid degassing of liquids occurs as coalescing bubbles of air are given an opportunity to rise to the surface of the liquid during the time the ultrasonic energy is off. At more rapid pulse rates the cleaning process may be enhanced as repeated high energy “bursts” of ultrasonic energy occur each time the energy source is turned on. Pulse operation may be especially useful in facilitating the cavitation of terpenes and petroleum based chemistries.

3.4 Dialysis (Rousseau, 1987)

Dialysis first was reported in 1861 by Graham, who used parchment paper as a membrane. His experiments were based on the observations of a school teacher, W.G. Schmidt, that animal membranes were less permeable to colloids than to sugar or salt. Over the next 100 years, dialysis became widely use as a laboratory technique for the purification of small quantities of solutes but, with minor exceptions, it realized on large-scale industrial applications. In the last 20 years, development of dialysis for the treatment of kidney failure has brought about a resurgence of interest in dialysis for a wide range of separations. Dialysis is a diffusion-based separation process that uses a semi-permeable membrane to separate species by virtue of their different mobilities in the membrane. A feed solution, containing the solutes to be separated, flows on one side of the membrane while a solvent stream, the dialysate, flows on the other side (Figure 3.12). Solute transport across the membrane occurs by diffusion driven by the difference in solute chemical potential between the two membrane-solution interfaces. In practical dialysis devices, an obligatory transmembrane hydraulic pressure may add an addition component of convective transport. Convective transport also may occur if one stream, usually the feed, is highly concentrated, thus giving rise to a transmembrane osmotic gradient down which solvent will flow. In such circumstances, the description of solute transport becomes more complex since it must incorporate some function of the transmembrane fluid velocity.

The relative transfer of two solutes across a dialysis membrane is a function of both their diffusivities in the membrane and their driving forces. Separation will be efficient only for species that differ significantly in diffusion coefficient. Since

diffusion coefficients are a relatively weak function of molecular size, dialysis is limited in practice to separating species that differ significantly in molecular size, for example, separation of crystalloids from polymers or colloids. In addition to this limitation, dialysis is a useful technique only when the solute to be separated is present in high concentration. This is because solute fluxes in dialysis are directly dependent on the tranmembrane concentration gradient, an intrinsic property of the feed and dialysate streams. Thus, if the transmembrane concentration gradient is low, practical solute recoveries can be obtained only by increasing membrane area, which may compromise the economics of the process. Because of these considerations, dialysis is characterized by low flux rates in comparison to other membrane separation techniques. However, the passive nature of dialysis may be an advantage in those circumstances where the species to be separated are sensitive to mechanical degraderation by high pressures or high shear rates. Dialytic separations can be enhanced under certain circumstances by taking advantage of charge repulsion effects between a solute and the membrane, by complexing one or more of the species to be separated, by chemical conversion of the permeating solute in the dialysate, or by staging dialytic cells.

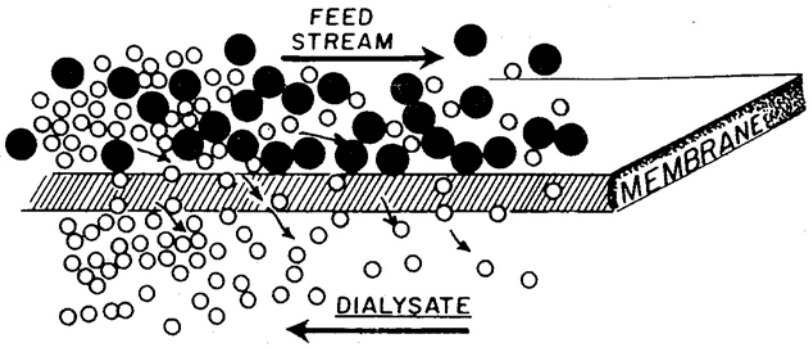


Figure 3.12 Schematic representation of dialysis (Rousseau, 1987)

Dialysis membranes fabricated from glassy polymers, such as methacrylates, polysulfones, polycarbonates and polyacrylonitriles, are not hydrogels and do not undergo irreversible alteration on drying. However, they are generally hydrophobic and their permeability may decrease after drying due to trapping of air in the membrane pores. The hydrophobic nature of most glassy polymers causes them to

adsorb proteins, if present, from the solutions in contact with the membrane. Such adsorption of protein can cause a decrease in membrane permeability and this may limit the role of such membranes for some applications in biotechnology. Yasuda and coworkers found the following relationships between diffusive permeability and membrane structure:

1. The permeability of solutes, whose size is small compared to the membrane pore size, is proportional to the degree of membrane hydration.
2. Membrane permeability decreases exponentially with increasing molecular size, where the latter is expressed in terms of molecular cross-sectional area.
3. Solute reflection coefficients change markedly when solute size approached the average pore size of the membrane.

Solutions adjacent to the membrane are rarely well mixed, and the resistance to transport resides not just in the membrane but also in the fluid regions, termed to boundary layers, on both the dialysate and feed side. Boundary layer effects typically account for from 25-75 % of overall resistance. They are minimized by rapid convective flow tangential to the surface of the dialyzing membrane. When fluid pathways are thin, juxtaposed to the membrane, flow is laminar, and boundary layer resistance decreases with increasing wall shear rate. Where geometry permits higher Reynolds numbers, flow become turbulent and resistance varies with net tangential velocity. Geometric turbulence promoters are often employed. All tactics to reduce boundary layer result in higher energy utilization. Quantitatively, the membrane resistance becomes part of an overall mass coefficient K_{ov} which, for conceptual purposes, is broken down into three independent and reciprocally additive components:

$$\frac{1}{K_{ov}} = \frac{1}{k_F} + \frac{1}{P_M} + \frac{1}{k_D} \tag{3.6}$$

where k_F is the feed side mass transfer coefficient (cm min^{-1}). k_D is the dialysate side mass transfer (cm min^{-1}). P_M is the membrane permeability (cm min^{-1}).

3.4.1 **Overall Mass Transfer in Dialyzer** (Rousseau, 1987)

In the previous section, mass transfer was discussed for a single point on the membrane. For practical applications, this analysis must be extended to describe mass transfer for the dialyzer as a whole. Neglecting convective contributions, we can write the following equation for the mass of solute dm transferred across an element of membrane of length *dx* and area dA_M per unit time (Figure 3.13)

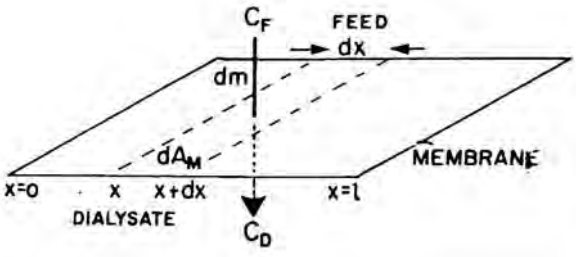


Figure 3.13 Diffusive mass transfers across an incremental length of membrane *dx* (Rousseau, 1987)

$$d\dot{m} = k_{ov}(C_F - C_D)dA_m \tag{3.7}$$

where C_F and C_D are he solute concentrations in the feed and dialysate, respectively, in element *dx*. Eq. 3.7 can be integrated along the length of the membrane to give the following equation for overall mass transfer:

$$\dot{m} = k_{ov}A_m \frac{(C_{Fo}-C_{Di})-(C_{Fi}-C_{Do})}{\ln[(C_{Fo}-C_{Di})/(C_{Fi}-C_{Do})]} \tag{3.8}$$

If ultrafiltration is negligible, inlet and outlet flows are equal for both the feed and dialysate streams. Using this assumption, the following overall mass balances may be written for each stream:

$$\dot{m} = Q_F(C_{Fi} - C_{Fo}) = Q_D(C_{Do} - C_{Di}) \tag{3.9}$$

Finally, the performance of a dialyzer can be described in terms of a "dialysance" D, which is defined as the rate of mass transfer divided by the concentration difference between inlet feed and inlet dialysate; that is

$$D = \frac{\dot{m}}{C_{Fi} - C_{Di}} \tag{3.10}$$

Combining Eqs. (3.8)-(3.10),

$$D = Q_F \left\{ \frac{exp[k_{0v} A_m (1 - Q_F/Q_D)/Q_F] - 1}{exp[k_{0v} A_m (1 - Q_F/Q_D)/Q_F] - Q_F/Q_D} \right\} \tag{3.11}$$

The foregoing assumes that the solute is distributed freely in the solvent phase. This assumption may not always be valid; for example, protein solutions, small solutes may bind to the protein molecules and exist in equilibrium between the free and bound states. In such circumstances, modifications of Eq. (3.11) must be used to determine dialysance. Eq (3.12) can be used to estimate the degree of separation of two solutes by a given dialyzer. For any solute, the fractional extraction into the dialysate, E, of solute from the feed stream is given by

$$E = \frac{Q_F C_{Fi} - (Q_F - Q_{UF}) C_{Fo}}{Q_F C_{Fi}} \tag{3.12}$$

If the inlet concentration of solute in the dialysate is zero (C_{Di} = 0) and negligible ultrafiltration, Eq. (3.12) further reduces to

$$E = \frac{D}{Q_F} \tag{3.13}$$

3.4.2 Dialysis as a Unit Operation (Rousseau, 1987)

Dialysis can be used as a unit operation in two basis configuration as a batch process or in a continuous process stream. Figure 3.14 depicts the flow diagram for batch dialysis. A well-mixed reservoir of solution volume V and solute concentration C_F is dialyzed continuously against a dialysate with an inlet solute concentration C_{Di}. The feed and dialysate streams enter the dialyzer with flow rates Q_F and Q_D, respectively. The feed stream exiting the dialyzer returns to the feed reservoir while the spent dialysate is discarded. Such a configuration could be used for stripping low-molecular-weight contaminants from a high-molecular-weight product. It represents, in essence, the practice of hemodialysis with the reservoir representing the body solute pool.

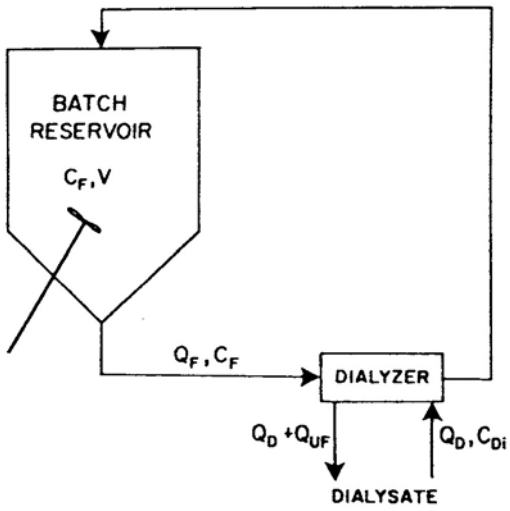


Figure 3.14 Schematic representation of batch dialysis (Rousseau, 1987)

A mass balance for the reservoir may be written

$$\frac{d(VC_F)}{dt} = -D(C_F - C_{Di}) \tag{3.14}$$

where the dialysance D is given by Eq. (3.11). A volume balance also may be written for the reservoir:

$$V = V^0 - Q_{UF}t \tag{3.15}$$

where V^0 is the initial reservoir volume and Q_{UF} is the rate of ultrasiltration from feed to dialysate. Substitution for V in Eq (3.14) and integration lead to the following expression for the concentration of solute in the reservoir as a function of time:

$$C_F^t = \frac{DC_{Di}}{D-Q_{UF}}\left[1 - \left(\frac{V^0 - Q_{UF}t}{V^0}\right)^{\beta}\right] + C_F^0\left(\frac{V^0 - Q_{UF}t}{V^0}\right)^{\beta} \tag{3.16}$$

where β is given by

$$\beta = \frac{D-Q_{UF}}{Q_{UF}} \tag{3.17}$$

CHAPTER IV

MATERIALS & METHODS

4.1 Chemicals

1. Tamarind kernel powder: G.M. Ichihara Co., Ltd., Thailand.
2. Tamarind seed polysaccharide, Sorbigum: G.M. Ichihara Co., Ltd., Thailand.
3. Protease (Esperase 8.0 L): The East Asiatic (Thailand) Public Co., Ltd.
4. 95% V/V food grade ethanol: SR Lab, Thailand.
5. 99.9% V/V isopropanol: AR, QRec.
6. Sodium hydroxide: Baker analytical reagent. J.T. Baker
7. Sulfuric acid: AR, J.T. Baker**.**
8. Boric acid: AR, QRec.
9. Phenol: AR, Panreac**.,** Spain
10. Selenium reagent mixture: AR, Merck, Germany**.**
11. Petroleum ether: AR, Fisher Scientific.
12. Bovine serum albumin, BSA**:** AR**,** Fluka**,** Switzerland.
13. Cazein: AR**,** Highmedia.
14. Copper Sulphate: AR, Carloerba, Italy**.**
15. Sodium potassium tartrate: AR, Ajax chemical, Australia.
16. Sodium carbonate: AR, Ajax chemical, Australia.
17. Folin-Ciocalteau reagent: AR, Carlo Erba, Spain**.**

4.2 Equipments

1. High-intensity ultrasound: 500 W Model with ½ in probe, 20 kHz**,** Sonic and Materials Inc.
2. Dialyzer: Hemoflow F70S, Fresenius**,** Thailand**.**
3. Rotary evaporator: N-1000, EYELA, Japan**.**
4. Spectrophotometer: Spectronic 20 Genesys, Spectronic instruments, USA.
5. Scanning electron microscopy: JSM-5410LV, JOEL, Japan.
6. Distillation Unit Nitrogen analyzer: BUCHI 339, Japan
7. Peristaltic pump: 505U, WATSON MARLOW, England.
8. Soxhlet apparatus.
9. Microscope**:** BH -2, OLYMPUS, Japan**.**
10. Rapid Visco Analyzer, RVA: RVA model super 3, Newport Scientific**,** Australia
11. Multi angle laser light scattering, MALLS: Wyatt technology, USA

4.3 Methods

4.3.1. Compositions & thermal properties of TKP

The compositions and thermal properties of raw material TKP as polysaccharide, protein and oil were firstly investigated in order to compare with the product obtained from each treatments. The analysis of each components was done according to section 4.4

4.3.2. De-oiled processing

4.3.2.1. Batch extraction

The TKP was mixed with solvent in a constant stirring beaker that was put into the controlled temperature water bath. The series of experiments were conducted and each trial was terminated at various extraction times. After each extraction

experiment, the de-oiled TKP (DTKP) was separated from solvents by Whatman filter paper No.1 under vacuum and was washed twice with fresh solvent (20 ml). In subsequently, DTKP was allowed to dry at room temperature. Then, the DTKP was kept in plastic desiccators at room temperature until used. The content of oil in final product was analyzed with Soxhlet method. Diagram for oil extraction was shown in Figure 4.1. The experiment conditions were summarized in Table 4.1.

Table 4.1 Extraction process parameters

Parameters	Conditions
Solvent type	95% ethanol, isopropanol or hexane
TKP to solvent ratio	1:3 – 1:10 g ml^{-1}
Extraction time	3 – 60 min
Extraction temperature	30 - 60°C

4.3.2.2. Batch extraction of the high-intensity ultrasound-assisted 95% ethanol extraction

For the high-intensity ultrasound-assisted extraction, the TKP was mixed with 95% ethanol in beaker. The ultrasonic probe was inserted into the mixture directly. The samples were extracted under continuous ultrasonic waves at 20 kHz. The energy input was controlled by setting the amplitude of the sonicator probe. The sonication amplitude range was 0-60% with 5 s “on” and 5 s “off”. The sonication time was counted only the “on” time. After extraction experiments, the extracted mixture was treated and analyzed as that mentioned in the batch extraction. Diagram for high-intensity ultrasound assisted 95% oil extraction was shown in Figure 4.2. The experiment conditions were summarized in Table 4.2.

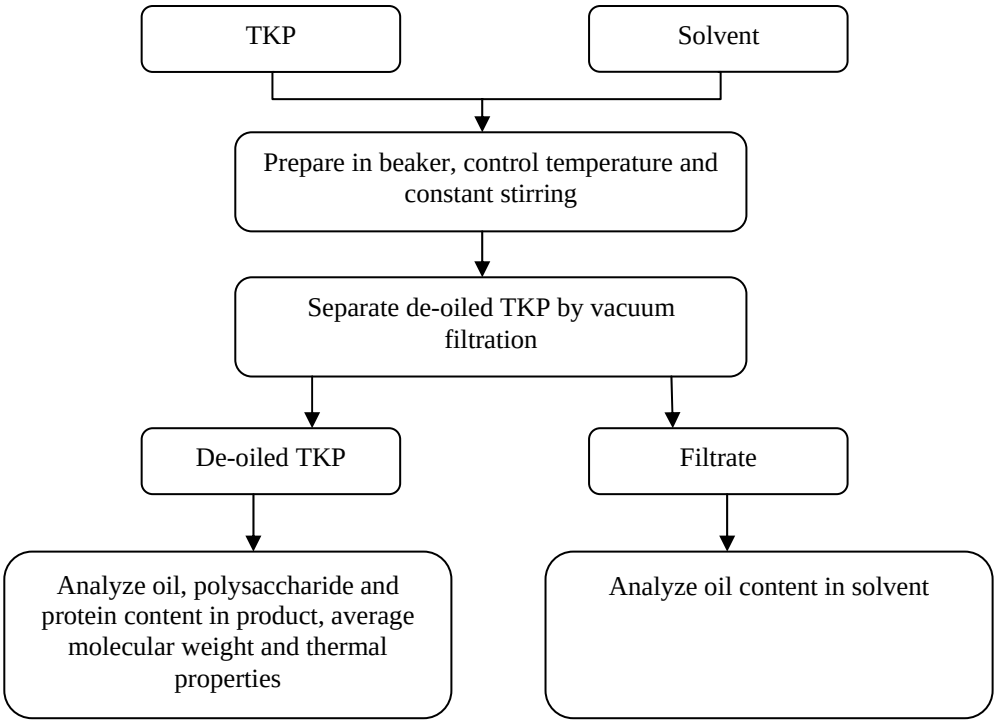


Figure 4.1 Diagram for oil extraction from TKP

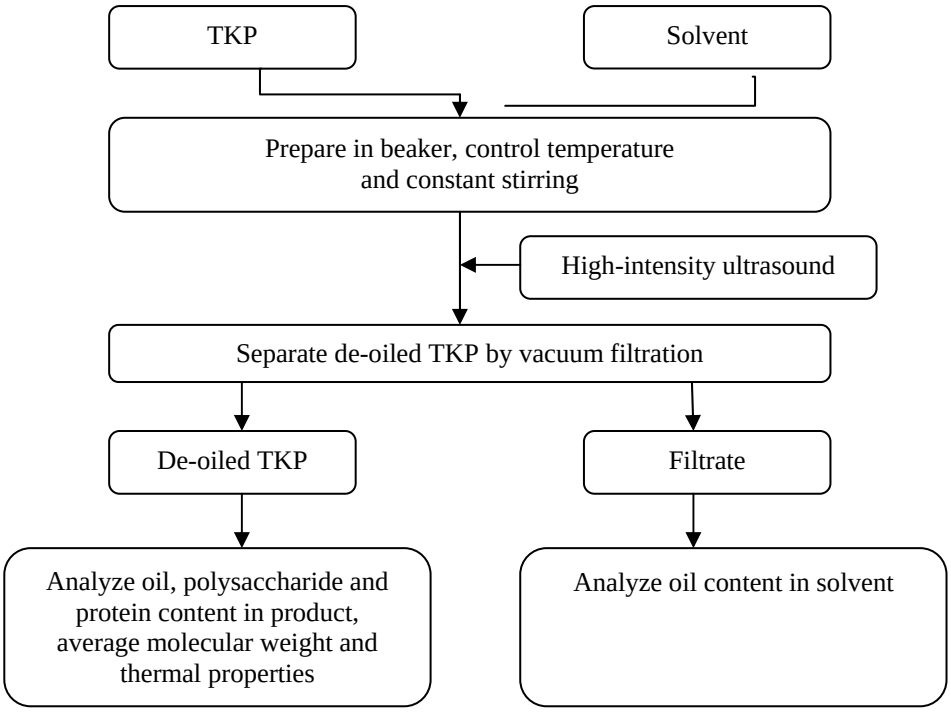


Figure 4.2 Diagram for high-intensity ultrasound assisted 95% ETOH oil extraction

Table 4.2 High-intensity ultrasound assisted extraction process parameters

Parameters	Conditions
Solvent type	95% ethanol
TKP to solvent ratio	1:3
Amplitude level	0-60% (0-2.5 W cm^{-3})
Extraction time	3 - 60 min
Extraction temperature	30 - 60°C

4.3.3. Isolated tamarind seed polysaccharide (ITSP) processing

The raw material (DTKP) in this experiment was chosen from the suitable condition of de-oiled processing.

4.3.3.1. Isolation of tamarind seed polysaccharide using protease

The 5g DTKP (dry basis) was mixed with 5 ml 95%ethanol and 245 ml deionized water in a beaker. The temperature was maintained at 37°C with constant stirring using magnetic stirrer. Neutral protease (Esperase 8.0L) was added to the slurry. After the protease digestion the suspension were separated by using centrifuge (at 1500 rpm, 10 min) and the supernatant (top layer) was removed carefully. Then, the sedimentation powder was mixed with 50 ml 95% ethanol and stirred 5 min. The powder was separated from solvent by Whatman filter paper No. 1 under vacuum. After that, isolated tamarind seed polysaccharide (ITSP) was allowed to dry at room temperature, pass through a 150 µm stainless sieve and stored in plastic desiccators at room temperature until analyzed. Diagram for isolation of tamarind seed polysaccharide using protease was shown in Figure 4.3. The experiment conditions were summarized in Table 4.3.

4.3.3.2. Isolation of tamarind seed polysaccharide using high-intensity ultrasound

For high intensity-ultrasound treatment, the 5 g DTKP (dry basis) was mixed with 5 ml 95%ethanol and 245 ml deionized water in a beaker. The temperature was

maintained at 37°C with constant stirring using magnetic stirrer. The energy input was controlled by setting the amplitude of the sonicator probe. The sonication amplitude was 25, 50, 75 or 100% with 5 s on and 5 s off for 15, 30, 45 or 60 minutes. The sonication time included only the on time. The isolation powder was treated and analyzed as described in the protease treatment. Diagram for isolation of tamarind seed polysaccharide using high-intensity ultrasound was shown in Figure 4.4. The experiment conditions were summarized in Table 4.4.

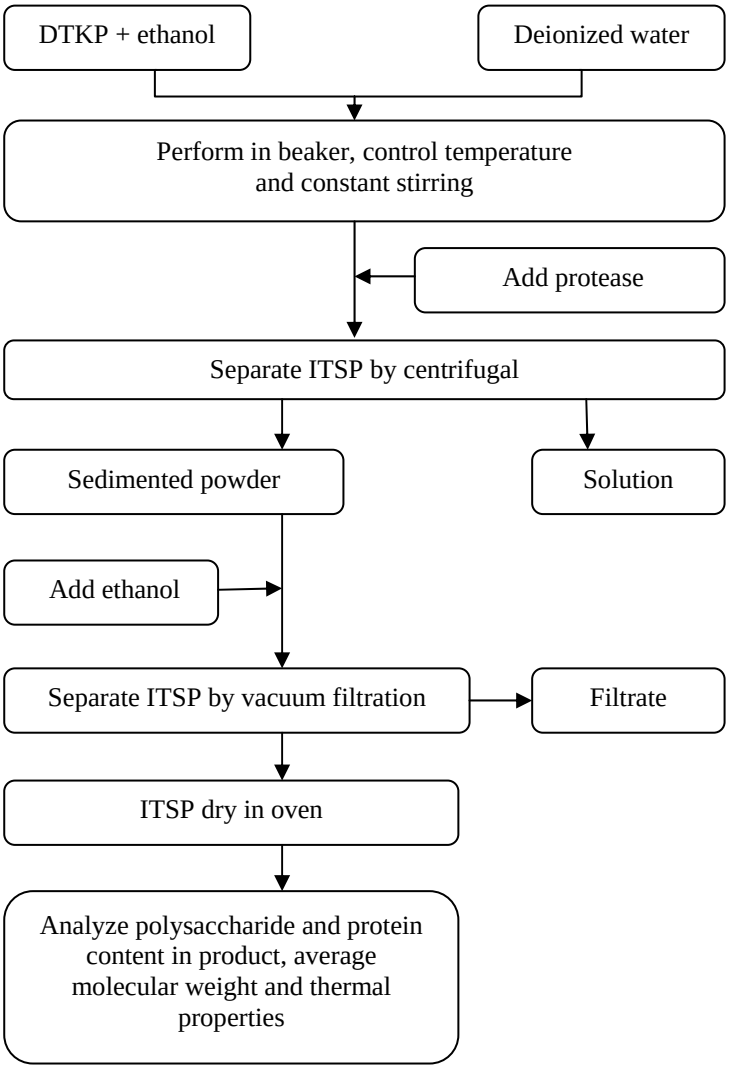


Figure 4.3 Diagram for isolation of tamarind seed polysaccharide using protease

Table 4.3 Protease digestion parameters

Parameters	Conditions
Protease type	Esperase 8.0 L
Enzyme level	0.1 – 0.5% (based on powder)
Digestion time	1 – 5 h
Digestion temperature	37°C

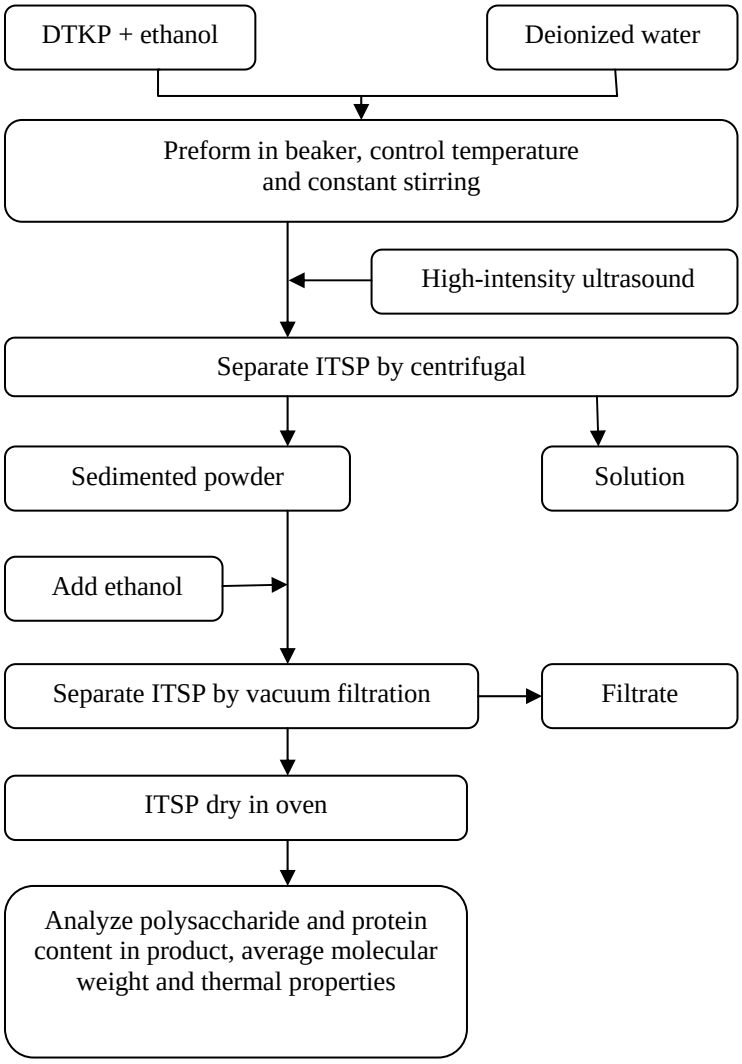


Figure 4.4 Diagram for isolation of tamarind seed polysaccharide using high-intensity ultrasound

Table 4.4 High-intensity ultrasound parameters

Parameters	Conditions
High-intensity ultrasound	500 W, ½ in. probe
Amplitude level (power)	0-100% (0-2 W cm^{-3})
Sonication time	15 – 60 min
Sonication temperature	37°C

4.3.3.3. Isolation of tamarind seed polysaccharide using combination of protease and high-intensity ultrasound

In this procedure, high-intensity ultrasound treatment was applied in 3 categories. The first method, high-intensity ultrasound was used before protease digestion. The second method, high-intensity ultrasound was used after protease digestion. And the last method, high-intensity ultrasound was used together with protease treatment. The level of protease and digestion time was chosen from suitable condition in 4.3.3.1. The level of amplitude of high-intensity ultrasound and sonication time were chosen from suitable condition in 4.3.3.2. The experiment condition was shown in Table 4.5. The isolation of powder was the same as described in the protease treatment. Diagram for isolation of tamarind seed polysaccharide using combination of protease and high-intensity ultrasound was shown in Figure 4.5.

Table 4.5 Combination of protease and high-intensity ultrasound (HIU) parameters

Parameters	Conditions
Protease level and digestion time	Suitable condition from 4.3.3.1
Amplitude level and sonication time	Suitable condition from 4.3.3.2
Sequent	HIU before protease digestion
	HIU after protease digestion
	HIU plus protease digestion

4.3.4. Purification of isolated tamarind seed polysaccharide using dialyzer

The purification of isolated tamarind seed polysaccharide (ITSP) using dialyzer was investigated in order to separate residual small molecule of protein in ITSP. Then, ITSP was dissolved in hot water (85°C) for 30 minute. The insoluble fraction

and clear supernatant was removed by centrifugation at 3000 rpm and 20 min. Then, the supernatant was passed into dialyzer apparatus (Figure 4.6) in order to remove residual protein. The arrangement of feed solution and dialysate (deionized water) flow pattern was in counter-current. The conditions of processing showed in Table 4.6. After that, the resulting solution was precipitated in 95% ethanol and dried in hot air oven at 80°C. Finally, the dried precipitated solid was milled and kept in plastic desiccators at room temperature until analyzed. Diagram for purification of isolated tamarind seed polysaccharide using dialyzer was shown in Figure 4.7.

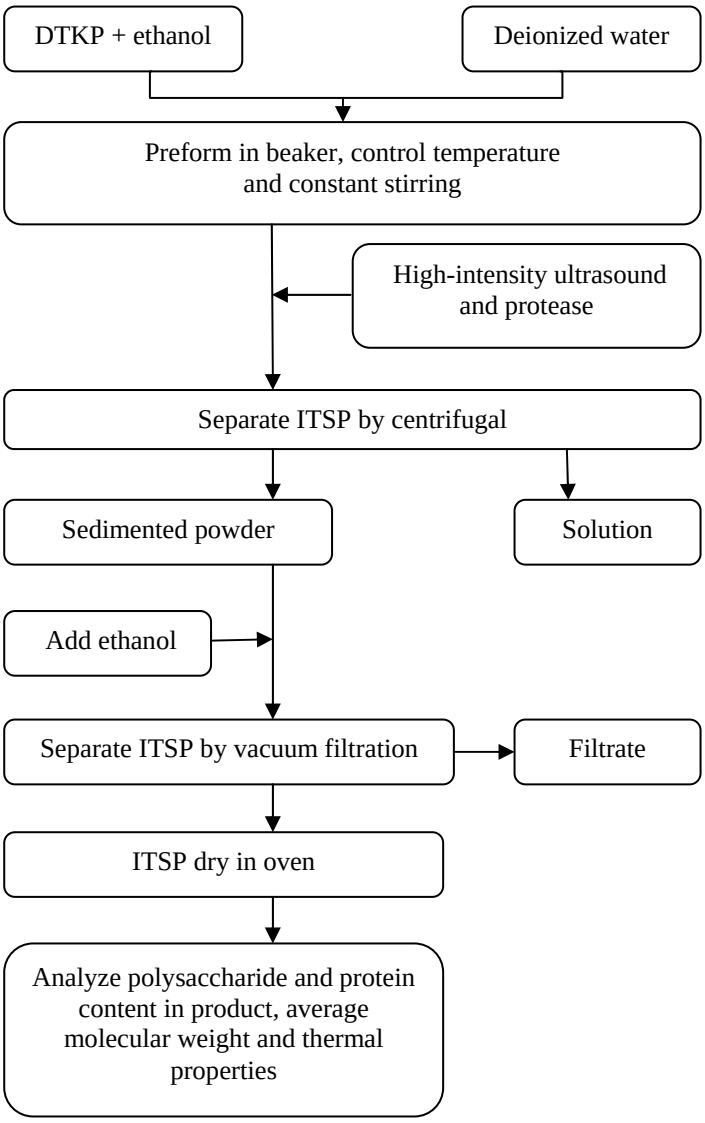


Figure 4.5 Diagram for isolation of tamarind seed polysaccharide using protease and high-intensity ultrasound

Table 4.6 Technical data on the hollow fiber dialyzer

Name	Hemoflow F70S
Manufacture	Fresenius
Material	Polysulfone
Membrane surface area (m^2)	1.6
Inner diameter (μm)	200
Wall thickness (μm)	40
Effective fiber length (mm)	250
Number of hollow fiber	10800
Shell diameter (mm)	45
Ultrafiltration coefficient (ml h^{-1} mm.Hg)	50
Feed flow rate (ml min^{-1})	150, 200 or 250
Dialysate flow rate (ml min^{-1})	900, 1,200 or 1,500
Porosity, ε	0.37
Tortousity	2.5
Flow direction	Counter current

Figure 4.6 Schematic of dialyzer apparatus

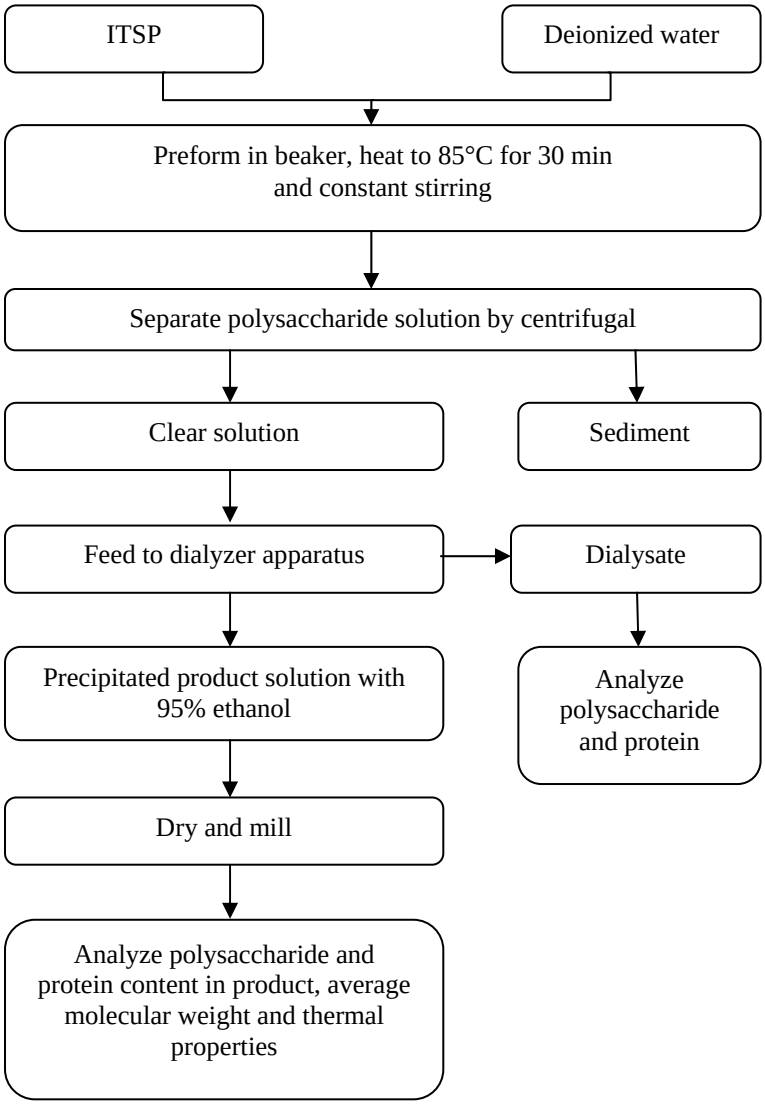


Figure 4.7 Diagram for purification of isolation of tamarind seed polysaccharide using dialyzer

4.4 Analyzed methods

4.4.1 Polysaccharide analysis

The polysaccharide content was determined according to Phenol-sulfuric method (Dubois et al, 1956). Tamarind seed polysaccharide that obtained from GM Ichihara Co., Ltd Thailand was used as a standard polysaccharide.

4.4.2 Protein analysis

The protein content in form powder was determined by Kjeldahl method (AOAC 955.04). The protein content in solution was measured by Lowry method (Lowry et al, 1951). The bovine serum albumin was used as a standard protein.

4.4.3 Oil analysis

The oil content in TKP and product was analyzed by Soxhlet extraction method (AOAC. 935.29).

4.4.4 Scanning electron microscopy (SEM) analysis

The microstructure and the surface character of TKP samples were observed and recorded on the scanning electron microscope (JSM-5410LV, JOEL, Japan).

4.4.5 Thermal properties analysis

The thermal properties of TKP and products were measured by using a Rapid Visco Analyzer (RVA, RVA model super 3, Newport Scientific) equipped with a cooling system. The viscosity of TKP sample (4% wt/wt) were observed at initial temperature 50°C for 0 to 1 minute, 1to 5 minute heated to 95 °C with heating rate 11.25 °C min^{-1}, 5 to 7 minute hold the temperature at 95°C, 7 to 11 minute cooled to 50°C with cooling rate 11.25 °C min^{-1} and hold the temperature at 50°C until stop the process at 13 minute.

4.4.6 Molecular weight analysis

Molecular weight of TKP and products were estimated by Zimm plot analysis using Multi angle laser light scattering (MALLS, Wyatt, 1992).

4.5 Calculation parameters

4.5.1 Percentage of oil removal (dry basis)

$$Oil\ removal(\%) = \frac{initial\ oil\ \ (g/g\ TKP) - residual\ oil\ (g/g\ TKP)}{initial\ oil\ (g/g\ TKP)} \times 100$$

4.5.2 Percentage of product yield (dry basis)

$$Yield\ (\%) = \frac{product(g)}{raw\ material\ (g)} \times 100$$

4.5.3 Percentage of protein eliminated

$$Protein\ eliminated\ (\%) = \frac{protein\ in\ raw\ material(g) - protein\ in\ product\ (g)}{protein\ in\ raw\ material\ (g)} \times 100$$

4.5.4 Percentage of polysaccharide loss

$$Polysaccharide\ loss\ (\%) = \frac{polysacchride\ in\ raw\ material(g) - polysaccharide\ in\ product\ (g)}{polysaccharide\ in\ raw\ material\ (g)} \times 100$$

4.5.5 Power intensity (W cm^{-2})

$$Power\ intensity = \frac{Amplitude\ (\%) \times 500\ W}{100\ \times (\pi/r^2)}$$

CHAPTER V

RESULTS & DISCUSSIONS

The general goal of this research was to produce highly purified TSP from TKP. Therefore, de-oil process using different solvents and HIU, protein removal by protease enzyme with HIU and finally by dialysis were consecutively performed.

5.1 Compositions & pasting properties of TKP

The compositions of TKP were shown in Table 5.1. TKP composed of polysaccharide (xyloglucan), protein, oil and other 69.84, 18.82, 8.00 and 3.34%, respectively. The particles size of TKP depended on milling process and sieving. TKP, in this research, passed through 200 meshes sieving, was obtained from GM Ichihara Co., Ltd., Thailand as raw material. The average particles size was 22.35 µm. Figure 5.1 shows the SEM image of TKP raw material (5.1a) and TKP disperse in water (5.1b). From Figure 5.1 (a), the powder composed of large particles (polysaccharide) and small globular particle (protein), while Figure 5.1(b) shows the blue color of polysaccharide in TKP dispersed in water with I_2 solution (iodine impregnation showing polysaccharide granules in blue).

Table 5.1 Compositions of tamarind kernel powder (dry weight)

Composition	%
Polysaccharide	69.84
Protein	18.82
Oil	8.00
Other	3.34
Average particle size (µm)	22.35

Solubility of TKP can be divided to three parts. About 10% is dissolved in water at temperature below 20°C, next 25% of TKP is soluble in water at room temperature (30°C) and most of TKP is soluble in water at temperature above 80°C. The temperature at the onset of the increase in the viscosity can be considered as the

starting point of the gelatinization and is call pasting temperature. When most TKP granules became swollen stage above pasting temperatures, a fast increase in viscosity occurred. While the system was at the holding temperature (95°C), the mixtures were subjected to mechanical or shear stress. Figure 5.2 shows the thermal properties of TKP that was measured with RVA. It was found that, when the temperature was raise, the viscosity of slurry increased because of the increasing of solubilization of TKP. The pasting temperature was about 68-70°C. After holding the temperature at 95°C, the viscosity slightly decreased (416 to 373 cp) and rise up to a final viscosity when the slurry was cooled down.

Protein oil and fiber in raw material TKP are unfavorable because it contributes little or nothing to intend in any applications. Moreover, oil or fat in TKP make the product tacky, rancidity, and non-free flowing. As a result, such TKP is difficult to convey and disperse. The water-insoluble fiber in TKP can build up in the processing equipment which is necessary to periodically shut-down for removal. Therefore, in this research, the experiment was applied in 3 categories. The first experiment (section 5.2), the de-oiled processing will be studied in order to get DTKP. The second experiment (section 5.3), polysaccharide isolated (ITSP) processing by protease and high-intensity ultrasound will be investigated. And finally, in last experiment (section 5.4), dialyzer was used to increase the purity of polysaccharide.

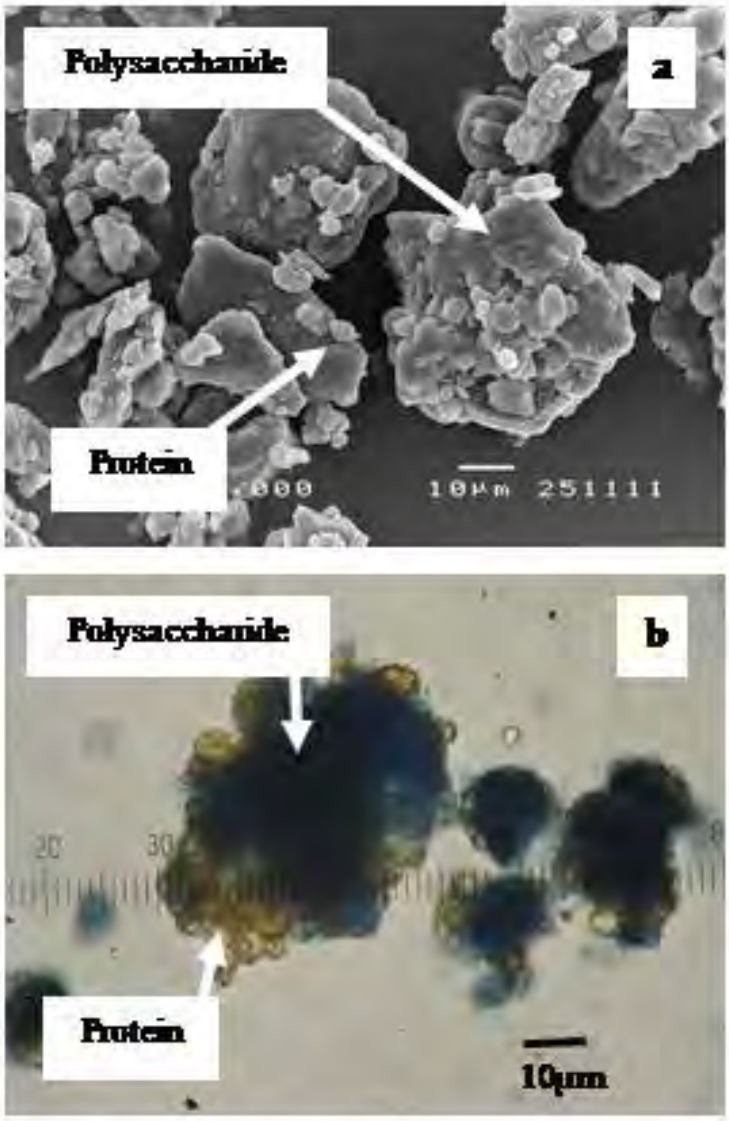


Figure 5.1 (a) Scanning electron microscope images of tamarind kernel powder, (b) Microscope images of tamarind kernel powder dispersed in water.

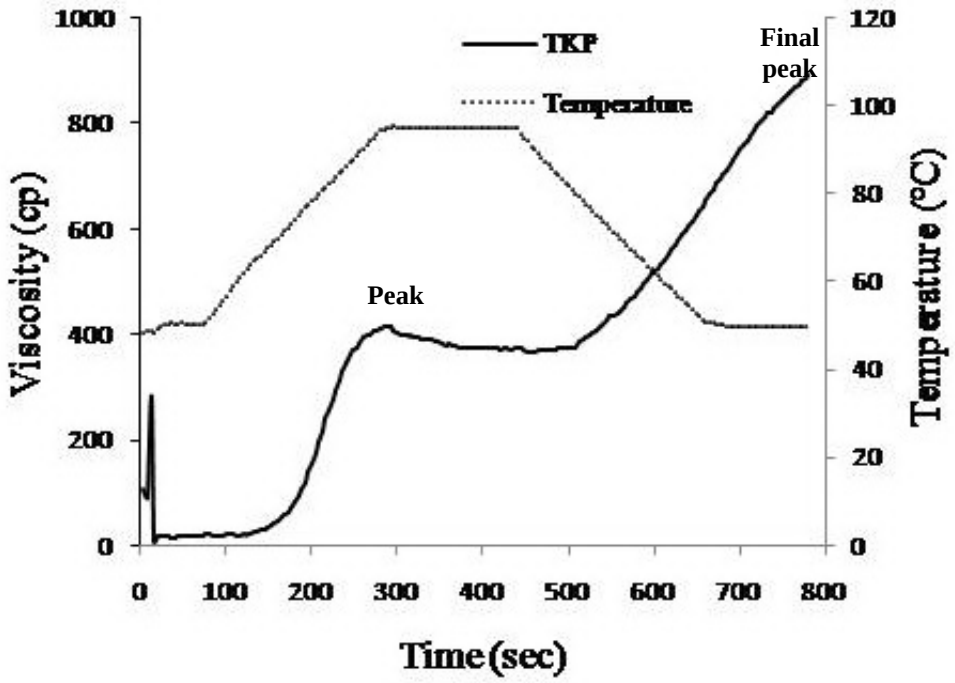


Figure 5.2 Typical RVA pasting profile of tamarind kernel powder (4% wt/wt)

5.2 De-oil TKP (DTKP) processing

At present, tamarind kernel oil is extracted from TKP by solvent extraction. The solvent such as hexane is widely used in oil extraction industry. However, the disadvantages of hexane and other organic solvents that used in industry are flammability, toxicity and restrictive to governmental regulations. Thus, the recent interest in alternative solvents (95% ethanol and isopropanol) has shifted towards finding a solvent that is less flammable and produces less noxious emissions.

5.2.1 Effect of different solvents on oil extraction

Figure 5.3 shows the percentage of oil removal in DTKP extracted by 95% ethanol, isopropanol and hexane (temperature 30°C, solvent to solid ratio 1: 3). The experimental results show that, when the extraction time was increased from 3 to 60 min, the percentage of oil removal extracted by 95% ethanol slightly increased from 35.02 to 39.94%. For isopropanol and hexane extraction, the percentage of oil removal was higher than 95% ethanol extraction from 87.75 to 94.38% and 90.21 to

94.54%, respectively. In addition, the experimental results found that at initial time period to 3 min. the percentage of oil removals rapidly increased from 0 to 35.02, 87.75 and 90.21%, for 95% ethanol, isopropanol and hexane extraction, respectively. After 3 min. to 60 min. of extraction, percentage of oil removals slightly increased. Thus, it showed that the longer extraction time did not make a significant difference to the percentage of oil removal (Proctor et al, 1996, Gandhi et al, 2003) . The driving force for extraction is the concentration gradient of the solute in the solid surface and that in the bulk of the solvent. For an initial time period, the powder is rich in oil and the surface is saturated with the solute. At this period, the rate of extraction remains constant while after this period, the diffusion of solute to the solid surface from the interior of the solid becomes the rate determining step. So, the rate of oil extraction decreases with time and reaches zero. Moreover, the initial rate of oil extraction was very fast in shorter time because the powder used in this study was the smallest size. In case of 95% ethanol, ethanol molecule was block dissolving lipid molecule that impregnation in polysaccharide helix (granule) by water molecule (Franco, 2007). Therefore, the percentage oil removal in powder was lower than isopropanol and hexane.

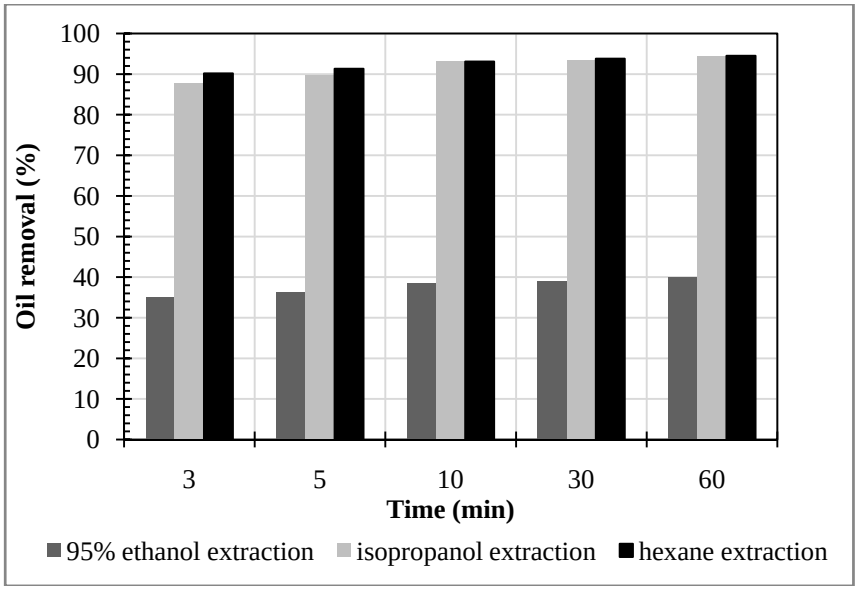


Figure 5.3 Effect of organic solvents on percentage oil removal from TKP (extraction temperature 30°C; solvent to solid ratio 3:1 ml g^{-1})

5.2.2 Effect of solvent ratio extraction

Figure 5.4 shows the effect of solvent to solid ratio (3:1 to 10:1, ml g^{-1}) on the percentage oil removal. At the solvent to solid ratio below 3:1, the mixture was not well mixed. For 95% ethanol extraction, the percentage oil removal increased with the increasing of the ratio of solvent to solid. The oil removal was increased from 42.34 to 78.23% for solvent to solid ratio 3:1 to 7:1, respectively. A slightly increase in oil extraction was observed for ratio more than 7:1 due to the limitation of diffusion mass transfer. On the other hand, the percentage of oil removal extracted by isopropanol and hexane for solvent to solid ratio 3:1 to 10:1 was found to slightly increase from 93.13 to 96.88% and 93.08 to 96.81% for isopropanol and hexane, respectively. It means that isopropanol and hexane has higher oil solubility than 95% ethanol. Therefore, the larger volume of 95% ethanol added the more oil extraction obtained due to solubility effect. However, the larger ratio of solvent to solid for isopropanol and hexane gained slightly oil extraction due to the limitation by diffusion mass transfer. Finally, regarding to the price of solvent, the suitable solvent to solid ratio should be 3:1 ml g^{-1}.

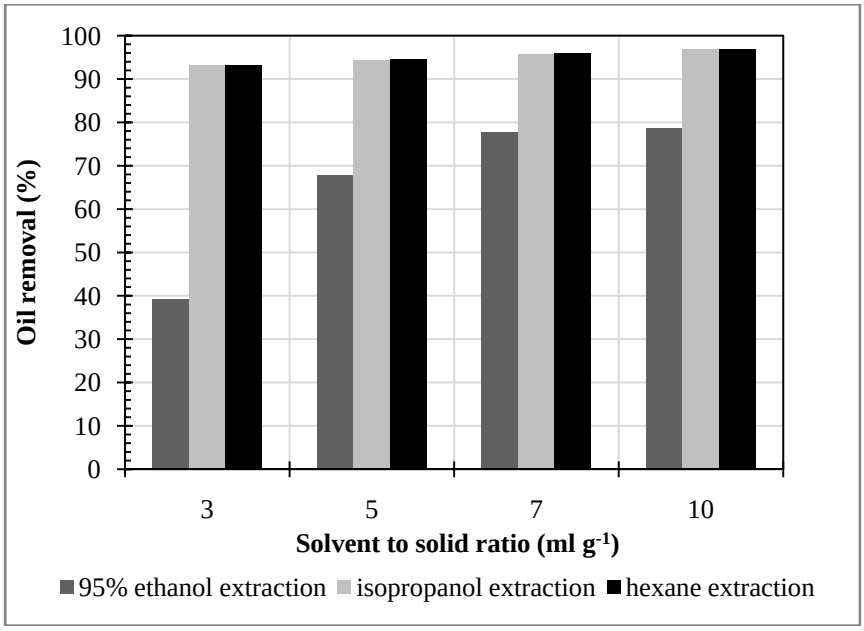


Figure 5.4 Effect of solvent to solid ratio on the percentage of oil removal from TKP (extraction temperature 30°C; extraction time 10 min)

5.2.3 Effect of extraction temperature

Figure 5.5 shows the effect of temperature on the percentage of oil removal from TKP. At the range of temperature between 30 to 60ºC, the percentage of oil removal for extraction by 95% ethanol, isopropanol and hexane increased from 42.34 to 89.13, 93.13 to 96.52 and 93.08 to 97.24%, respectively. In case of 95% ethanol, the rising of temperature strongly enhanced the solubility rather than diffusivities of solute to solvent, so the oil removal at high temperature treatment was increased more than low temperature treatment (Kwiatkowski et al, 2002). However, the temperature from 50 to 60ºC, oil removal was limited by diffusion mass transfer. In contrary, in case of isopropanol and hexane extraction, increasing of temperature could slightly enhance the oil removal due to slightly enhancement of solubility and consecutively diffusion mass transfer limitation.

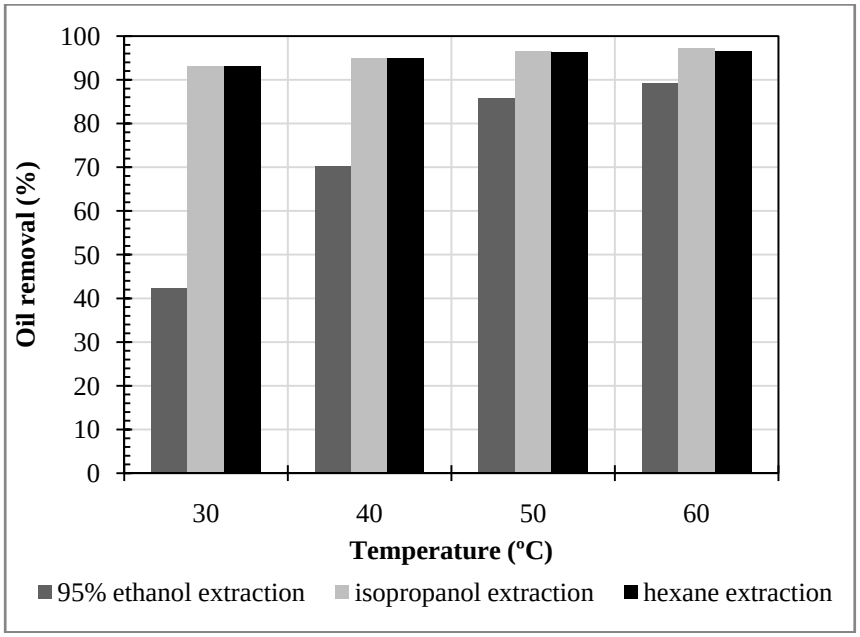


Figure 5.5 Effect of temperature on the percentage of oil removal from TKP (extraction time 10 min; solvent to solid ratio 3:1 ml g^{-1})

5.2.4 Effect of ultrasonic amplitude power assisted 95% ethanol extraction

From previous experiments, the experimental results indicated that oil extraction using 95% ethanol had lower effectiveness than isopropanol and hexane

extraction (Zhang, 2002, Franco, 2007, Liauw, 2008). Nevertheless, the 95% ethanol is so interesting that the price of 95% is cheaper than both solvents. To improve the efficiency of 95% extraction, high-intensity ultrasound was applied to enhance extraction of lipophilic compounds from TKP.

The effect of high-intensity ultrasound-assisted 95% ethanol extractions on the percentage of oil removal are shown in Figure 5.6, 5.7 and 5.8. Figure 5.6 shows the effect of the amplitude level of high-intensity ultrasound on oil removal. The report showed that the percentage of oil removal from TKP increased linearly from 42.34 to 82.88% with increasing ultrasonic amplitude level from 0 to 60%. As the greater amplitude ultrasonic wave travel through a liquid medium, more bubbles were created and collapsed. Since the temperature and pressure inside the bubbles were very high and the collapse of bubbles chanced over very short time, the violent shock wave was generated which could enhance the penetration solvent into the group of solid particles and destroyed the interactive force between particles. Moreover, the violent shock wave has caused the molecule to mix better enhancing the mass transfer rate. However, it was also found that when the amplitude was applied more than 50%, solvent was rapidly evaporated (Zhang, 2008). Thus, base on these results, the suitable ultrasonic amplitude level of high-intensity ultrasound was approximately 50%, thus ensuring nearly maximum oil removal in TKP.

The effect of sonication time at 50% amplitude level was shown in Figure 5.7. The percentage of oil removal strongly increased from 61.94 to 80.79% at time interval of 3 to 10 min and slightly increased after 10 to 60 min sonication time. The process indicates that ultrasound is more effective in the first 10 min. It means that applying ultrasonic wave or input energy in the first stage could destroy interactive force between particles or destroy hydrogen force between water and polysaccharide. These phenomena leads to enhance the contact area between solvent and particles, so ethanol molecule could adhere to lipid molecule in polysaccharide and diffused out of granule. This is in good agreement with previous works published by Li et al.(2004), Zhang et al. (2008), Hemwimol et al. (2006) and Zhoa et al. (2005). They had confirmed that ultrasound was very effective through an enhancement of the internal

diffusivity, even during the later stage of extraction which was controlled by diffusion mass transfer.

Figure 5.8 shows the effect of temperature-assisted 50% amplitude level extraction using 95% ethanol. The increasing of temperature from 30 to 60°C improved the percentage of oil removal from 78.76 to 95.07%. Comparing with 95% ethanol extraction, at low temperature, 30 and 40°C, the percentage of oil removal was increased from 42.34 to 78.76% and 70.13 to 89.07%, respectively. However, at high temperature 50 and 60°C, the percentage of oil removal slightly increased from 85.80 to 93.56% and 89.13 to 95.97%, respectively. The high-intensity ultrasound had more effective at low temperature than at high temperature because acoustic wave from high-intensity ultrasound cause to strongly increase diffusivities of oil to 95% ethanol over solubility. However, at high temperature, high-intensity ultrasound had slightly improvement on oil extraction over solubility. Based on this experimental, the suitable temperature for high-intensity ultrasound should be 50°C.

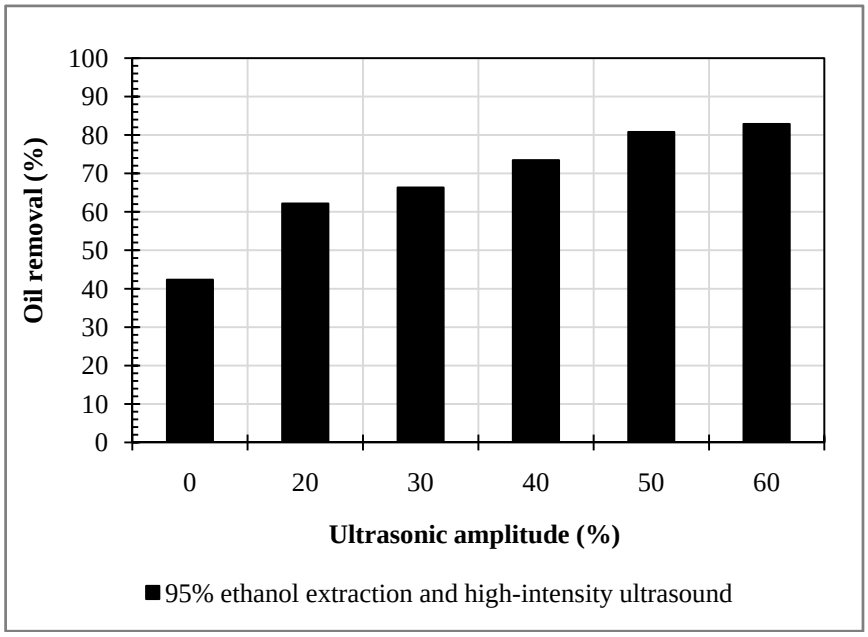


Figure 5.6 Effect of percentage amplitude level of high-intensity ultrasonic on the percentage of oil removal from TKP (extraction time 10 min; solvent to solid ratio 3:1 ml g^{-1}; extraction temperature 30°C)

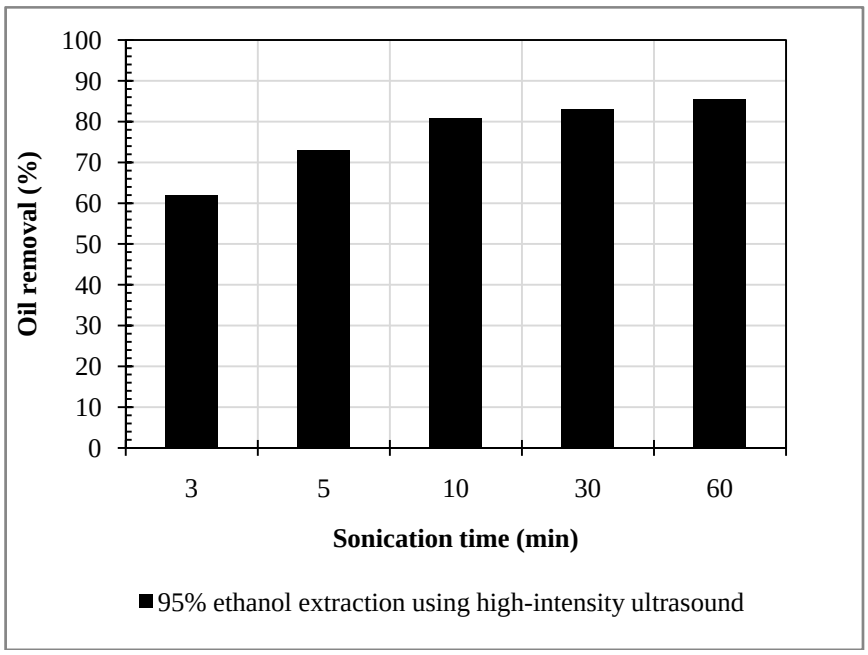


Figure 5.7 Effect of sonication time on the percentage of oil removal from TKP (amplitude level 50%; extraction temperature 30°C; solvent to solid ratio 3:1 ml g^{-1})

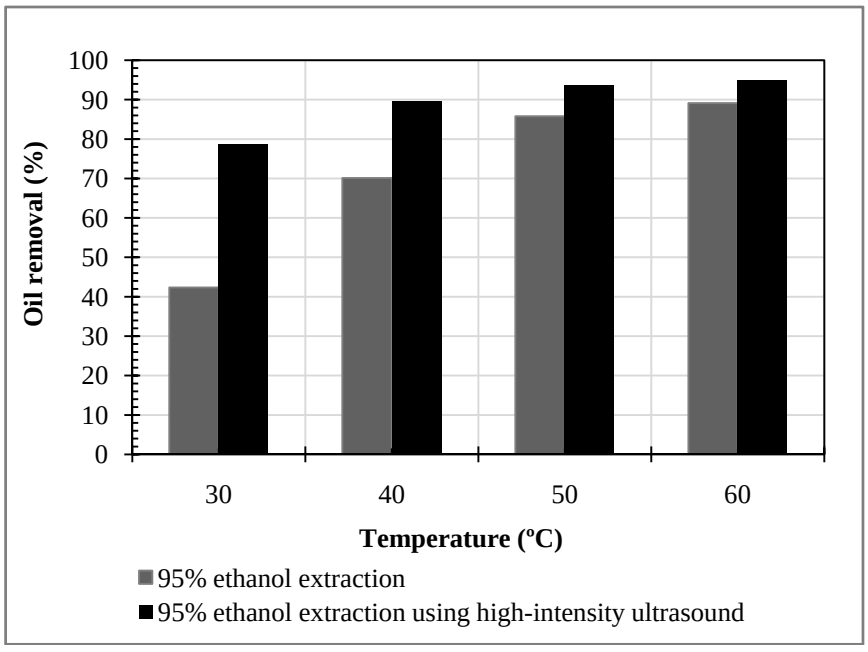


Figure 5.8 Effect of temperature on the percentage of oil removal from TKP using ultrasound (amplitude level 50%; extraction time 10 min; solvent to solid ratio 3:1 mlg^{-1})

5.2.5 Effect of ultrasound on the physical properties

Figure 5.9 shows the SEM images of TKP (5A) and DTKP (5B and 5C) at a magnification factor 1000x. There was a slightly significant on the size of particles. After 10 min ultrasound-assisted extraction (5C), most of particles are smaller than un-treat (5A) and un-used ultrasound (5B). The high-intensity ultrasound had an effect on particle size of DTKP. Besides, the average molecular weight of polysaccharide was reduced by high-intensity ultrasound too. The molecular weight of polysaccharide in DTKP from different types of extraction was shown in Figure 5.10. The increasing of amplitude level broke the polysaccharide granule to smaller size than un-used ultrasound.

Table 2 summarized the percentage of oil removal and the pasting properties of DTKP using different treatments. The pasting properties were measured by RVA. The decreasing of oil in DTKP makes a higher viscosity and easier to dispersed in water. Regarding to the effect of ultrasonic on viscosity, the peak, breakdown and final peak values of the product produced by isopropanol, hexane and 95% ethanol using 50% amplitude level of high-intensity ultrasound at 50°C (U50T50) extraction at nearly the same value of oil removal 93.13, 93.34 and 93.13%, respectively, showed the strongly improvement of 95% ethanol extraction using high-intensity ultrasound. Based on this experiment, the suitable condition for DTKP processing was selected by the percentage of oil removal in term of solvent type, solvent to solid ratio and used energy. So, isopropanol extraction at the solvent to solid ratio of 3:1 ml g^{-1}, extraction time 10 min and the temperature of 30 °C are suitable for production and for further study in protein elimination.

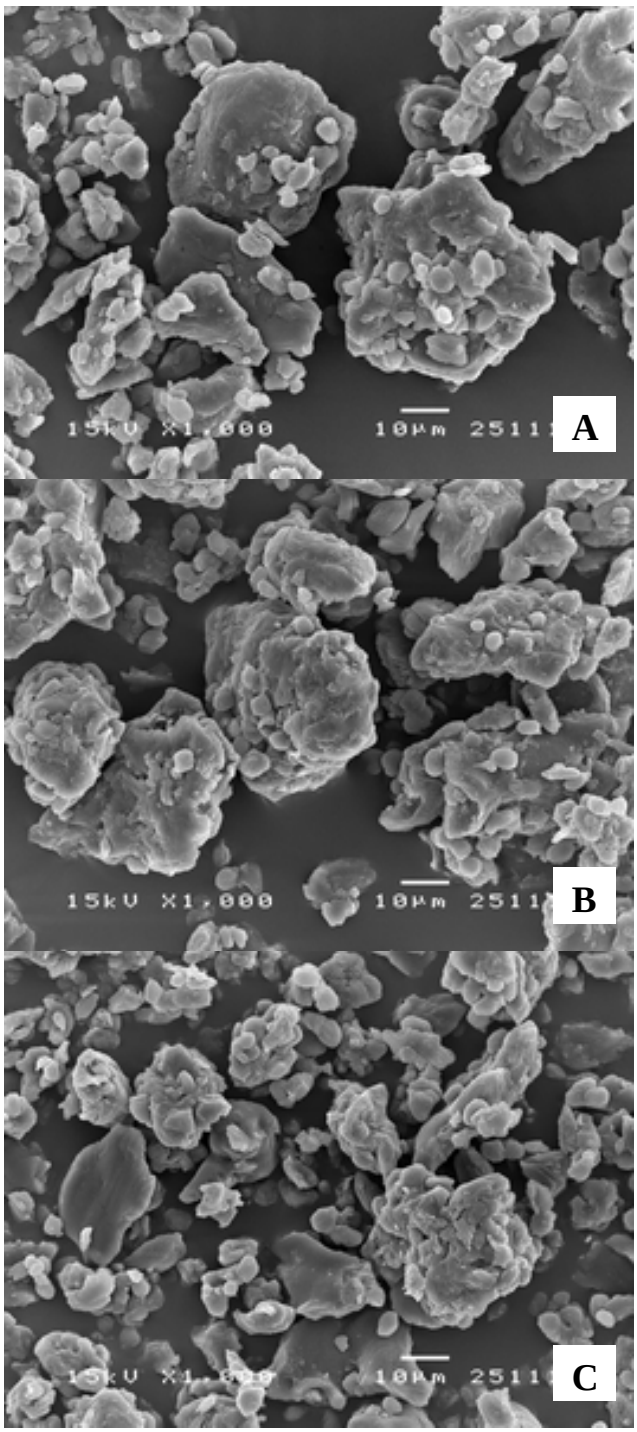


Figure.5.9 Scanning electron microscope images of TKP.

(A) Fresh TKP.

(B) DTKP (95% ethanol extraction)

(C) DTKP (95% ethanol extraction using high-intensity ultrasound 10 min at 50% amplitude.)

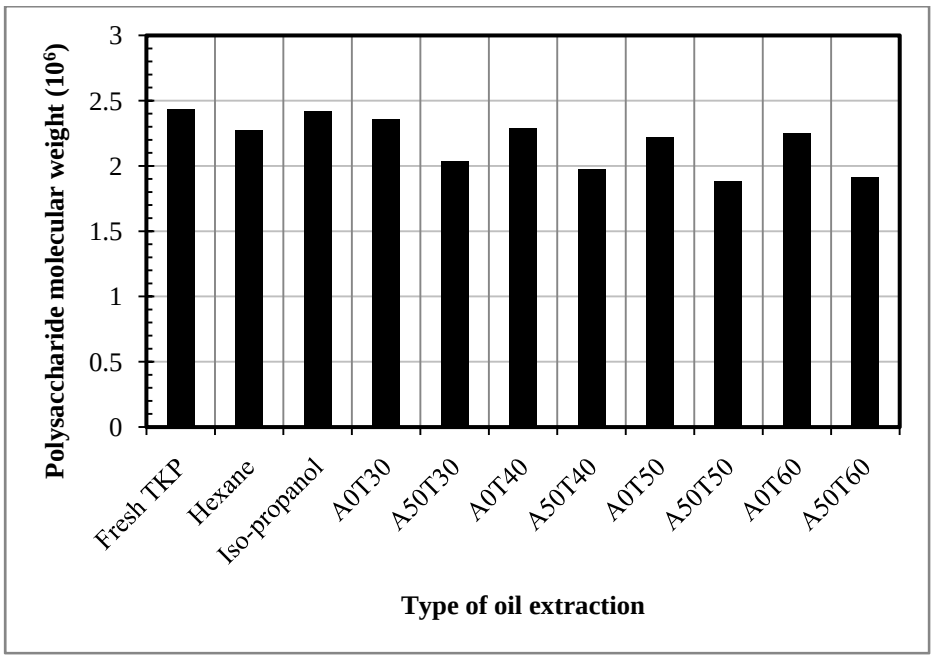


Figure 5.10 Effects of type of extraction on polysaccharide molecular weight of DTKP (isopropanol and hexane = extraction time 10 min; solvent to solid ratio 3:1 ml g^{-1}; A0T30 = 0% amplitude level, temperature 30ºC, extraction time 10 min; solvent to solid ratio 3:1 ml g^{-1})

Table 5.2 The pasting properties of DTKP

Treatment	Oil removal (%)	Viscosity (cp)			Dispersion in water
		Peak	Breakdown	Final peak	
Fresh TKP	0	416	43	892	-
Hexane[a]	93.34	858	47	1797	++
Isopropanol[a]	93.13	847	46	1831	++
A0T30[b]	42.34	501	28	1142	-
A50T30[b]	77.5	597	48	1303	+
A0T40[b]	68.38	591	33	1323	+
A50T40[b]	89.13	747	56	1640	++
A0T50[b]	84.88	641	41	1473	+
A50T50[b]	93.13	883	83	1820	++
A0T60[b]	88.5	731	57	1559	+
A50T60[b]	94.75	902	80	1932	++

[a] extraction time 10 min; solvent to solid ratio 3:1 ml g^{-1}

[b] 0% amplitude level, temperature 30ºC, extraction time 10 min; solvent to solid ratio 3:1 m g^{-1}

5.3 Isolation of tamarind seed polysaccharide (ISTP)

The aim of this section was to study the use of protease enzyme and high-intensity ultrasound in order to digest protein and break the association polysaccharide granules and protein matrix in DTKP. DTKP as raw material consisted of polysaccharide, protein, oil and others, 75.40, 20.32, 0.65 and 3.63, respectively (Table 5.3). Protease enzyme used in this experiment is Esperase 8.0 L (8.0 KNPU, 1 KNPU = 1000 NPU, 1 Novo Protease Unit (NPU) = the amount of enzyme which hydrolyzes casein at such a rate that the initial rate of formation of peptides/minute corresponds to 1 micromole of glycine min^{-1}).

Table 5.3 Compositions of de-oiled TKP

Composition	%
Polysaccharide	75.40
Protein	20.32
Oil	0.65
Others	3.63

5.3.1 Isolation of tamarind seed polysaccharide using protease

In this experiment, the levels of protease were chosen in the range of 0.1 to 0.5% protease solution based on the powder weight. The low level of enzyme was insufficient to digest the tamarind kernel protein for long time operation whereas the high level resulted in increased protein contamination in the ITSP (Lumdubwong et al., 1998, Wang et al., 2004). The amounts of protease added were 0.1, 0.3 or 0.5% and the reaction times were 1, 3 or 5 h. The percentages of protein eliminated of ITSP by protease were shown in Figure. 5.11. In the case of non-enzyme digestion, the elimination of protein increased with long digestion time because some of the protein matrix can be swollen and dissolved in aqueous system. At all three levels of protease used, the protein eliminated in ITSP increased as digestion time from 1 to 5 h and the increase of protease level gave a high amount of protein eliminated. The protease could attach and digest protein matrix on the surface of polysaccharide granule. However, at long time digestion, 5 h, the protein eliminated of 0.3 and 0.5% protease

were slightly different. It may be that protease could not attack and digest the protein inside the group of polysaccharide granules.

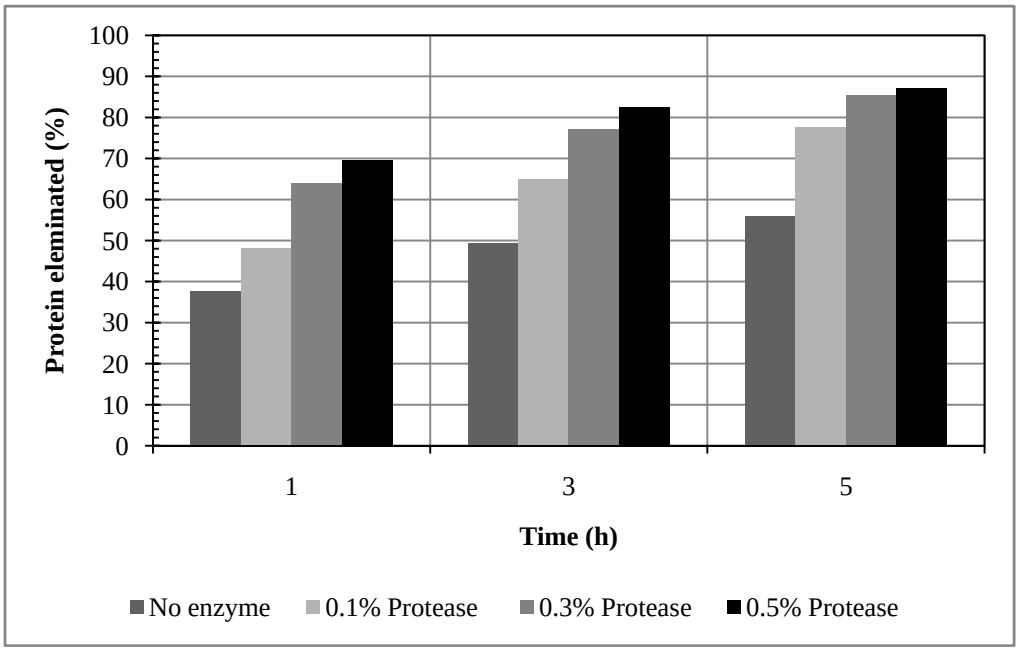


Figure 5.11 Effect of protease levels and digestion time on percentage of protein eliminated from de-oiled TKP.

The percentages of yield of ITSP by protease were shown in Figure 5.12. The yield of ITSP reduced as digestion time and protease level increased. The main composition in ITSP was polysaccharide, thus the loss of powder yield in ITSP increased with the loss of polysaccharide in ITSP (Nitayanont, 1978, Sangyon, 2002, Poommarinvarakul, 2004). It caused the small polysaccharide granules and some of polysaccharide granules swollen and dissolved in the aqueous system at room temperature. After the protease digestion, the protein matrix at the surface of polysaccharide granule became smaller. As a result, the water molecule could invade into polysaccharide granule, so the polysaccharide granule swell and dissolve to the aqueous system. It should be note that the loss of polysaccharide in protease digestion process was higher than non-enzymatic process 3 to 15%. The values of average molecular weight of polysaccharide were shown in Figure 5.13. The average molecular weight of ITSP by protease digestion was larger than that of the raw material because of the loss of small polysaccharide granules in powder.

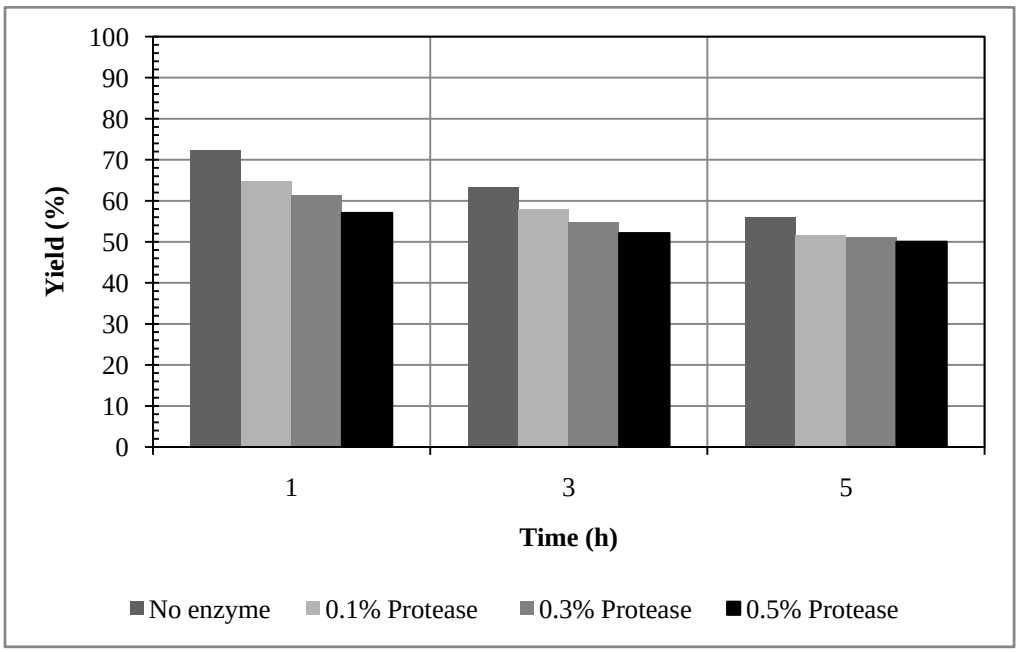


Figure 5.12 Percentage of yield of ITSP by protease digestion.

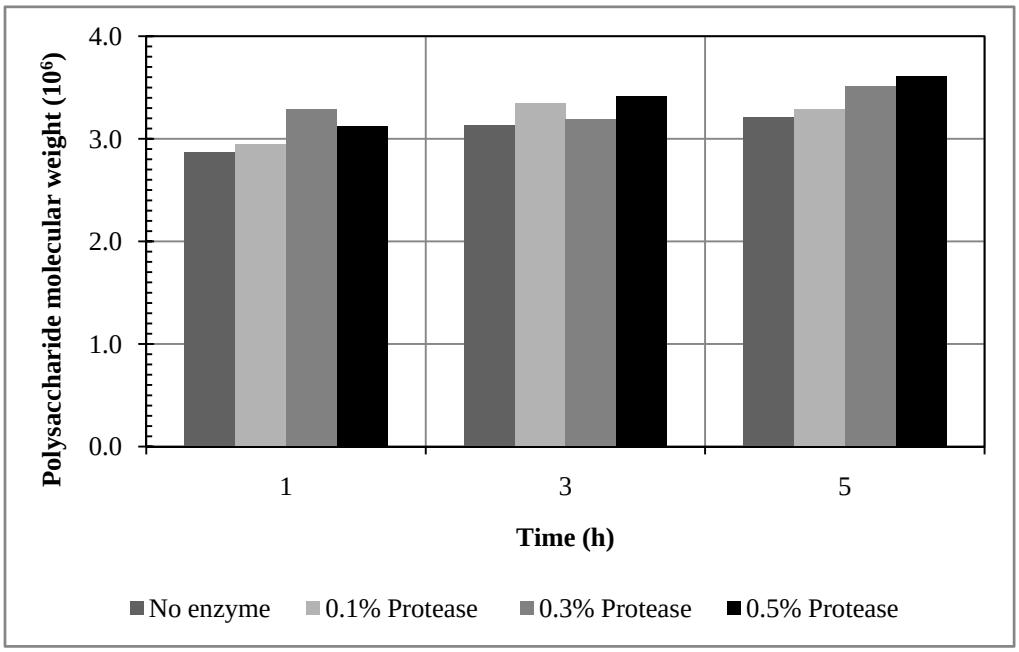


Figure 5.13 Average molecular weight of polysaccharide after protease digestion.

The rate of protein elimination can be applied with Michaelis-Menten equation (M-M equation) (5.1) (Levenspiel. O., 1999)

$$-r_A = k_E \frac{C_{E0} C_A}{C_M - C_A} \tag{5.1}$$

where $-r_A$ is the rate of protein hydrolysis. k_E is the rate constant (h^{-1}). C_A is the protein content after digestion for the time period. C_{E0} is the total of enzyme. C_M is a Michaelis constant. For batch process system, integration of the M-M equation gives

$$\frac{C_{A0} - C_A}{\ln (C_{A0}/C_A)} = -C_M + \frac{k_E C_{E0} t}{\ln (C_{AO}/C_A)} \tag{5.2}$$

$$A = -C_M + B k_E C_{E0} \tag{5.3}$$

where $A = \frac{C_{A0} - C_A}{ln(C_{A0}/C_A)}$, $B = \frac{t}{ln(C_{A0}/C_A)}$

The plots of *A* versus *B* in this experiment were shown in Figure 5.14. The plots were received by calculation from the protein content of ITSP at the three concentration levels. The rate constant k_E was calculated from the slope (*m*) of the graph. Table 5.4 shows the rate constant k_E as a function of protease concentration.

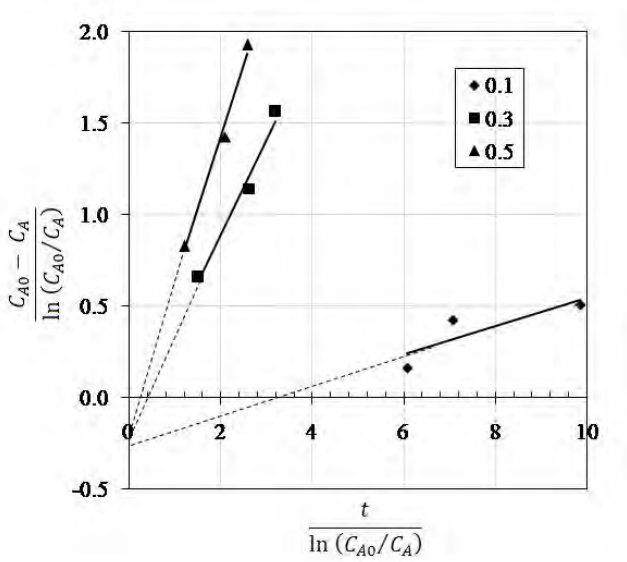


Figure 5.14 Protein digestion fitted with the Michaelis-Menten equation (◆ 0.1% protease, ■ 0.3% protease and ▲ 0.5% protease)

Table 5.4 Protein digestion rate constant k_E calculated from the slopes (m) of Michaelis-Menten plots for 2% DTKP dispersions hydrolyzed by Esperase 8.0L at three different enzyme concentrations.

Protease %	Protease (g)	m (g h^{-1})	C_M (g)	k_E (h^{-1})
0.1%	0.0002	0.077	0.230	0.000385
0.3%	0.0006	0.523	0.157	0.000872
0.5%	0.0010	0.779	0.140	0.000779

The treatment with 0.3 or 0.5% protease concentrations for 1 or 3 h yielded the comparable results. Thus, the treatment with 0.3% protease concentration for 1 h digestion time was chosen for the further study where the combination of protease and high-intensity ultrasound was exploited.

5.3.2 Isolation of tamarind seed polysaccharide using high-intensity ultrasound

In this experiment, the effects of the amplitude levels of high-intensity ultrasound and sonication time on isolated tamarind seed polysaccharide were studied. The rising of amplitude levels and sonication time increased the protein eliminated in aqueous system (Figure 5.15). The increasing of protein eliminated was because the generated energy of high-intensity ultrasound destroyed the interactive bond between polysaccharide granule and protein matrix, so the protein matrixes were freely dispersed in aqueous and separated from ITSP by centrifugal method. On the other hand, the yields of ITSP were reduced by the rising of amplitude levels and sonication time because the generated energy of high-intensity ultrasound could damage the polysaccharide granules to become smaller (Wang et al, 2004). So, the small polysaccharide granules could easily swell and dissolve in the aqueous system (Figure 5.16).

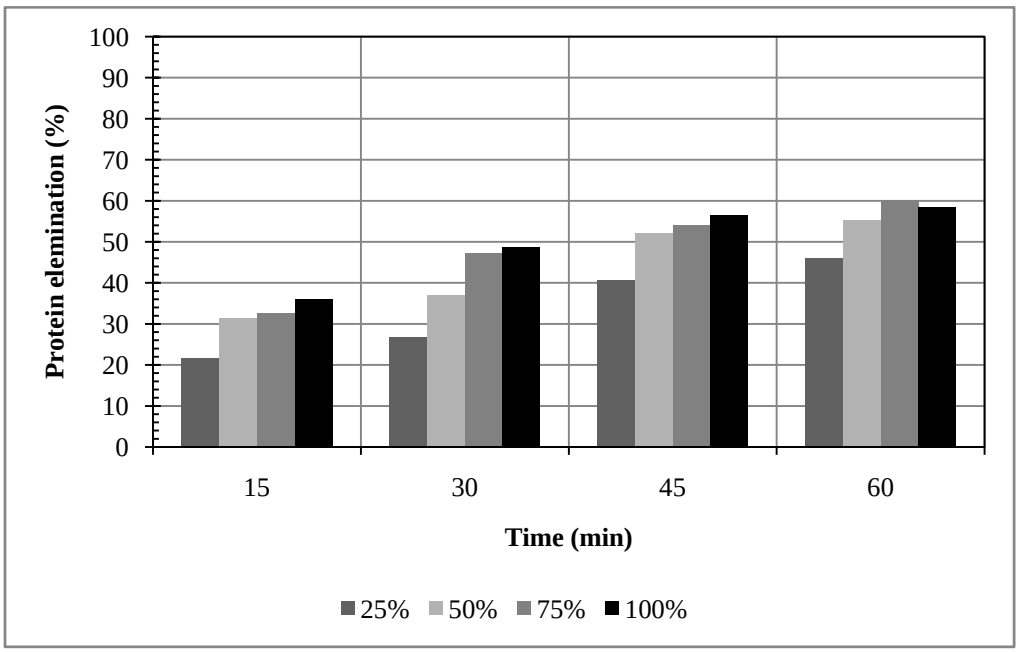


Figure 5.15 Effect of the amplitude levels of high-intensity ultrasound and the sonication time on percentage of protein eliminated from DTKP.

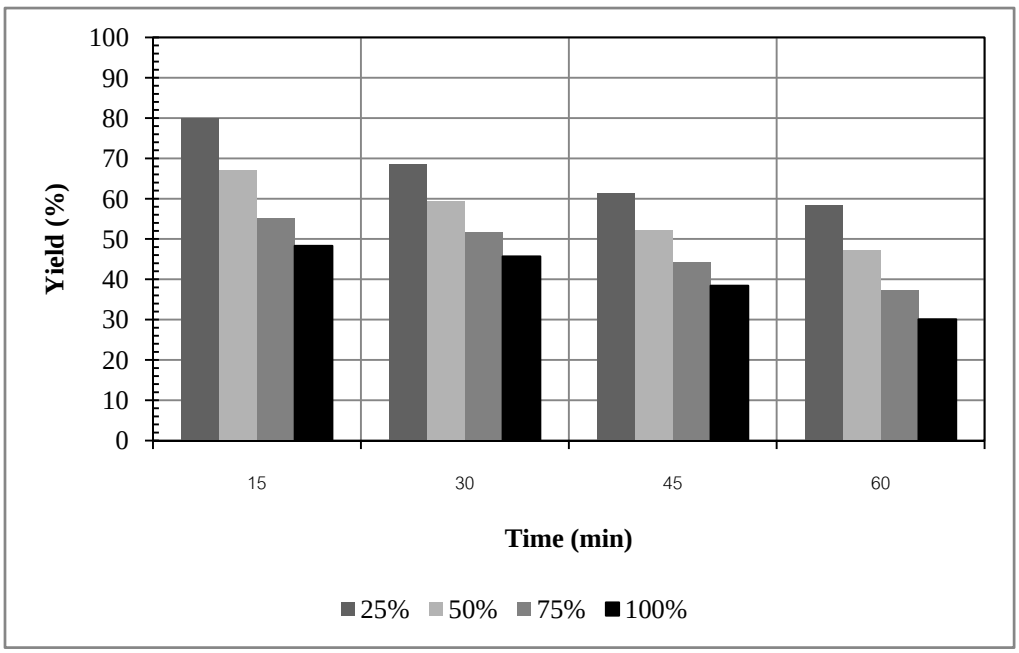


Figure 5.16 Percentage of yield of ITSP by high-intensity ultrasound.

Acoustic wave of ultrasound cannot be absorbed by protein and polysaccharide molecules, it must be transformed to a chemically usable form via the indirect, complex phenomenon known as cavitaion. Beyond an intensity threshold, the acoustic waves can break the cohesion of a liquid and create microcavities. From an initial submicron size, gas bubbles trapped in the liquid or on solid surface grow to a few tens of microns and become unstable. A collapse occurs rapidly for a short time. The collapse also generates shock waves which induces mechanical effects, even in homogenous media. If the phenomenon is too fast to alter small molecules, it is likely to increase the splitting of less mobile polymers to smaller fragments. Beside to the interface of solid-liquid system, the collapse occur dissymetrically, with a liquid jet crossing the cavity at a velocity reaching several hundreds of ms^{-1}. Small particles, solid or liquid, are ejected and cracked by the impact. Some of the particles between polysaccharide granules and protein complex were disassociated by acoustic wave. The energy from high-intensity ultrasound can also break polysaccharide granule to smaller size. When the size of polysaccharide granules decreased, they could be easily dissolved in aqueous system.

The risings of amplitude level and sonication time affected to decrease the average molecular weight of polysaccharide from 2.414 x 10^6 to 0.830 x10^6 g $mole^{-1}$ (Figure 5.17). Madras et al., (2000, 2001) presented a model to investigate depolymerization in the factor of sonication time. The introduction of the rate on a limiting molecular weight is shown in Eq. 5.4.

$$k(M) = k_U(M - M_e) \tag{5.4}$$

They developed the model with a quasi first order reaction kinetics in the form of

$$\ln H = \ln\left(\frac{M_e - M_0}{M_e - M_t}\right) = k_U M_e t \tag{5.5}$$

Where M_0 is the molecular weight of the polymer prior to sonication and M_t is the molecular weight after sonication for time t. M_e is the final molecular weight of polymer after sonication for long time, $M_e = \lim_{t\to\infty} M_t$. Extrapolation of molecular weight versus time data using a simple exponential decay function predicts that the

molecular weight changes less than 5% after sonication for time longer than 60 min. k_U is a rate constant of depolymerization.

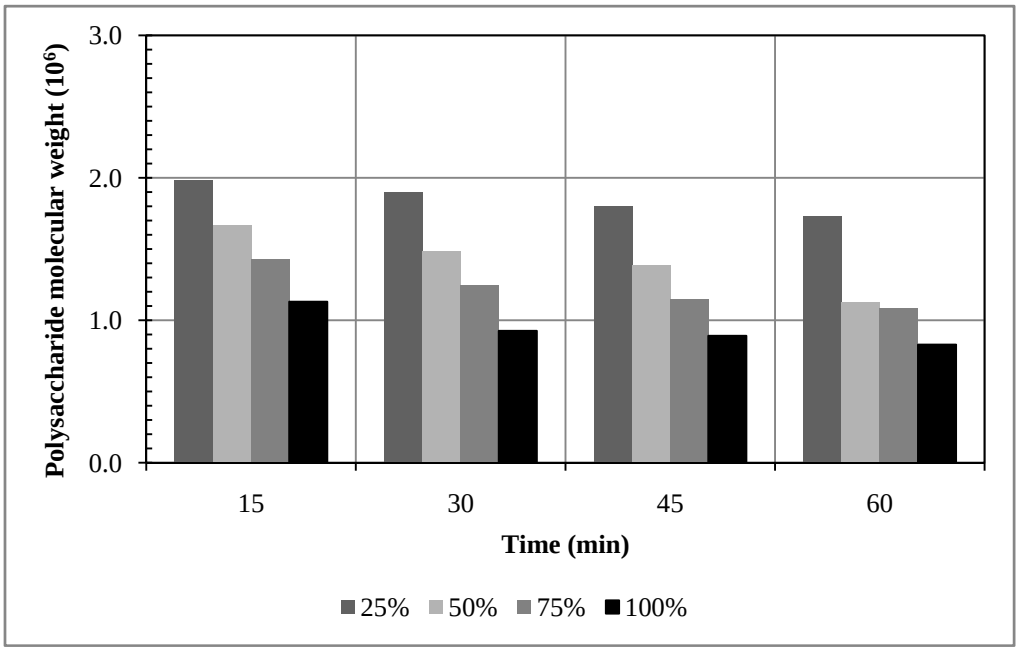


Figure 5.17 Average molecular weight of polysaccharide after sonication.

The plot of ln *H* versus time in this experiment is non-linear (Figure 5.18). It agrees with Baxter et al., (2005) that the polysaccharide chains do not most likely spit into exactly two equimolar chains. Instead, the spitting may be much more random and produce more than two chains from one initial molecule. Schmid declination factor was applied to develop degradation model Eq. 5.6 by Baxter et al., (2005). They assumed that the number of moles generated per unit volume decreased with the degree of polymerization. The susceptibility of a polymer to high-intensity ultrasound increased with the number of monomers in the polymer.

$$-\frac{M_e}{M_t} - ln\left(1 - \frac{M_e}{M_t}\right) = \frac{k}{c} P_e^2 t + C \qquad (5.6)$$

where P_e is the final degree of polymerization given by $P_e = M_e / M_{monomer}$. (this experiment P_e = 4610 was calculated from M_e = 0.83 x 10^6 g mole^{-1} and $M_{monomer}$ assumed to be 180 g mole^{-1}). Figure 5.19 shows a plot of the Schmid declination factor calculated from the molecular weight data of ITSP dispersions with the

sonication at four amplitude levels. The rate constant k_U was calculated from the slope of Schmid declination factor versus time:

$$k_U = \frac{c}{P_e^2} m \qquad (5.7)$$

Table 5.5 shows the rate constant as a function of high-intensity ultrasonic amplitude. The rate constant of depolymerization increased with the ultrasonic amplitude.

From these results, the suitable condition of ITSP by high-intensity ultrasound was selected by focusing on the yield and the average molecular weight of polysaccharide. Thus, 25 and 50% amplitude levels and 15 and 30 min sonication times were chosen for the study of combination of protease and high-intensity ultrasound.

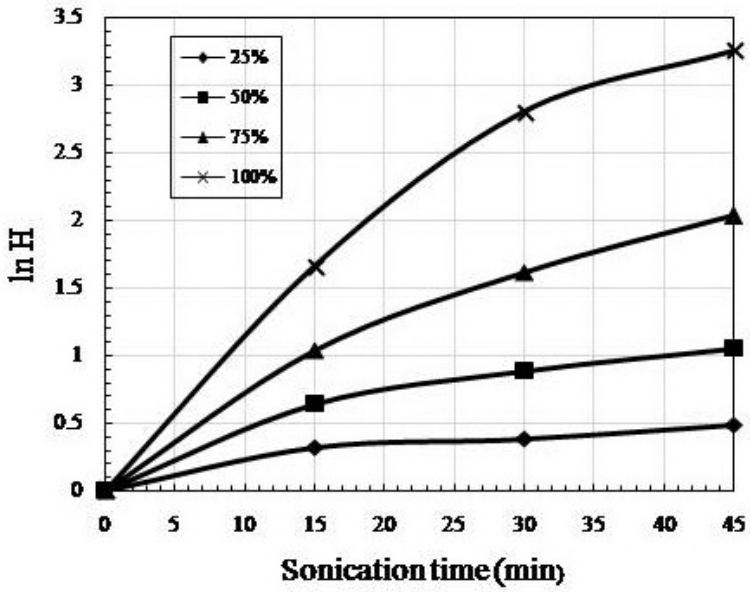


Figure 5.18 Quasi first order reaction kinetics as a function of treatment time and polysaccharide dispersion from ultrasonication at amplitude 25, 50, 75 and 100%

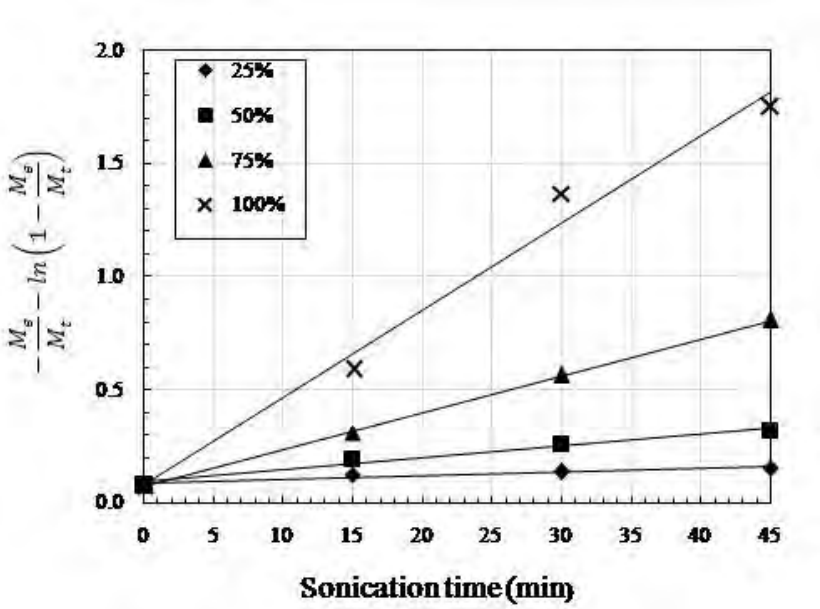


Figure 5.19 Schmid declination factor as a function of treatment time and polysaccharide dispersion from ultrasonication at amplitude 25, 50, 75 and 100%

Table 5.5 Depolymerization rate constant k_U calculated from the slopes m of Schmid plot for 2% de-oiled TKP dispersions from ultrasonication at four different intensities: 25, 50, 75 and 100%

Amplitude (%)	Intensity W cm^{-2}	m (min^{-1}) x 10^{-2}	k_U (mol min^{-1}) x10^{-14}
25	49.32	0.170	0.050
50	98.64	0.527	0.155
75	147.95	1.631	0.479
100	197.27	3.868	1.137

5.3.3 Isolation of tamarind seed polysaccharide by a combination of protease and high-intensity ultrasound

From the previous experiment, it was found that the protease hydrolyzed protein on polysaccharide granule surface and the ultrasonic disassociated the complex of polysaccharide granule and protein matrixes. Thus, we hypothesized that the combination of enzyme and high-intensity ultrasound reduced protein contamination in a shorter time than using solely protease. It is assumed that the

cavitation effect from high-intensity ultrasound in aqueous medium would destroy the associations of the complex between polysaccharide granules and protein matrixes. When the protein matrix was loosened, the protease would be more effective in digesting the protein from polysaccharide granule. In this procedure, the protease concentration was chosen at 0.3% and the amplitude levels of high-intensity ultrasound were chosen at 25 and 50%. The percentages of yield and protein eliminated of ITSP were shown in Figure 5.20.

The use of sonication before protease treatment is shown in bar graph (1)-(4). The ranges of protein elimination and yield are 57.84 to 72.64% and 60.33 to 50.88%, respectively. The simultaneous sonication and protease treatment is shown in bar graph (5)-(8). The ranges of protein elimination and yield are 75.48 to 95.45% and 65.23 to 53.12%, respectively. The use of sonication after protease treatment is shown in bar graph (9)-(12). The ranges of protein elimination and yield are 83.77 to 95.62% and 57.88 to 45.78%, respectively. The results found that the increase of sonication time and amplitude level increased the protein eliminated but reduced the yield. The simultaneous sonication and protease treatment gave high protein eliminated but a slightly loss of yield. Compared with other treatments, it is possibly due to a short reaction time and better protease activation by sonication (Wang et al., 2004, Imai et al., 2004). The use of sonication after protease treatment generally made high protein eliminated. These results demonstrated that protease was more effective in loosening the protein matrixes around the polysaccharide granule and was more activated by high-intensity ultrasound. However, the yield was reduced by long time digestion. The average molecular weights of polysaccharide in ITSP with the combination of high-intensity ultrasound and protease treatment are shown in Figure 5.21. They were similar to the high-intensity ultrasound treatment. The increase of amplitude level and sonication time gave the short polysaccharide molecule. Thus, the preferred combination of protease and high-intensity ultrasound for high yield and high protein eliminated was protease digestion and sonication at 50% amplitude level used together for 30 min.

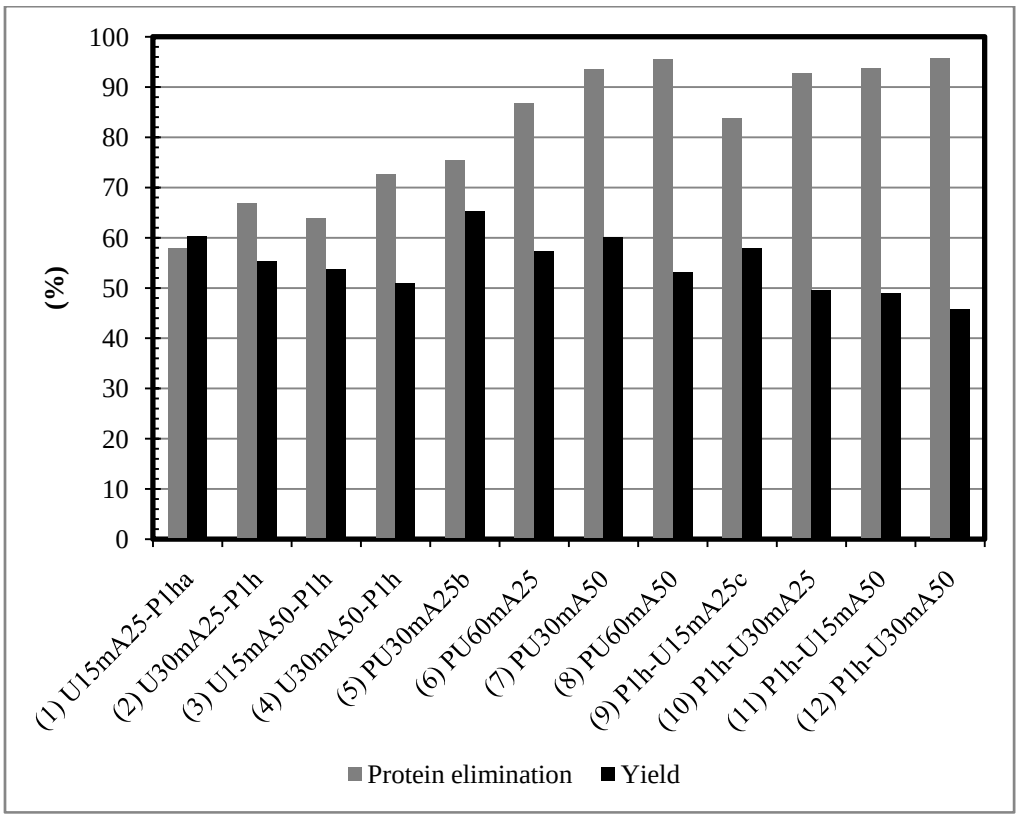


Figure 5.20 Percentages of protein eliminated and yield of ITSP by combination of 0.3% protease and 25 or 50% amplitude levels of high-intensity ultrasound. (U15mA25-P1h = Sonication 15 min at 25% amplitude and then 1 h protease digestion; PU30mA25 = Protease digestion and sonication 30 min at 25% amplitude concurrently; P1h-U15mA25 = Protease digestion 1 h and then sonication 15 min at 25% amplitude)

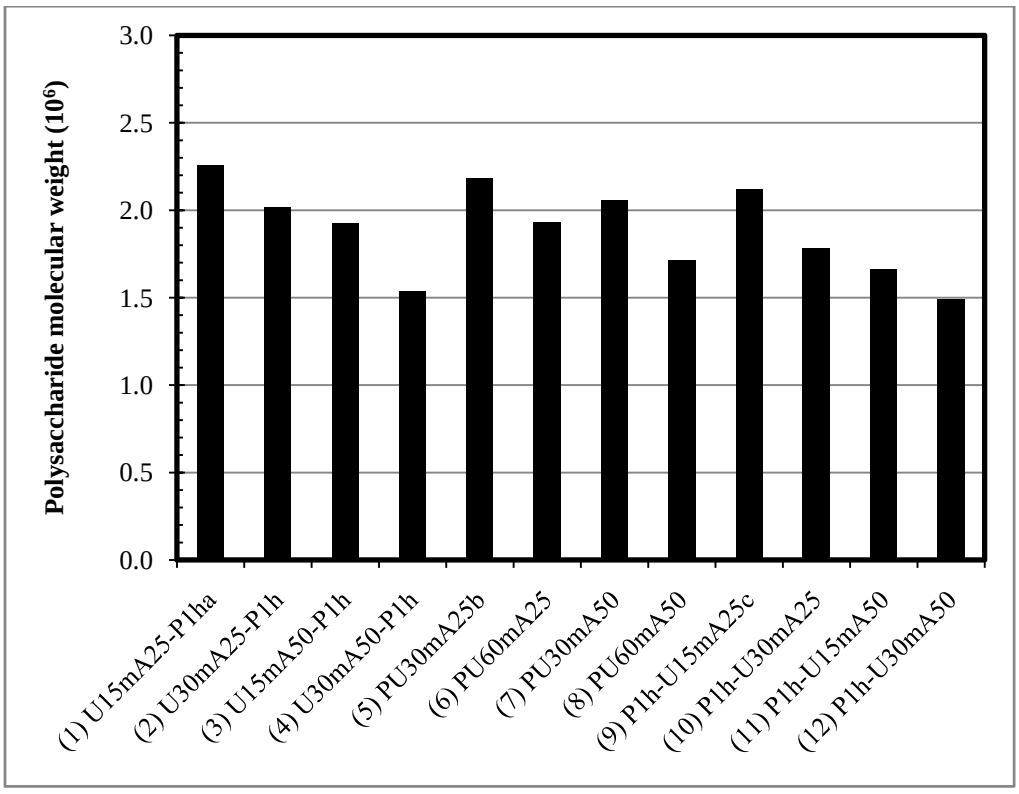


Figure 5.21 Average molecular weights of polysaccharide of ITSP by combination of 0.3% protease and 25 0r 50% amplitude levels of high-intensity ultrasound (U15mA25-P1h = Sonication 15 min at 25% amplitude and then 1 h protease digestion; PU30mA25 = Protease digestion and sonication 30 min at 25% amplitude concurrently; P1h-U15mA25 = Protease digestion 1 h and then sonication 15 min at 25% amplitude).

5.3.4 Pasting properties of isolated tamarind seed polysaccharide

The pasting properties of all treatments on ITSP are summarized in Table 5.6, 5.7 and 5.8. The peak, final viscosities of ITSP by using protease alone were higher than that by using other treatments. However, the different viscosity values between peak and breakdown were lower than those of the combination treatment and high-intensity ultrasonic treatment, respectively. The pasting properties could also be related to the residual protein content and the molecular weight of polysaccharide. The high purity and long chain of polysaccharide made high viscosity. The discharging of protein matrix from polysaccharide granule was assumed to be caused

by disulfide bond cleavage by dilute alkali. In another way, a reduced amount of protein matrix attached on polysaccharide granule produced high viscosity polysaccharide.

Table 5.6 RVA pasting properties of ITSP by the protease digestion

Treatment	Viscosity (cp)		
	Peak	Breakdown	Final
No enzyme 1 h	2417	275	4538
0.1% Protease 1 h[a]	2619	286	4913
0.3% Protease 1 h	2901	274	5264
0.5% Protease 1 h	2931	301	6253
No enzyme 3 h	2382	342	4742
0.1% Protease 3 h	2614	370	5038
0.3% Protease 3 h	3031	355	5719
0.5% Protease 3 h	3385	380	6714
No enzyme 5 h	2493	399	4900
0.1% Protease 5 h	2718	419	5534
0.3% Protease 5 h	3091	421	6109
0.5% Protease 5 h	3670	443	7193

[a] Protease at 0.1% powder level and digestion for 1 hr

Table 5.7 RVA pasting properties of ITSP by high-intensity ultrasound at different levels and sonication time.

Treatment	Viscosity (cp)		
	Peak	Breakdown	Final
A25T15[b]	1920	1739	2171
A50T15	1735	1514	2320
A75T15	1438	1093	2193
A100T15	1117	891	2247
A25T30	1831	1635	2213
A50T30	1729	1370	2314
A75T30	1750	1320	2029
A100T30	1412	1131	1903
A25T45	1825	1613	2730
A50T45	1631	1298	2271
A75T45	1414	935	2019
A100T45	1093	597	1871
A25T60	1735	1439	2224
A50T60	1525	1144	2015
A75T60	1210	743	1933
A100T60	1031	531	1648

[b] amplitude 25% sonicate 15 min

Table 5.8 RVA pasting properties of ITSP by the combination of 0.3% protease and high-intensity ultrasound

Treatment	Viscosity (cp)		
	Peak	Breakdown	Final
U15m25%a-P1h[c]	2432	354	3984
U30m25%a-P1h	2489	479	3790
U15m50%a-P1h	2127	558	3871
U30m50%a-P1h	2216	713	3550
PU30m25%a[d]	2443	303	4379
PU60m25%a	2639	481	4424
PU30m50%a	2695	733	4795
PU60m50%a	2725	920	4109
P1h-U15m25%[e]	2981	632	4201
P1h-U30m25%	2823	840	4337
P1h-U15m50%	2990	1235	4541
P1h-U30m50%	2975	1470	4109

[c] Sonication 15 min at 25% amplitude and then 1 h protease digestion

[d] Protease digestion and sonication 30 min at 25% amplitude concurrently

[e] Protease digestion 1 h and then sonication 15 min at 25% amplitude

5.4 **Purification of isolated tamarind seed polysaccharide using dialyzer**

After protein removal with protease and high-intensity ultrasound, the protein contamination was above 2% in powder product. Thus, to improve the purity and the properties of isolated tamarind seed polysaccharide, dialysis technique was chosen. The raw material was ITSP from a combination of 0.3% protease and 50% amplitude level of high-intensity ultrasound for 30 min sonication time. The compositions were shown in Table 5.9. The feed solution was prepared by the method in experiment 4.3.4.

Table 5.9 Compositions and average molecular weight of polysaccharide of isolated tamarind seed polysaccharide using protease and high-intensity ultrasound.

Compositions	%
Polysaccharide	95.86
Protein	2.06
Others	2.08
Avg. MW. polysaccharide	1.573×10^6

The dialyzer module was selected by the molecular weight of protein. The protein molecular weight in feed solution was determined by MALDI-TOF MASS spectrometry and the data was shown in Table 5.10. The molecular weight of protein was below 8000 Da, so the dialyzer F70S, molecular weight cut-off (MWCO) 18,000 Da, was selected to use in this experiment. The reasons to use this dialyzer were its hydrophilicity, suitable MWCO and ease to buy from the market.

Table 5.10 Molecular weight of protein (Da) determined by MALDI-TOF MASS spectrometry

Feed solution		Dialysate
Before process	After process	
1,917	3,236	1,932
3,234	7,607	2,739
7,606		3,262

Nine experiments (F150D900, F150D1200, F150D1500, F200D900, F200D1200, F200D1500, F250D900, F250D1200 and F250D1500) were carried out by varying counter-currently between feed flow rate from 150, 200 to 250 ml min^{-1} and dialysate flow rate from 900, 1200 to 1500 ml min^{-1}. The initial volume of both feed side and dialysate side were fixed at 1000 ml. Both fluids arrangement were recycled to feed and dialysate tanks in order to eliminate protein from feed side to dialysate side. The effect of feed and dialysate flow rates on feed tank volume was shown in Figure 5.22. The figure shows three types of volume changes in feed tank. The first type (F150D900, F200D1200, F250D1500) reveals slightly volume reduction while the second (F200D900, F250D900, F250D1200) and the third (F150D1200, F150D1500, F200D1500) reveal large volume reduction and large volume increased in feed tank, respectively. This is due to ultrafiltration occurred during water from both solutions permeated through the membrane. Table 5.11 shows the related ultrafiltration (UF) and back-ultrafiltration (back-UF) rates for different feed and dialysate flow rates. The higher dialysate flow rates at low feed flow rate promote back-UF (volume in feed tank increased) while the lower dialysate flow rates at higher feed flow rate promote UF (volume in feed tank decreased). Small UF values indicate that pressure different across the membrane has slightly effect to volume changes. The changes of volume in feed tank have impact to protein concentration in feed side and also protein elimination. Figure 5.23, 5.24 and 5.25 show protein concentrations in feed side, dialysate side and percentage of protein eliminated, respectively.

For the analysis of protein in dialysate, the results showed that the molecular weight 7607 Da was not found in dialysate side (Table 5.10). It means that the protein of large molecular weight could not pass through the membrane. The results were found that, an increase of flow rates in feed and dialysate made more feed recycles and the protein in feed and dialysate could be exchanged at a high rate. Therefore, the percentage of protein eliminated increased. Moreover, the different protein concentration in feed and dialysate made the diffusion of protein from feed to dialysate. In addition, the back-UF reduced the protein eliminated because the protein concentration in feed decreased due to the transfer of water from dialysate. Thus, the exchange rate decreased. In the case of F250D900 and F250D1200, the protein was rapidly eliminated than other treatments because the ultrafiltration made the protein concentration in being processed feed higher than the protein concentration in initial feed. So the considerable difference of protein concentrations in feed and dialysate gave the high exchange rate.

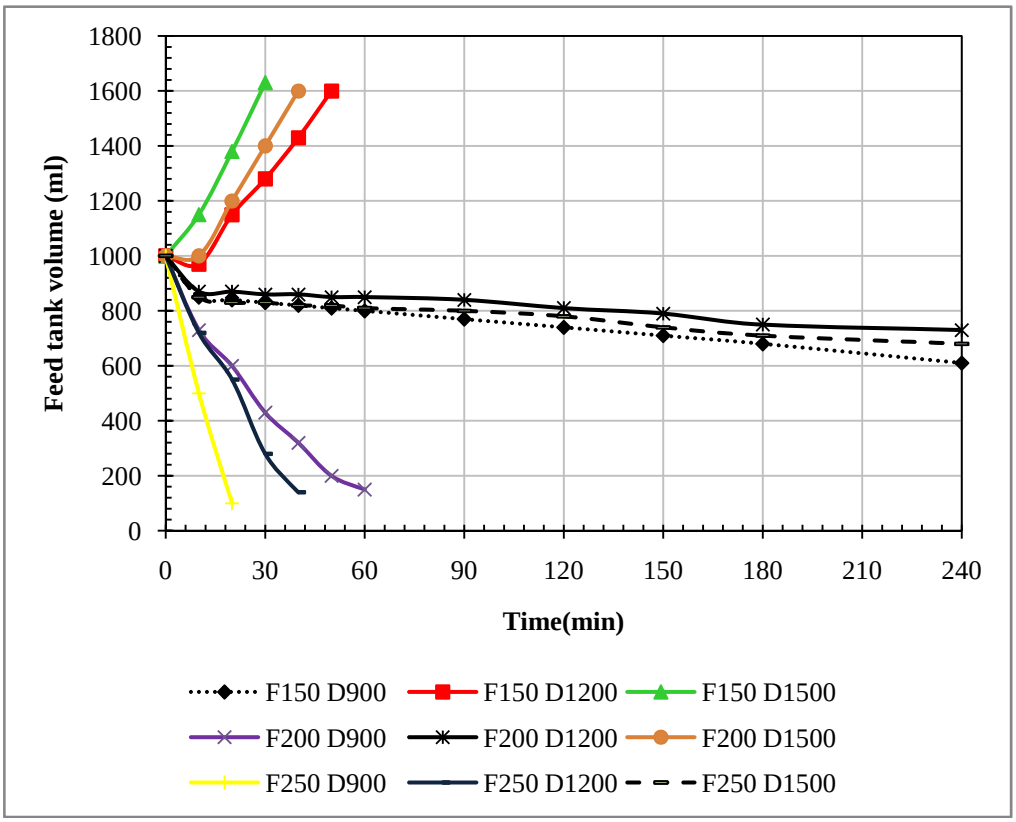


Figure 5.22 Effect of feed and dialysate flow rates on feed tank volume

Table 5.11 Ultrafiltration rate

Feed (ml min^{-1})	Dialysate (ml min^{-1})	Ultrafiltration (ml min^{-1})
150	900	1.04
	1200	-15.75 (back-UF)
	1500	-24.00 (back-UF)
200	900	11.60
	1200	0.74
	1500	-20.00 (back-UF)
250	900	40.00
	1200	19.33
	1500	0.61

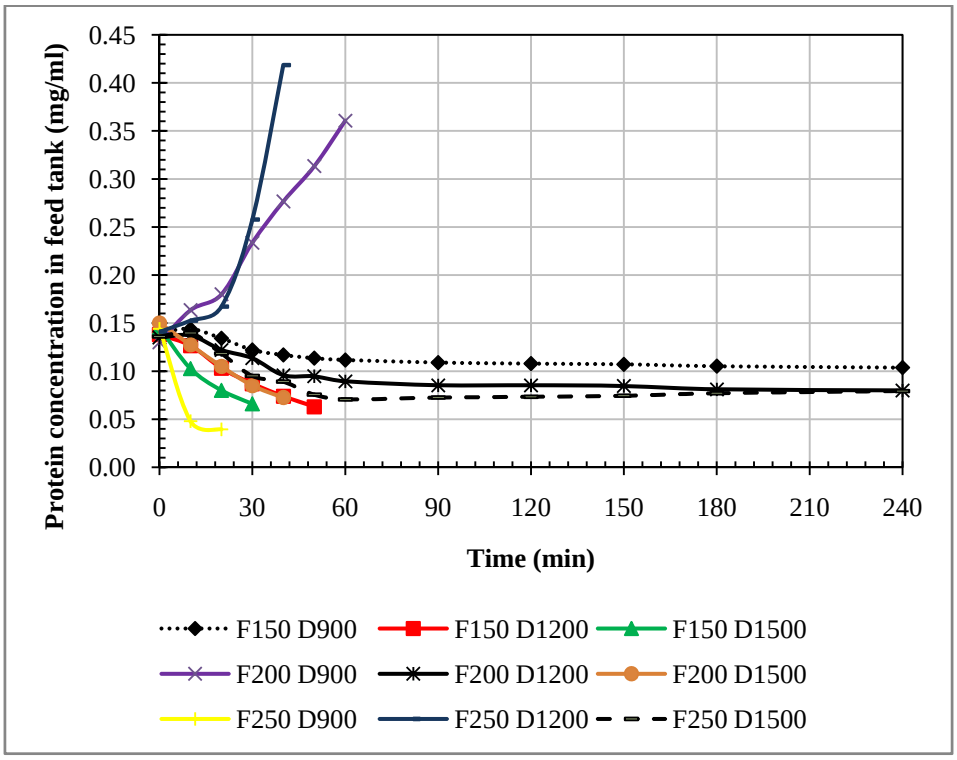


Figure 5.23 Protein concentration in feed tank

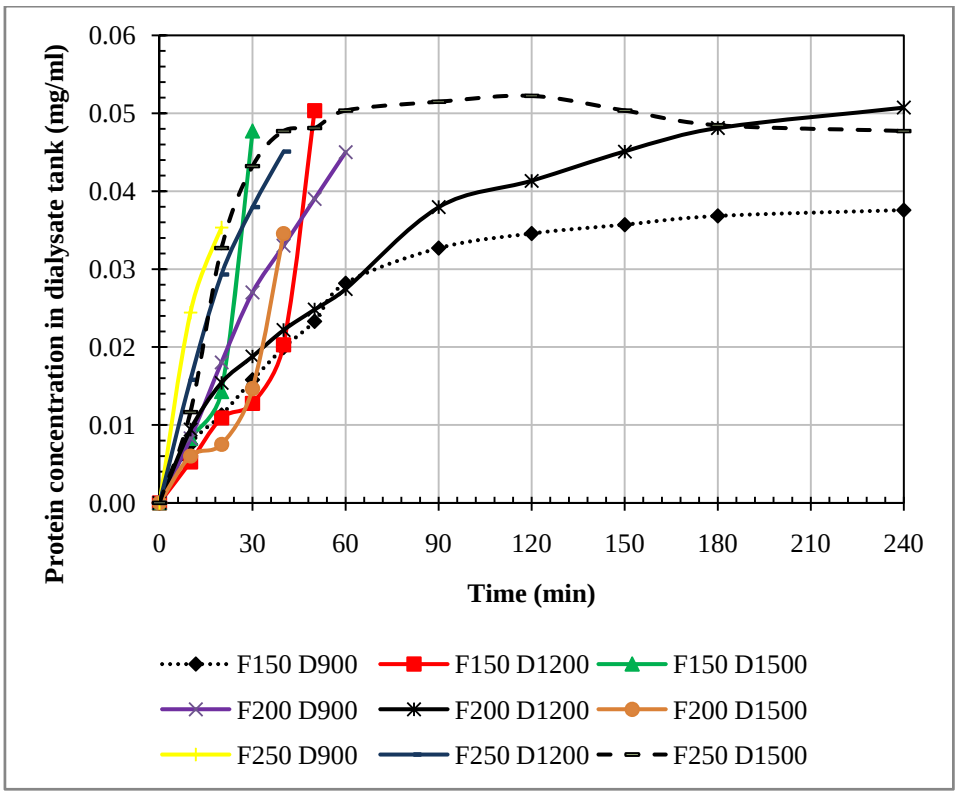


Figure 5.24 Protein concentration in dialysate tank

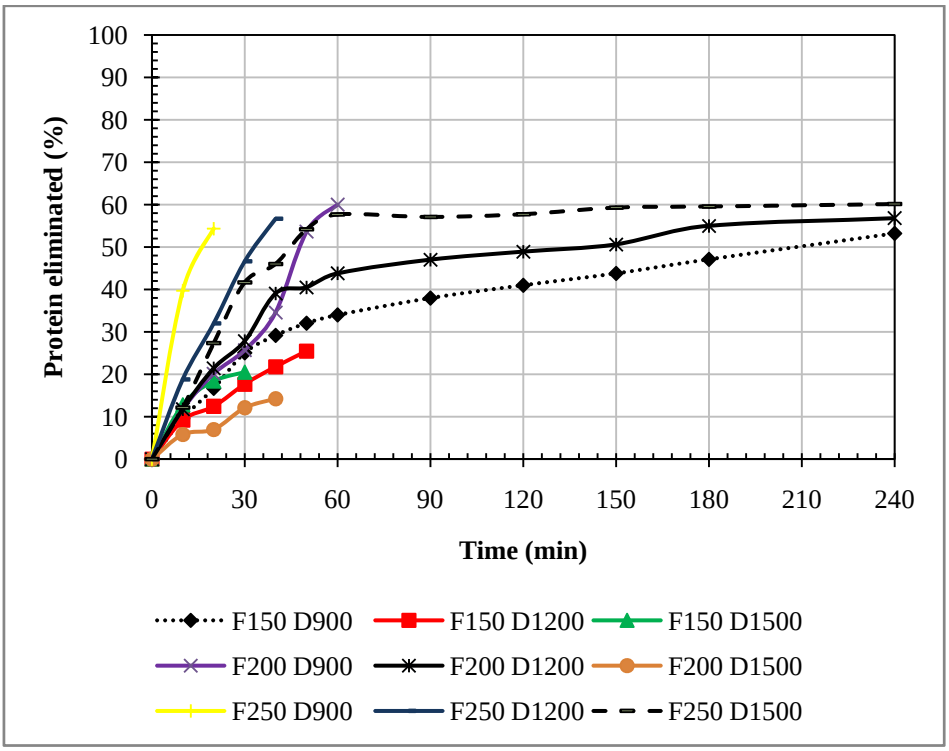


Figure 5.25 The percentage of protein eliminated in feed tank

Figure 5.26 shows the percentage of yield and protein eliminated and also figure 5.27 shows the purity of polysaccharide after dialysis process. The yields of F200D900, F250D900 and F250D1200 were lower than other treatments because when the water in feed solution passed through the dialysate side, the polysaccharide concentration in feed tank increased and made the viscosity higher than the starting feed. Thus, the polysaccharides attached inside the feed tube in dialyzer and were lost in dialyzer. The high back-UF rate (F150D1200 and F200D1500) gave a higher yield than other treatments but the purity of polysaccharide decreased. The back-UF could decrease the viscosity in feed, so the polysaccharide loss in dialyzer decreased. The rising of protein eliminated could increase the product purity. Considering purity, the condition of 250 ml min^{-1} feed flow rate and 1500 ml min^{-1} dialysate flow rate was chosen for production.

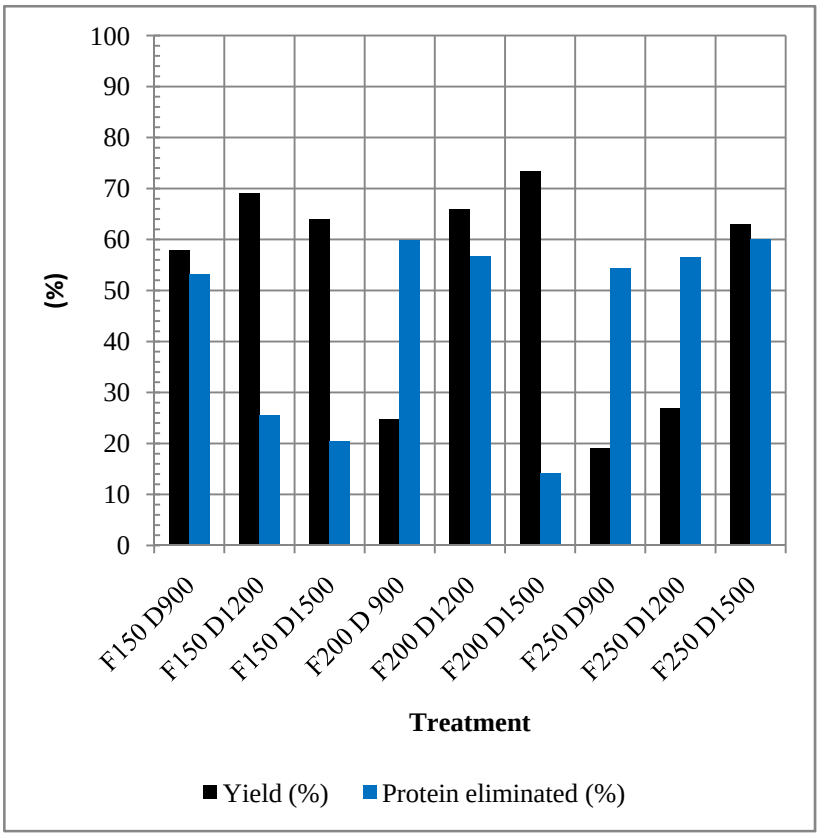


Figure 5.26 Percentage of yield and protein eliminated

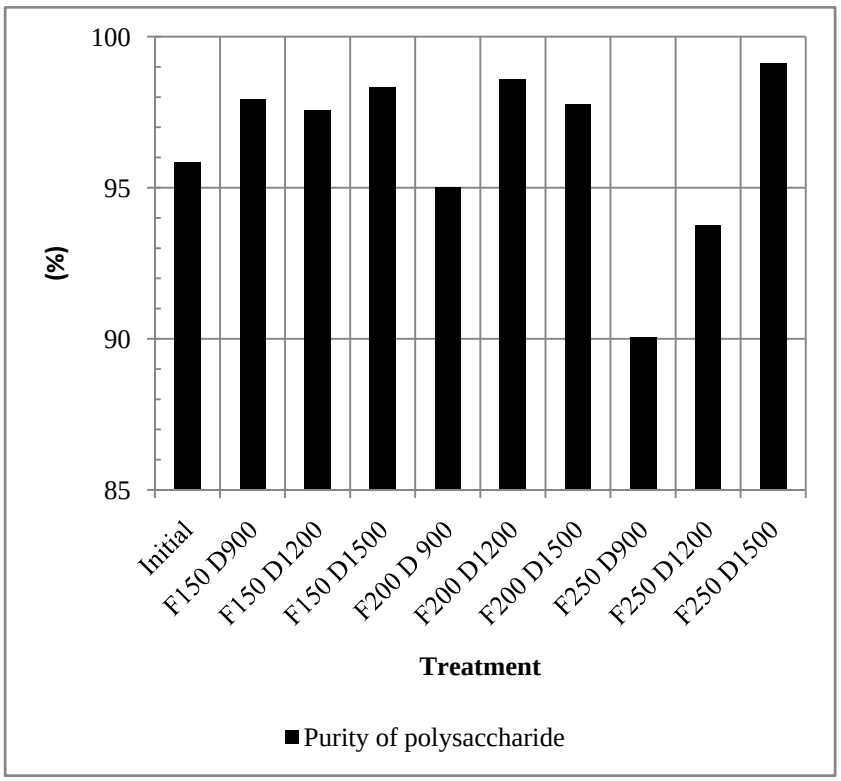


Figure 5.27 Purity of polysaccharide

Table 5.12 shows the dialysis parameter calculated from experiments. The report found that the increase of feed flow rate increased the dialysance (D, the performance of dialyzer) and the overall mass transfer coefficient (k_{OV}). Moreover, it decreased the rejection coefficient (R) of solute (Table 5.13). β represents the feed and dialysate cycling effectiveness. A high value means the ability of dialyzer to work for a long time and to work at high exchanging round numbers. Moreover, the reject coefficient of polysaccharide calculated from polysaccharide concentrations (Figure 5.28 and 5.29) were very high. It means this membrane is possible to use in recovery of polysaccharide.

Table 5.12 Dialysis parameters calculated from experiments

Feed (ml min^{-1})	Dialysate (ml min^{-1})	E	D (cm^3 min^{-1})	k_{ov} (cm s^{-1})	β
150	900	0.235	53.333	7.093E-05	9.363
	1200	0.484	33.333	3.985E-05	1.588
	1500	0.433	53.333	6.999E-05	1.462
200	900	0.141	71.111	9.564E-05	0.087
	1200	0.409	80.000	1.105E-04	14.589
	1500	0.405	53.333	6.585E-05	1.487
250	900	0.014	213.333	5.946E-04	0.037
	1200	0.221	142.222	2.353E-04	0.217
	1500	0.417	106.667	1.508E-04	18.716

Table 5.13 Rejection coefficient of protein and polysaccharide

Feed (ml min^{-1})	Dialysate (ml min^{-1})	Protein rejection	Polysaccharide rejection
150	900	0.563	0.999
	1200	0.963	0.998
	1500	0.965	0.999
200	900	0.484	0.999
	1200	0.581	0.999
	1500	0.969	0.999
250	900	0.569	0.999
	1200	0.547	0.999
	1500	0.467	0.999

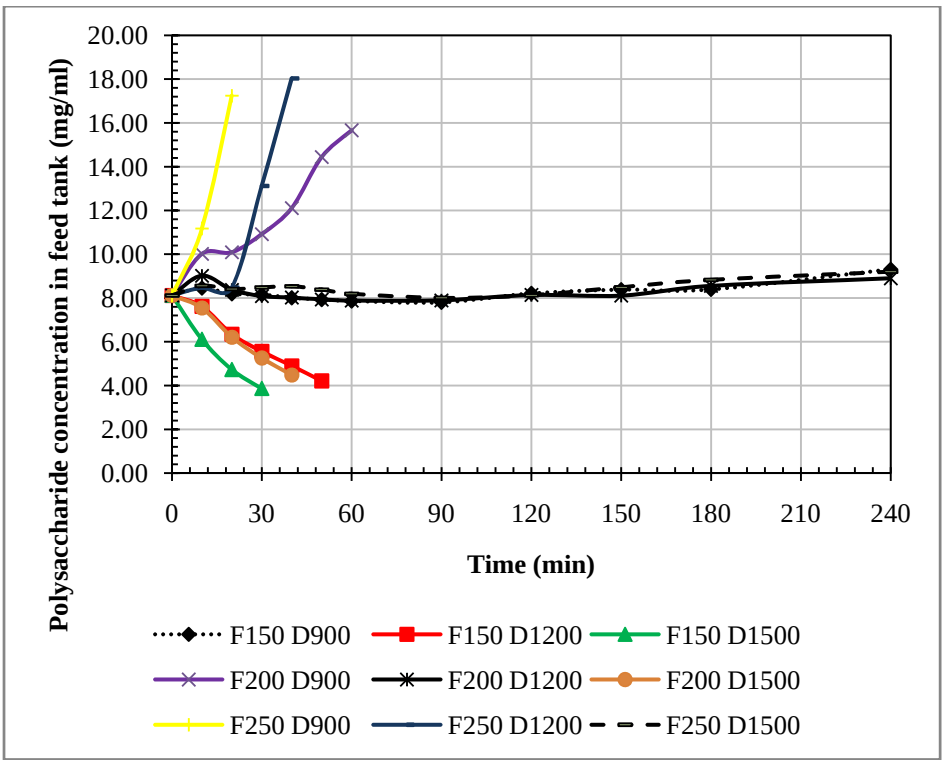


Figure 5.28 Polysaccharide concentration in feed tank

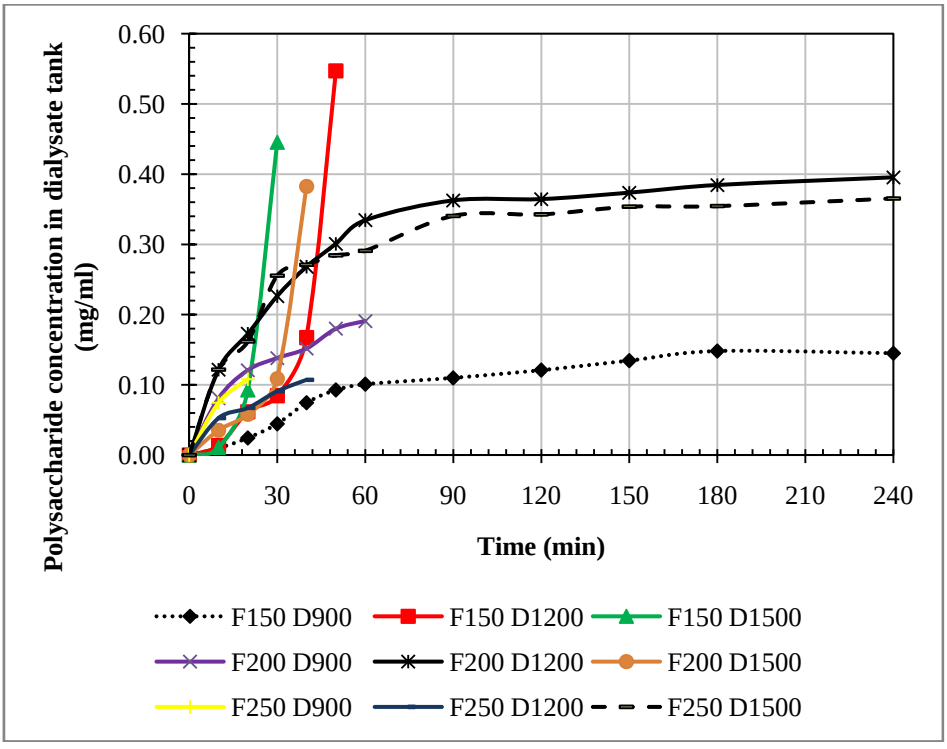


Figure 5.29 Polysaccharide concentration in dialysate tank

Table 5.14 shows the summary of tamarind polysaccharide compositions. MTSP is the modified tamarind seed polysaccharide that obtains from dialysis process. The loss of polysaccharides and the removal of protein occurred in aqueous system. The average molecular weight of MTSP polysaccharide was smaller than other polysaccharides. After the polysaccharides dissolved in water, they changed the granule structure to the chain form. When precipitating the polysaccharide solution using 95% ethanol, the polysaccharide could not reform to granule. Thus, the milling of precipitated polysaccharide can break the chains of polysaccharide to be smaller.

The pasting properties of polysaccharide were shown in Figure 5.30. The increase of polysaccharide purity could increase the viscosity and the thermal properties. For MTSP, the report showed that the initial viscosity was higher than that of other polysaccharides and it slightly decreased when the temperature rose. It presented that the powder could rapidly dissolve in water at low temperature. The viscosity profile of MTSP was similar to that of the modified starch.

Table 5.14 Summary table of polysaccharide processing from tamarind kernel powder

Product	Yield (%)	Avg. MW ($x10^{-6}$)	Polysaccharide (%)			Protein (%)			Oil (%)			Remark
			Content	Loss	Total loss	Content	Removal	Total removal	Content	Removal	Total removal	
TKP	100	2.437	69.84	-	-	18.82	-		8	-	-	
DTKP	92.55	2.414	75.4	-	-	20.32	-		0.55	93.13	93.13	using IPA
ITSP	55.64	1.537	95.86	23.63	23.63	2.06	93.91	93.91	0.05	94.53	99.65	E+A
MTSP	35.12	0.557	99.13	34.72	50.15	0.86	73.65	98.39	0	100	100	F250 D1500

DTKP: de-oiled processing using IPA ratio 1:3, 10 min, 30 °C

ITSP: combination 0.3% protease 50% high-intensity ultrasound amplitude 30 min

MTSP: feed 250 ml min^{-1} dialysate 1500 ml min^{-1}

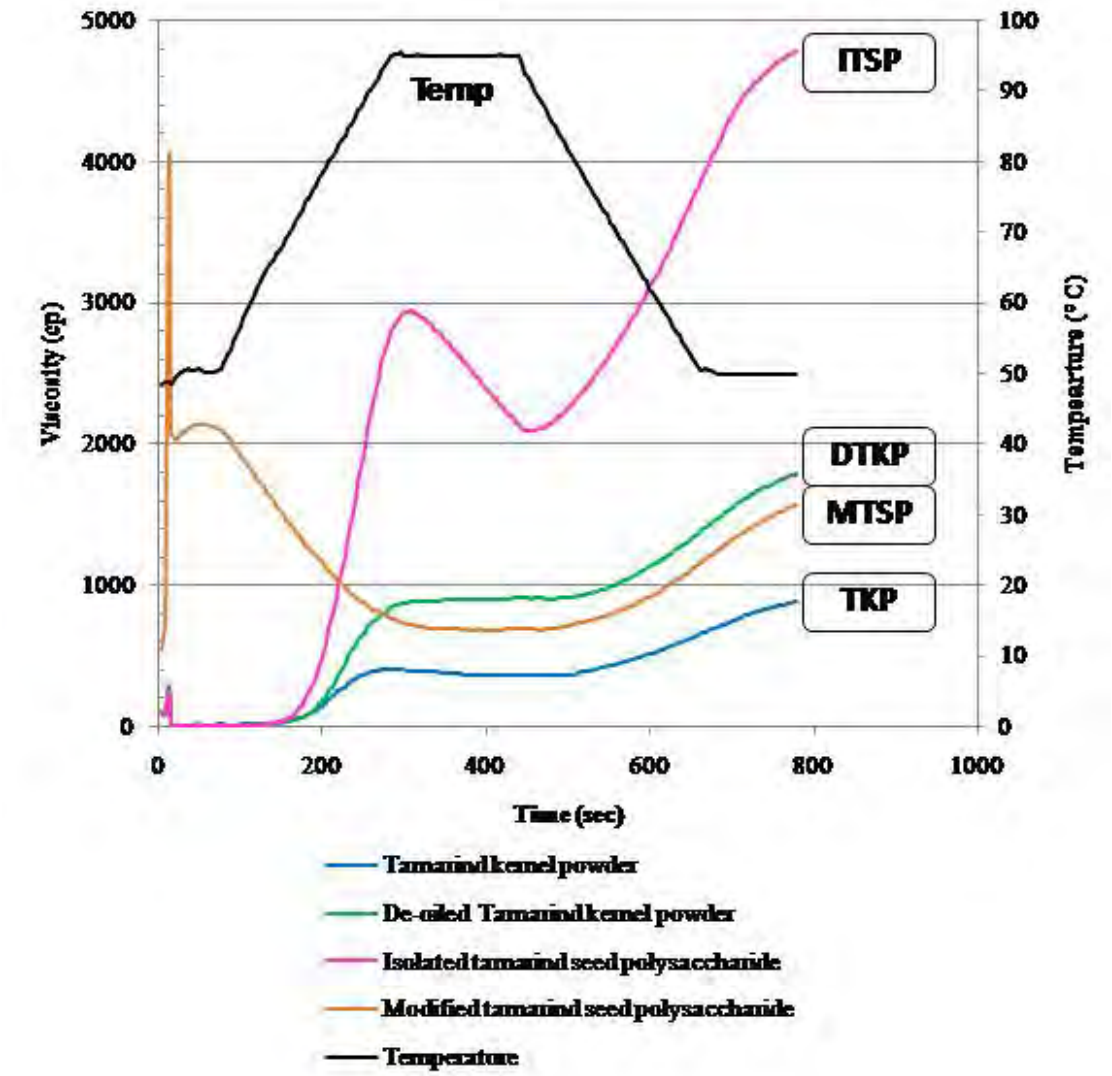


Figure 5.30 Typical RVA pasting profile of tamarind kernel powder, de-oiled tamarind kernel powder, isolated tamarind seed polysaccharide and modified tamarind seed polysaccharide (4% wt/wt)

CHAPTER VI

CONCLUSIONS & RECOMMENDATION

6.1 **Conclusions**

6.1.1. Compositions & thermal properties of TKP

i. The TKP composes of polysaccharide, protein, oil and others 69.84, 18.82, 8.00 and 3.34%, respectively.

ii. The pasting temperature was about 70°C. The viscosity of 4% (wt/wt) TKP was 416 cp at 95°C and 892 cp at 50°C.

6.1.2. The de-oiled processing

i. Isopropanol and hexane have similar extraction efficiency on the basis of extraction time, extraction temperature and solvent to solid ratio.

ii. The water content in 95% ethanol decreased the effective of oil removal.

iii. The use of high-intensity ultrasound in the 95% ethanol extraction increased the percentage of oil removal but reduced the size of TKP and the average molecular weight of polysaccharide.

iv. The decreasing of oil content in DTKP (de-oiled TKP) made the good dispersions in water.

v. The isopropanol extraction at the solvent to solid ratio of 3:1 mlg^{-1}, extraction time 10 min and the temperature of 30°C was a suitable condition for DTKP production.

6.1.3 The isolated tamarind seed polysaccharide processing

i. The solely utilization of protease digestion for 3 to 5 hours and 0.3 to 0.5% enzyme concentrations produced low protein ITSP with good thermal properties.

ii. The kinetics of protein digestion could be fit with Michaelis-Menten equation.

iii. The high-intensity ultrasound could not decrease the protein contamination in ITSP. The increasing of amplitude level and sonication time was declining the average molecular weight of polysaccharide.

iv. The Schmid polymer degradation model was used to fit the kinetics of molecular weight of polysaccharide decay as a function of time.

v. A combination of protease and high-intensity ultrasound treatment has a potential to produce ITSP in high yield and low protein contaminated in short time.

vi. A 0.3% protease and 50% amplitude level at 30 min sonication time gave the 93.49% protein eliminated and 60.12 % yield for ITSP.

6.1.4. Purification of isolated tamarind seed polysaccharide using dialyzer.

i. The increase of feed flow rate increased the dialysance (D, the performance of dialyzer) and the overall mass transfer coefficient (k_{OV}) and also decreased the rejection coefficient (R) of protein.

ii. The highest purity of polysaccharide, 99.13%, at 63.12% yield was achieved at 250 ml min^{-1} feed flow rate and 1500 ml min^{-1} dialysate flow rate.

6.1.5. Compositions & thermal properties of product.

i. From solvent extraction, DTKP composes of polysaccharide, protein, oil and others 75.40, 20.32, 0.65 and 3.63% respectively. The pasting temperature was about 70°C. The viscosity of 4% (wt/wt) DTKP was 847 cp at 95°C and 1,831 cp at 50°C.

ii. From HIU and protease combination, ITSP composes of polysaccharide, protein and others 95.86, 2.06 and 2.08 respectively. The pasting temperature was about 66°C. The viscosity of 4% (wt/wt) ITSP was 2,695 cp at 95°C and 4,795 cp at 50°C.

iii. From dialysis membrane separation, MTSP composes of polysaccharide and protein, 99.13 and 0.86 %, respectively. The pasting temperature was below 50°C. The viscosity of 4% (wt/wt) MTSP was 780 cp at 95°C and 1578 cp at 50°C.

6.2 Recommendation

Regarding to the dialysis experiment, the molecular weight of protein in feed solution was below 8000 Da. so the dialyzer F70S, molecular weight cut-off (MWCO) 18,000 Da, was selected to use in this experiment. The experiments found that 60 % protein in feed was eliminated to dialysate but above 99% polysaccharide was rejected. However, the protein molecular weight 7607 Da was not found in dialysate. It means that the protein of large molecular weight could not pass through the membrane. Thus, new membranes having larger MWCO should be applied in order to improve protein elimination.

Moreover, the volume in feed tank was changed at the large different between feed and dialysate flow rates. In case of UF effect, we have some remark that about 50% protein was eliminated couple with UF action. Therefore, it is interesting to apply Ultrafitration process in the next research.

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APPENDICES

APPENDIX A

Analytical methods

1. Protein analysis

The amount of protein in TKP was determined by Kjeldahl method (AOAC.920.87).

- **Chemicals**

1. 6.25 M Sodium hydroxide (NaOH)
2. 2% wt/v Boric acid (H_3BO_3)
3. Concentrated sulfuric acid (H_2SO_4)
4. Selenium reagent mixture

- **Method**

The Kjeldahl method was divided into three main steps:

Digestion – added 0.5 g of selenium reagent mixture as a catalyst to 0.2 g of TKP sample and digested with concentrated sulfuric acid solution by boiling for 90 min. After that a homogeneous sample in concentrated sulfuric acid solution was obtained. An ammonium sulfate solution was formed in the end result.

Distillation – adding excess 6.25 M NaOH to the acid digestion mixture to convert NH_4^+ to NH_3, followed by boiling and condensation of the NH_3 gas in a receiving solution.

Titration – The amount of ammonia in the receiving solution was determined by titration with standard sulfuric acid.

The percentage of protein in TKP was calculated through:

$$\text{Protein (\%)} = \frac{1.4007 \times 6.25 \times N_{H_2SO_4} \times V_{H_2SO_4}}{Wt_{sample}}$$

$N_{H_2SO_4}$ = Normality of standard sulfuric acid

$V_{H_2SO_4}$ = Volume of sulfuric acid for titration

Wt_{sample} = Weight of used sample

2. Polysaccharide analysis

The amount of polysaccharide in TKP was determined by Phenol-sulfuric method (Dubois et al., 1956).

- **Chemicals**

1. 5% wt/v Phenol

2. Concentrated sulfuric acid (H_2SO_4)

- **Method**

TKP sample solution was prepared having polysaccharide in range of 10-70 µg/ml. 1 ml of phenol and 5 ml of concentrated sulfuric acid was added into 1 ml of sample solution. Solution was mixed by vortex and placed to form a reaction for 10 min. After that the reaction was stopped by placing in water bath at 25-30ºC for 10-20 min. The color intensity was measured by spectrophotometer at 483 nm. The measured absorbance was recorded and calculated amount of polysaccharide in TKP through standard calibration curve of TSP as showed in figure A-1.

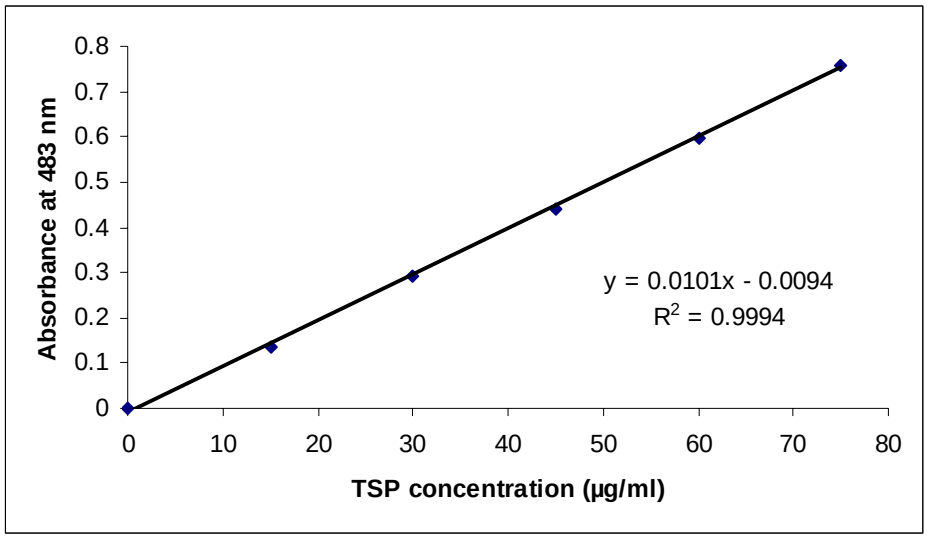

Figure A-1 Standard calibration curve of tamarind seed polysaccharide (TSP)

3. Oil analysis

The amount of fat in TKP was determined by soxhlet extraction method (AOAC. 920.39).

- **Chemicals**

1. Petroleum ether

- **Method**

Two grams of tamarind kernel powder was placed into a thimble of a soxhlet apparatus and extracted with 150 ml of petroleum ether. Extraction was carried out for 3 hours. After that solvent was poured into weighted evaporing flask. Solvent was evaporated by a rotary-evaporator and the last traces were removed by placing the flask with the extract in an oven at 110 ^{0}C for one hour. The flask was cooled in a dessicator and weighed.

APPENDIX B

Protease

Protease enzyme used in this experiment is Esperase 8.0 L.

- protease form *Bacillus* sp.,
- minimum 8.0 KNPU
- A product of Novozyme corp.

Unit definition: 1 KNPU = 1000 NPU, 1 Novo Protease Unit (NPU) is the amount of enzyme which hydrolyzes casein at such a rate that the initial rate of formation of peptides/minute corresponds to 1 micromole of glycine min^{-1}

APPENDIX C

Experimental data of ITSP

Table C-1 Powder yield, polysaccharide content, polysaccharide loss, protein content and protein eliminated of TKP isolated by protease at different levels and durations time.

Treatment	Powder yield (%)	Polysaccharide content (%)	Polysaccharide loss (%)	Protein content (%)	Protein eliminate (%)	Mw (10^{-6})
No enzyme 1 h	72.35	79.81	17.97	16.38	37.66	2.673
0.1% Protease 1 h[a]	64.73	80.81	25.70	15.25	48.07	2.746
0.3% Protease 1 h	61.32	85.10	25.88	11.15	64.03	2.791
0.5% Protease 1 h	57.06	86.18	30.15	10.14	69.56	2.625
No enzyme 3 h	63.22	80.64	27.58	15.24	49.32	2.721
0.1% Protease 3 h	57.81	84.27	30.80	11.49	65.05	2.798
0.3% Protease 3 h	54.82	88.19	31.41	7.72	77.12	3.193
0.5% Protease 3 h	52.14	90.23	33.30	6.11	82.43	3.329
No enzyme 5 h	55.89	80.52	35.14	15.01	55.87	2.610
0.1% Protease 5 h	51.58	87.40	36.99	8.23	77.67	2.887
0.3% Protease 5 h	51.11	90.48	38.22	5.41	85.45	3.014
0.5% Protease 5 h	50.04	91.22	39.34	4.88	87.15	3.217

a Protease at 0.1% powder level and digestion for 1 hr

Table C-2 Powder yield, polysaccharide content, polysaccharide loss, protein content, protein eliminated and average molecular weight of isolated TKP by high-intensity ultrasound at different levels and sonication time.

Treatment	Powder yield (%)	Polysaccharide content (%)	Polysaccharide loss (%)	Protein content (%)	Protein eliminate (%)	Mw (10^{-6})
A25T15[a]	80.11	79.48	9.56	18.59	21.66	1.981
A50T15	67.22	78.53	25.02	19.39	31.44	1.668
A75T15	55.14	74.29	41.81	23.26	32.53	1.392
A100T15	48.33	71.78	50.72	25.22	35.88	1.131
A25T30	68.57	77.58	24.44	20.31	26.74	1.902
A50T30	59.38	77.44	34.68	20.20	36.90	1.485
A75T30	51.83	77.90	42.65	19.40	47.11	1.145
A100T30	45.71	75.37	51.06	21.35	48.66	0.926
A25T45	61.33	78.89	31.27	18.42	40.57	1.801
A50T45	52.22	79.50	41.03	17.44	52.09	1.383
A75T45	44.39	77.05	51.41	19.68	54.05	1.038
A100T45	38.44	75.10	58.99	21.52	56.48	0.891
A25T60	58.39	79.96	33.68	17.56	46.06	1.733
A50T60	47.37	78.85	46.94	17.98	55.20	1.125
A75T60	37.30	76.12	59.67	20.39	59.99	0.924
A100T60	30.11	70.18	69.98	26.33	58.30	0.830

a amplitude 25% sonicate 15 min

Table C-3 Powder yield, polysaccharide content, polysaccharide loss, protein content and protein eliminated of TKP isolated by the combination of 0.3% protease and high-intensity ultrasound

Treatment	Powder yield (%)	Polysaccharide content (%)	Polysaccharide loss %)	Protein content (%)	Protein eliminate (%)	Mw(10^{-6})
UT15A25-P1h[a]	60.33	83.98	28.03	13.28	57.84	1.981
UT30A25-P1h	55.38	85.48	32.75	11.36	66.92	1.835
UT15A50-P1h	53.74	84.43	35.55	12.78	63.87	1.614
UT30A50-P1h	50.88	87.12	37.03	10.22	72.64	1.389
PUT30A25[b]	65.25	90.25	16.35	7.14	75.48	1.924
PUT60A25	57.39	92.97	24.21	4.41	86.67	1.749
PUT30A50	60.12	95.86	18.14	2.06	93.49	1.573
PUT60A50	53.12	96.30	27.34	1.63	95.45	1.285
P1h-UT15A25[c]	57.88	92.25	24.15	5.33	83.77	1.631
P1h-UT30A25	49.61	94.17	33.64	2.81	92.67	1.478
P1h-UT15A50	48.93	95.22	33.82	2.43	93.75	1.316
P1h-UT30A50	45.78	96.54	37.22	1.82	95.62	1.011

a Sonication 15 min at 25% amplitude and then 1 h protease digestion

b Protease digestion and sonication 30 min at 25% amplitude concurrently

c Protease digestion 1 h and then sonication 15 min at 25%

APPENDIX D

Molecular weight of protein determined by MALDI-TOF MASS spectrometry

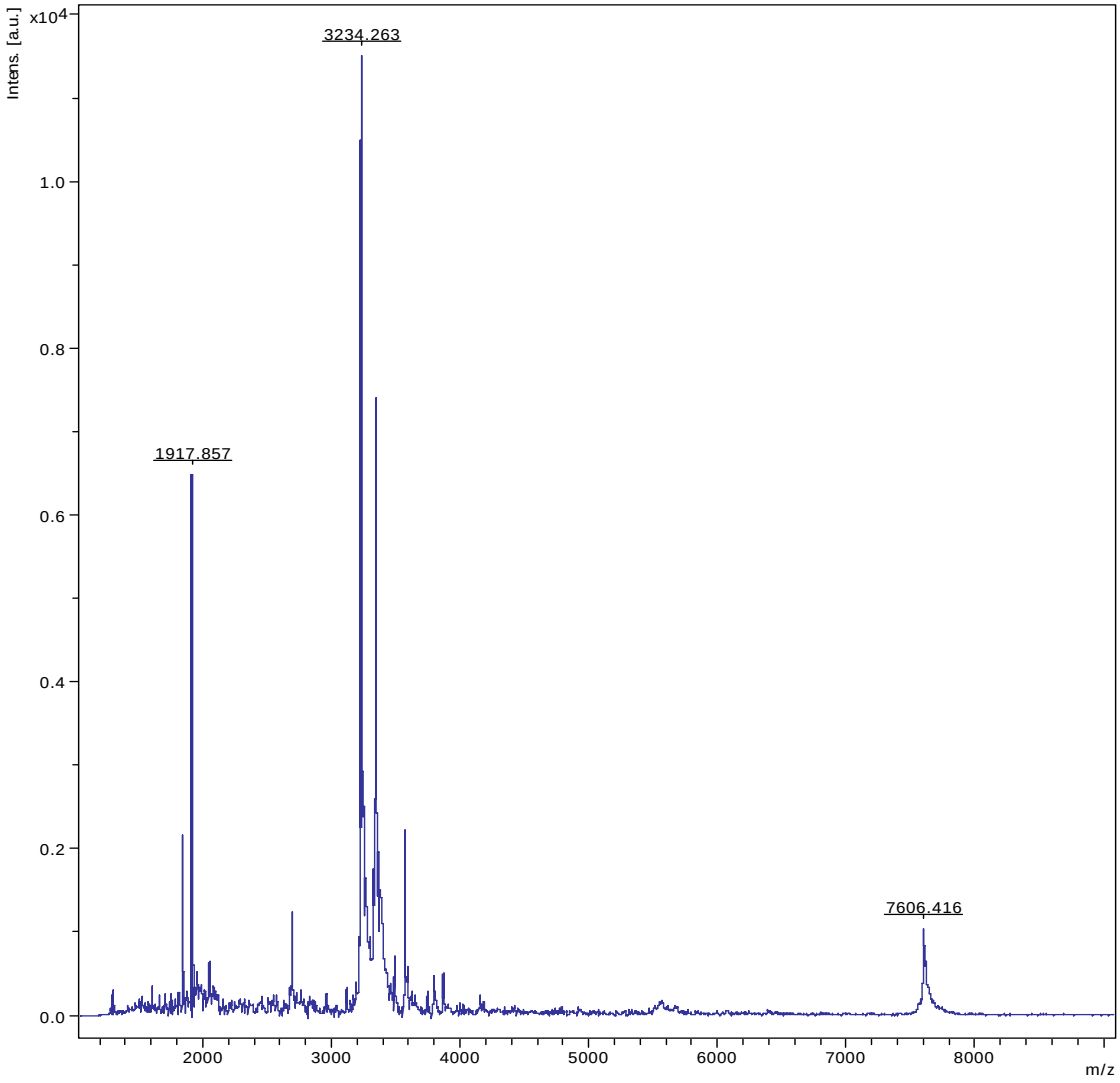

Figure D-1 Mass spectrometer of protein in feed solution determination by MALDI-TOF MASS spectrometry.

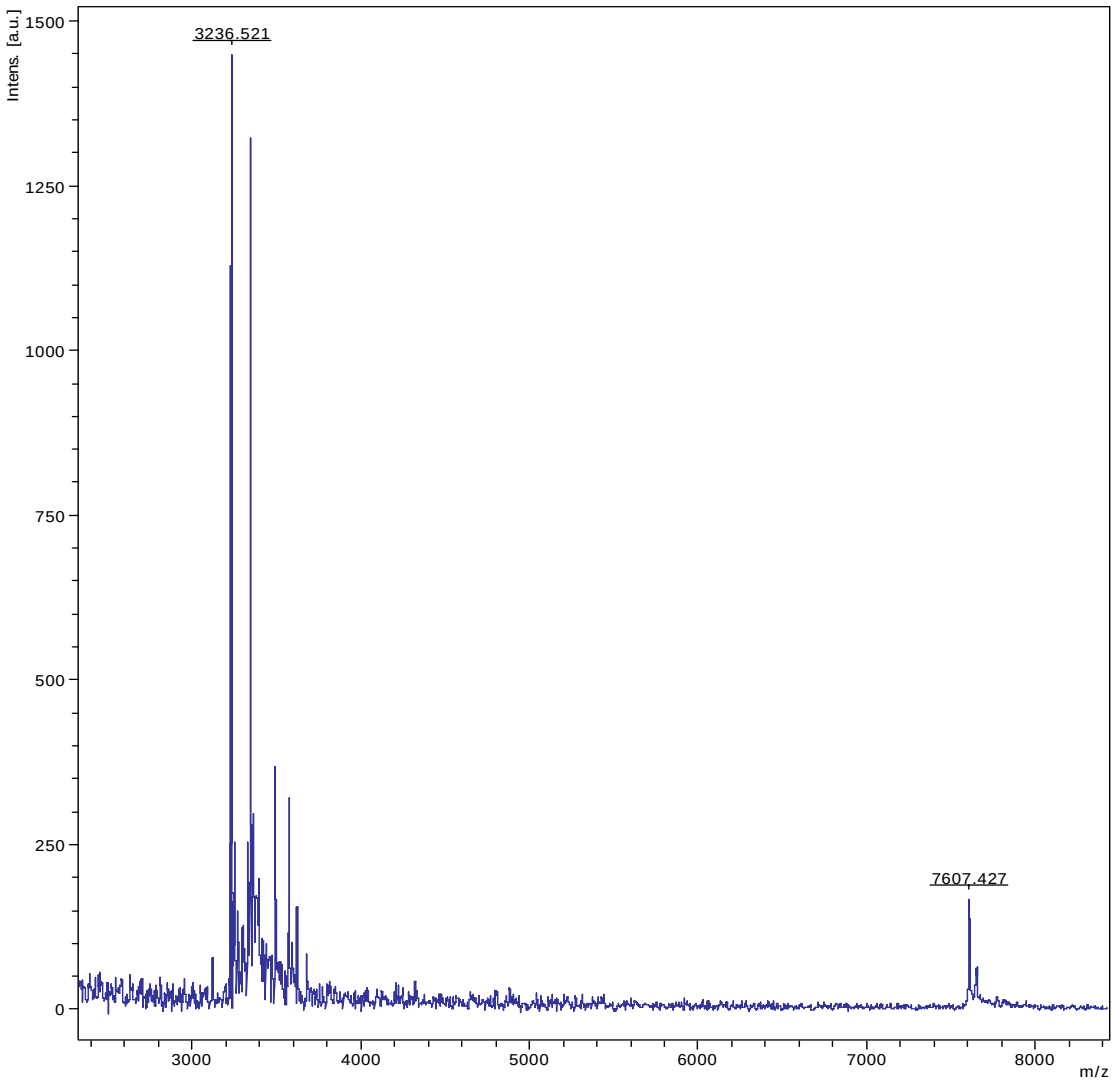

Figure D-2 Mass spectrometer of protein in feed solution after processing determination by MALDI-TOF MASS spectrometry.

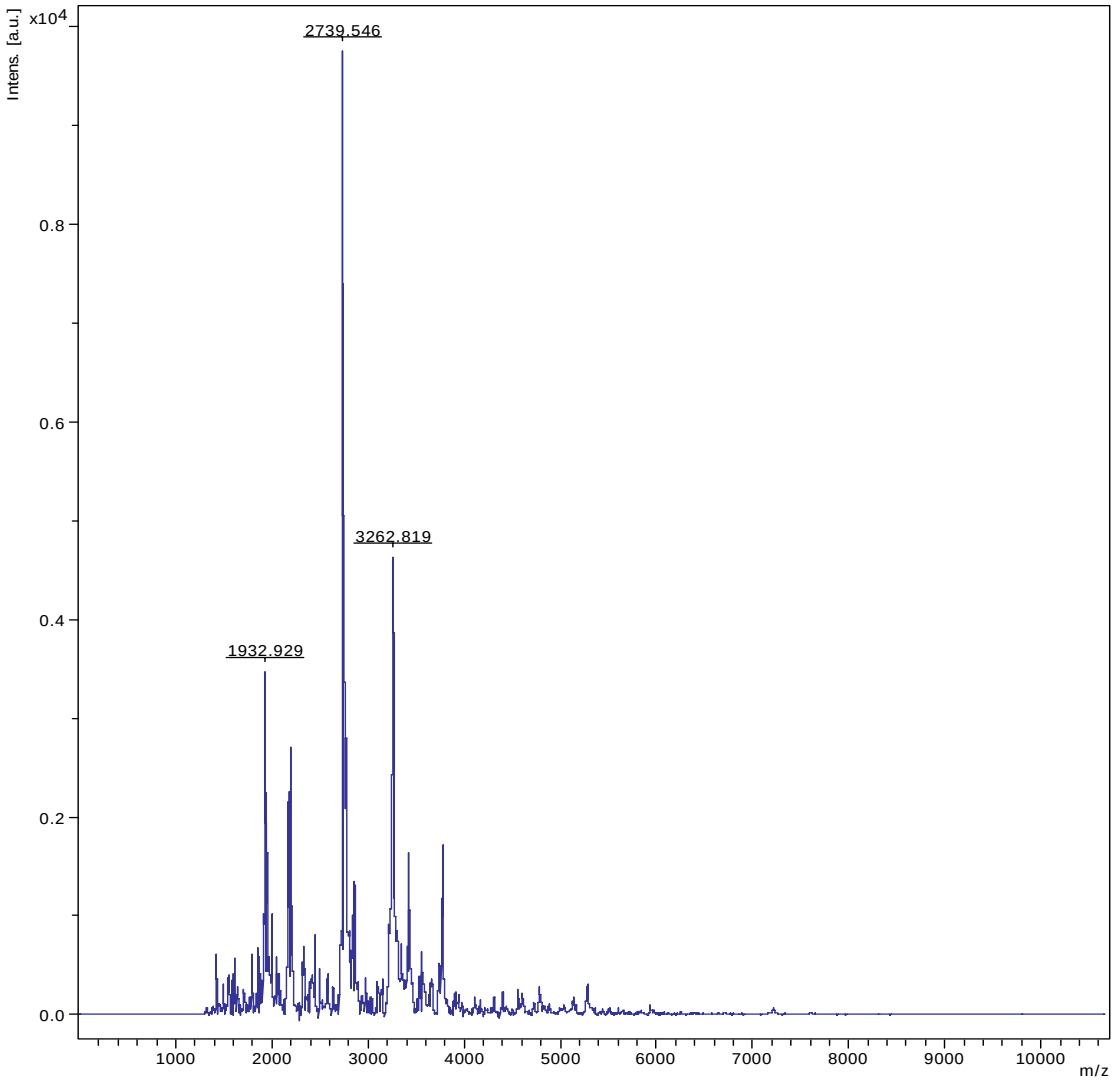

Figure C-3 Mass spectrometer of protein in dialysate determination by MALDI-TOF MASS spectrometry.

VITA

Mr. Sukhum Poommarinvarakul was born on December 27th in Bangkok, Thailand. He finished his secondary course from Rajavinit Mathayom School, Bangkok. He received the Bachelor's Degree of Science with a major in Department of Food technology, Faculty of Science, Chulalongkorn University in 1998. After graduation, he continued his further study for master's degree at the Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University. He participated in the Biochemical Engineering Research Group and achieved his Master's degree in 2004. He entered his Doctoral Degree in Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University.