การแยกและการทำให้บริสุทธิ์ของยางอนุภาคเล็กจากสกิมลาเท็กซ์

นางสาวกนกวรรณ จุมที

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ หลักสูตรปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2543

ISBN 974-346-587-1

RECOVERY AND PURIFICATION OF SMALL RUBBER PARTICLES FROM SKIM LATEX

Miss Kanokwan Jumtee

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Petrochemistry and Polymer Science
Program of Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2000

ISBN 974-346-587-1

Thesis Title	RECOVERY AND PURIFICATION OF SMALL RUBBER PARTICLES FROM SKIM LATEX
By	Miss Kanokwan Jumtee
Program	Petrochemistry and Polymer Science
Thesis Advisor	Professor Pattarapan Prasassarakich, Ph.D.
Thesis Co-advisor	Professor Yasuyuki Tanaka, Ph.D.
	Assistant Professor Jitladda Sakdapipanich, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree.

……..Dean of Faculty of Science
(Associate Professor Wanchai Phothiphichitr, Ph.D.)

Thesis Committee

……..Chairman
(Associate Professor Supawan Tantayanon, Ph.D.)

……..Thesis Advisor
(Professor Pattarapan Prasassarakich, Ph.D.)

……..Thesis Co-advisor
(Professor Yasuyuki Tanaka, Ph.D.)

……..Thesis Co-advisor
(Assistant Professor Jitladda Sakdapipanich, Ph.D.)

……..Member
(Associate Professor Wimonrat Trakarnpruk, Ph.D.)

กนกวรรณ จุมที : การแยกและการทำให้บริสุทธิ์ของยางอนุภาคเล็กจากสกิมลาเท็กซ์ (RECOVERY AND PURIFICATION OF SMALL RUBBER PARTICLES FROM SKIM LATEX) อ. ที่ปรึกษา : ศ.ดร.ภัทรพรรณ ประศาสน์สารกิจ; อ. ที่ปรึกษาร่วม : ศ.ดร.ยาสุยูกิ ทานากะ และ ผศ.ดร.จิตต์ลัดดา ศักดาภิพาณิชย์; 120 หน้า, ISBN 974-346-587-1.

สกิมลาเท็กซ์เป็นผลิตภัณฑ์พลอยได้จากการผลิตน้ำยางข้น สกิมลาเท็กซ์มีเนื้อยางประมาณ 3-10 เปอร์เซ็นต์ และประกอบด้วยยางอนุภาคขนาดเล็กเป็นส่วนใหญ่ ยางสกิมที่กลับมาโดยการจับก้อนด้วยกรดซัลฟุริก มีองค์ประกอบที่ไม่ใช่ยางมากกว่ายางธรรมชาติทั่วไป ดังนั้นยางสกิมจึงถูกจัดเป็นยางเกรดต่ำ งานวิจัยนี้มีวัตถุประสงค์เพื่อให้ได้ยางสกิมที่มีความบริสุทธิ์สูง โดยใช้วิธีการทำให้บริสุทธิ์หลายวิธี ดังนี้ 1) การย่อยน้ำยางสกิมด้วยเอนไซม์ โดยมีโซเดียมคลอไรด์ 2) สะพอนิฟิเคชันของน้ำยางสกิมด้วยโซเดียมไฮดรอกไซด์ 3) การแยกเนื้อยางโดยการบ่มน้ำยางสกิมกับยีสต์ และ 4) สะพอนิฟิเคชันของยางสกิมแห่งในสารละลายโทลูอีน

การย่อยน้ำยางสกิมด้วยเอนไซม์โดยมีโซเดียมคลอไรด์และสะพอนิฟิเคชันของน้ำยางสกิมด้วยโซเดียมไฮดรอก-ไซด์ ทำให้เกิดการแยกชั้นเป็นชั้นครีมและชั้นซีรัม ชั้นครีมที่ได้จากทั้งสองวิธีมีเนื้อยางเพิ่มขึ้น 2-3 เท่า ขนาดของอนุภาคยางเฉลี่ยเพิ่มขึ้น จาก 0.1 ไมครอน เป็นประมาณ 3 ไมครอน ภายหลังการย่อยด้วยเอนไซม์ ขณะที่ขนาดของอนุภาคยางไม่เปลี่ยนแปลงหลังสะพอนิฟิเคชัน ปริมาณไนโตรเจนของยางบริสุทธ์ที่ได้จากการย่อยด้วยเอนไซม์และสะพอนิฟิเคชันลดลงจาก 2.7 เป็น 0.6 และ 0.3 เปอร์เซ็นต์, ตามลำดับ ยางบริสุทธ์นี้มีปริมาณเถ้าสูง และปริมาณเอสเทอร์ลดลงเล็กน้อย หลังจากการล้างชั้นครีมด้วยการปั่นแยก พบว่า ปริมาณไนโตรเจนลดลงจนถึง 0.04 และ 0.03 เปอร์เซ็นต์ สำหรับวิธีการย่อยด้วยเอนไซม์และสะพอนิฟิเคชัน ตามลำดับ ส่วนปริมาณเถ้า เอสเทอร์และเจลน้อยกว่ายางเริ่มต้นเล็กน้อย การบ่มน้ำยางสกิมด้วยยีสต์ ที่ pH 7 โดยมีปริมาณ SDS 0.2 เปอร์เซ็นต์ ก่อให้เกิดการเพิ่มขนาดของยางอนุภาคเล็กในน้ำยางสกิม จาก 0.1 ไมครอน เป็น ประมาณ 1-5 ไมครอน ภายหลังบ่ม 48 ชั่วโมง และปริมาณไนโตรเจนไม่เปลี่ยนแปลง การปั่นแยกน้ำยางที่บ่มด้วยยีสต์ ทำให้เกิดการแยกชั้น เป็นชั้นครีม ชั้นซีรั่ม และยีสต์ที่ก้นหลอด เนื้อยางสามารถได้กลับคืนประมาณ 45 เปอร์เซ็นต์ สะพอนิฟิเคชันของชั้นครีมลดปริมาณไนโตรเจนลงได้ถึง 0.71 เปอร์เซ็นต์ การกำจัดโปรตีนด้วยสะพอนิฟิเคชันของสารละลายยางสกิมความเข้มข้นสูงในโทลูอีน (10 เปอร์เซ็นต์) ด้วยโซเดียมไฮดรอกไซด์ที่อุณหภูมิ 70 องศาเซลเซียส ปริมาณไนโตรเจนและเถ้าลดลงได้ถึง 0.03 และ 0.3 เปอร์เซ็นต์ ตามลำดับ ยางสกิมบริสุทธ์ที่ได้จากวิธีนี้มีความแข็งแรงดึงของยางดิบต่ำกว่ายางสกิมตั้งต้น ความหนืดมูนนีไม่เปลี่ยนแปลง ค่าดัชนีความอ่อนตัวเริ่มแรกและค่าดัชนีความอ่อนตัวดีขึ้น

ภาควิชา -
สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
ปีการศึกษา 2543

ลายมือชื่อนิสิต .....
ลายมือชื่ออาจารย์ที่ปรึกษา .....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

4172203123 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEY WORD: PURIFICATION / RECOVERY / SAPONIFICAION / DEPROTEINIZATION / SKIM RUBBER / SKIM LATEX

KANOKWAN JUMTEE : RECOVERY AND PURIFICATION OF SMALL RUBBER PARTICLES FROM SKIM LATEX. THESIS ADVISOR : PROF. PATTARAPAN PRASASSARAKICH, Ph.D., THESIS COADVISOR : PROF. YASUYUKI TANAKA, Ph.D. and ASSIST. PROF. JITLADDA SAKDAPIPANICH, Ph.D., 120 pp. ISBN 974-346-587-1.

Skim latex is a by-product of the production of concentrated latex. Skim latex contains about 3-10%DRC (dry rubber content) and composes of mainly small rubber particles. The rubber fraction recovered by coagulation of skim latex with sulfuric acid contains higher amounts of non-rubber component than ordinary solid natural rubber. Therefore, skim rubber is evaluated to be a low-grade rubber. In this work, an attempt was made to get the highly purified skim rubber using various purification methods: 1) Enzymatic deproteinization of skim latex in the presence of NaCl, 2) Saponification of skim latex with NaOH, 3) Phase separation of rubber by incubation of skim latex with dry yeast, 4) Deproteinization of skim rubber by saponification in toluene solution.

Enzymatic deproteinization in the presence of NaCl and the saponification of skim latex with NaOH gave a clear phase separation as cream phase and serum phase. The resulting cream phase of both methods was concentrated to 2-3 times DRC. The average particle size increased from 0.1 μm to about 3 μm after enzymatic treatment, while it was unchanged after the saponification. The nitrogen content of the purified rubber by enzymatic deproteinization and saponification was reduced from 2.7 to 0.6 and 0.3%, respectively. The purified rubber contained high ash content and slightly lower in ester content. After the washing of cream phase by centrifugation, the nitrogen content was further reduced to 0.04 and 0.03% for the enzymatic treatment and the saponification, respectively. Ash, ester and gel contents were lower than that of the original rubber. The incubation of skim latex with dry yeast at pH 7 in the presence of 0.2% SDS caused an increase in particle size of small rubber particles in skim latex from 0.1 μm to about 1-5 μm after 48 h and an unchange in the nitrogen content. Centrifugation of yeast-treated lattices resulted in phase separation as cream, serum and yeast at bottom. The recovery of rubber particles of 45% can be achieved. Saponification of cream phase reduced the nitrogen content to 0.71%. Deproteinization by saponification of skim rubber in toluene solution (10% rubber concentration) was carried out with NaOH at 70°C. The nitrogen and ash contents decrease to 0.03% and 0.3%, respectively. This purified skim rubber showed the lower green strength than original skim rubber. Mooney viscosity was unchanged. Wallace plasticity (P_o) and Plasticity retention index (PRI) were improved.

Department -	Student's signature
Field of study Petrochemistry and Polymer Science	Advisor's signature
Academic year 2000	Co-advisor's signature
	Co-advisor's signature

ACKNOWLEDEGMENTS

The author would like to acknowledge her heartfelt gratitude and appreciation to her advisor, Prof. Pattarapan Prasassarakich, and her co-advisor, Prof. Yasuyuki Tanaka and Assistant Prof. Jitladda Sakdapipanich, for their kind supervision, in valuable guidance and generous encouragement throughout the course of this research. The author is also sincerely grateful to the members of the thesis committee for their comments, suggestions, and time to read the thesis.

Many thanks also go to the Department of Chemical Technology, the Department of Biochemistry and the Department of Chemistry, Faculty of Science, Chulalongkorn University, the Rubber Research Institute of Thailand and the National Metal and Materials Technology Centers (MTEC) for providing research facilities throughout the research work. Special thanks are also expressed to N.Y. Rubber Co., Ltd. for material supply and to the Graduate School of Chulalongkorn University and TJTTP (OECF) for the partial financial research support.

The author also would like to especially thank the following: Dr. Seiichi Kawahara, Miss Tippawon Kowitteerawut, and her friends whose names are not mentioned here, but contributed their assistance, suggestions, advice concerning the experimental techniques and the encouragement during the period of this study.

Finally, and most of all, the author would like to express her deep gratitude to her family for their love, inspiration, and endless encouragement throughout this entire study.

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

CHAPTER 1

INTRODUCTION

1.1 Scientific Rationale

The world production of natural rubber (NR) has increased at an average rate of 3.74 percent over the five years period (1994-1998). The average production of natural rubber is 6.22 million tons per year, while the world consumption during that period is 6.17 million tons per year [1]. The three major producers, Thailand, Indonesia and Malaysia constitute 70.59 percent of world output in 1998. Being the largest producer of natural rubber, the quality of Thai rubber is comparable to the best in the world nowadays. With the production base of 2 million tons per annum, Thailand is poised to be a dominant force in the rubber market in the future. Natural rubber production in Thailand is expected to reach 2.12 million tons in the year 2001. The global natural rubber production is expected to grow at 1.1 percent during 1999-2001, reaching about 8,535 thousand metric tons in 2010 [2].

The production of the concentrate latex in Thailand has been estimated at around 220 thousand tons per annual. Most of the concentrate latex is produced by the centrifugation technique. Large volumes of skim latex are also produced as a by-product from this technique. The approximately 10-12 percent of latex, which enters the centrifuge, effluxes as skim latex. Therefore, it is estimated that about 13 thousand tons of skim latex were produced each year.

Skim latex is considered to be a liquid waste portion of rubber latex, which contains approximately 3-10 percent rubber fraction, and a much higher proportion of natural non-rubber substances than normal rubber [3]. The recovery of skim rubber from skim latex has been carried out by the coagulation of rubber particles by sulfuric acid. Skim rubber is evaluated to be a low-grade natural rubber due to

presence of a number of impurities in it, which decrease the physical and mechanical properties [4].

However, it was disclosed recently that small rubber particles with average diameter of 0.1 μm in skim latex showed the properties perfectly different from ordinary natural rubber or the rubber from large particles in the cream phase [4,5]. The rubber from skim latex is composed of linear molecules, whereas that from cream phase is a branched molecule. The molecular weight and molecular weight distribution (MWD) as well as nitrogen and fatty acid ester content are also quite different. The rubber from skim latex shows a unimodal MWD with almost the same $\overline{M}_w$ and higher $\overline{M}_n$ compared with the rubber from cream phase [5]. Skim latex has a high nitrogen and lower ester content compared with those in the ordinary natural rubber. In addition, the mechanical and physical properties of skim rubber are also differed from those of normal rubber [4]. The properties of skim rubber were varied in rubber hydrocarbon content and in the nature of the non-rubber constituents.

Therefore, the development of a new method for the recovery and purification of the small rubber particles from skim latex is required to produce more valuable skim rubber. The purified skim rubber should contain less non-rubber components. It is also expected to contain almost no gel fraction and no branch points, which are derived from fatty acid ester groups. These characteristics will result in low green strength and high processability of purified skim rubber [6]. The purified skim rubber is expected to be a appropriate material for the production of rubber products such as adhesive base, chewing gum etc., which require these characteristics, as well as for blending with ordinary rubber.

1.2 Objectives of the Research Work

1.2.1 To find the appropriate conditions to recover and purify the small rubber particles from skim latex by deproteinization, saponification and incubation with yeast.

1.2.2 To find the appropriate conditions to purify solid skim rubber by saponification in toluene solution.

1.2.3 To investigate the properties of purified skim rubber.

1.3 Scopes of the Research Work

The present study is aimed to establish a novel method of recovery and purification of the small rubber particles from skim latex. This work is focused to the separation and purification of the skim latex to make the deproteinized skim rubber. The procedures to achieve the goal are as follows:

1.3.1 Literature survey and in-depth study of this research work.

1.3.2 Studying the conditions to recover and purify the small rubber particles from skim latex by the following methods:

a) Enzymatic deproteinization of skim latex in the presence of sodium chloride.

b) Saponification of skim latex with sodium hydroxide.

c) Incubation of skim latex with baker's yeast and saponification of cream phase with sodium hydroxide.

1.3.3 Studying the conditions to purify the solid skim rubber in toluene solution.

1.3.4 Studying the properties of purified skim rubber, particle size distribution, nitrogen, ash, ester, and gel contents.

1.3.5 Summarizing the results.

CHAPTER 2

THEORETICAL BACKGROUND

2.1 Natural Rubber Latex

Natural rubber latex (NRL) is obtained as a cloudy white liquid, harvested from many different species of plant growing mainly tropical zone. The most common source of NRL is *Hevea brasiliensis* or Para rubber, originated from Brazil. *Hevea rubber* is an important natural source of rubber due to the high yielding capacity and good growth characteristics, as well as its resistance to leave diseases and wind damage [7,8].

2.1.1 General Properties

Natural rubber latex, collected by tapping from *Hevea rubber* trees, is a colloidal suspension of rubber particles in an aqueous serum phase. The latex that exuded from the cut is called the fresh natural rubber latex or fresh latex. Fresh latex consists of approximately 25-40% dry rubber content (DRC) and 5-10% non-rubber substances. The non-rubber components include proteins, carbohydrates, lipids, and inorganic salts. Its composition varies according to the clones of rubber, age of rubber tree, and tapping method [9]. The composition of typical fresh latex is presented in Table 2.1

Table 2.1 Composition of latex sap [10].

Constituent	% Composition
Total solid	36
Dry rubber	33
Proteinaceous substances	1-1.5
Resinous substances	1-2.5
Ash	Up to 1
Sugar	1
Water	Add to 100

In general, fresh latex has the density of 0.975-0.980 g/cm^3 with the pH of 6.5-7.0 and a refractive index of 1.5910. Rubber does not dissolve in water, alcohol, nor acetone, but it swells and disperses or partly solubilizes in benzene, toluene, gasoline, carbon disulfide, turpentine, chloroform, carbon tetrachloride, and other halogen containing solvents. The chemical and physical properties of latex are influenced by clone, age of rubber [11], tapping intensity, soil characteristics [12] and season of tapping.

2.1.2 Latex Stability

Rubber particles in fresh natural rubber latex are spherical droplets of hydrocarbon, which are stabilized by the negative charge of surface-adsorbed proteins and phospholipids [13,14] as shown in Figure 2.1.

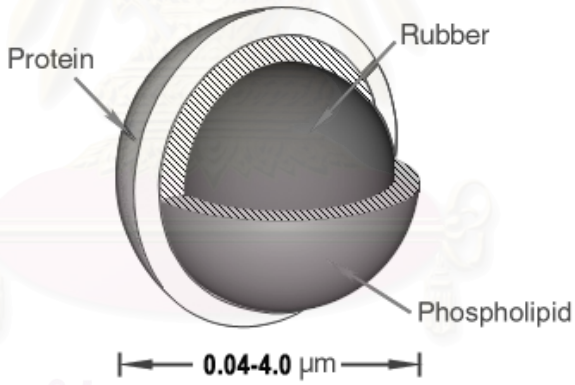


Figure 2.1 Rubber particle [13].

After exuding from the trees, latex will be coagulated within a few hours. This process occurs as a result from biochemical reactions with microorganisms and non-rubber components, e.g., sucrose and fructose. Consequently, the preservation is necessary. The preservative of natural rubber latex by ammonia was first recorded in patents to Johnson [15] and to Norris [16] published in 1853.

Although there are several disadvantages, which accompany the use of ammonia as preservative, ammonia is satisfactory and the most popular preservative

until now. When fresh latex is concentrated to the concentrated latex having ca. 60% DRC, it can be preserved in two ways. One is known as low-ammonia (LA) preservatives (0.2% w/w), the other is high-ammonia (HA) preservatives (0.7% w/w).

The colloidal stability of freshly prepared latex concentrate is always low. During storage at ambient temperature, the stability of latex increases rapidly due to the increasing content of free higher fatty acid soap (HFA), arising from the hydrolysis of phospholipids on the surface of the rubber particles [17,18]. On the other hand, Hasma (1991) found that the formation of HFA is not the major factor contributing to the increase in mechanical stability of HA-latex concentrate [19]. Since the increasing presence of HFA has a broad relation to the rise in mechanical stability.

The particle size of rubber particles in fresh latex depends on the age and clones of rubber trees. Natural rubber latex from the mature trees contains the rubber particles with particle sizes in the range from 0.04 to 4 μm [20] with mean diameter of 1.03 μm (Figure 2.2). The peak of the small rubber particle distribution below 0.2 μm is not clear in the distribution due to its low rubber content [5].

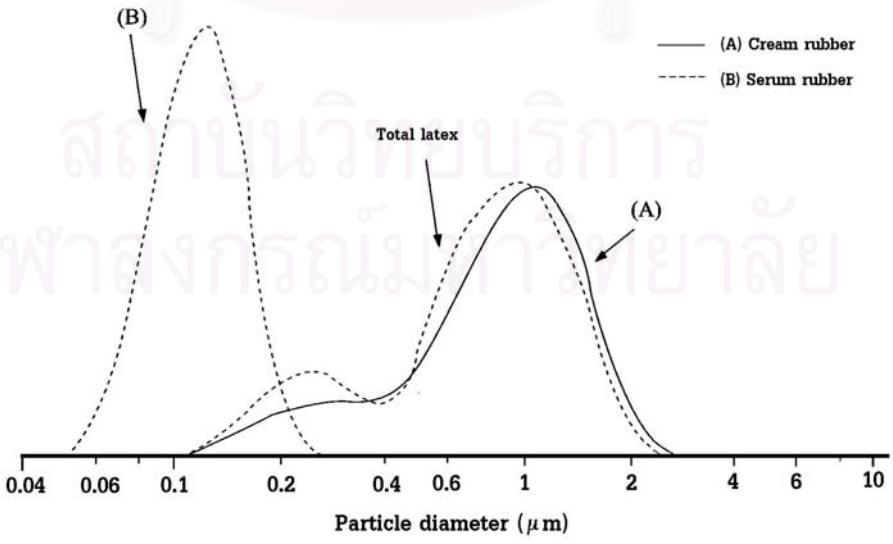


Figure 2.2 Particle size distribution of fresh latex [5].

After centrifugation, fresh latex is separated into four major components, i.e., rubber phase, C-serum, Frey-Wyssling particles and bottom fraction as illustrated in Figure 2.3.

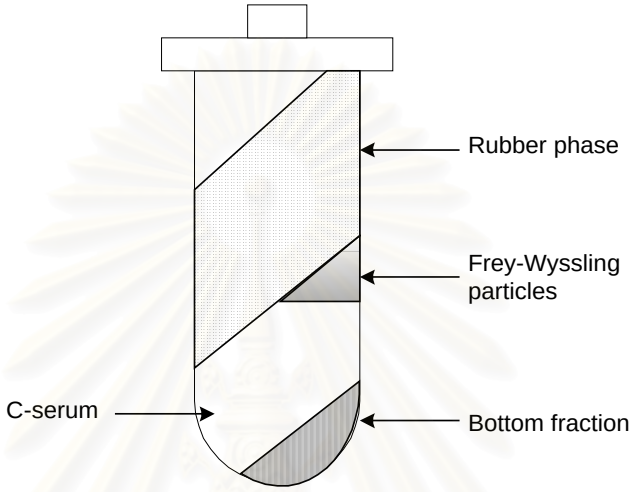


Figure 2.3 Four major components of fresh latex after ultra centrifugation [21].

Rubber phase

Rubber phase, the white upper layer, consists of rubber hydrocarbon particles stabilized by an adsorbed layer of proteins and phospholipids

C-serum

The serum comprises mostly soluble substances such as amino acid, proteins, carbohydrates, organic acid, inorganic salts and nucleotidic materials.

Frey-Wyssling particles

Frey-Wyssling particles comprise 2-3% of latex volume. It is spherical with encapsulation by two carotenoid layers, which make rubber dark yellow.

Bottom fraction

Bottom fraction or lutoid consists largely of the lutoid particles and non-rubber particles.

2.2 Concentration of Natural Rubber Latex [10]

Fresh field latex from *Hevea brasiliensis* tree has a rubber content of ca. 30-40% DRC, dispersed in water or dispersion medium. The concentration of latex is necessary not only to reduce the volume of latex for transportation, but also to reduce the ratio of non-aqueous substances to dry rubber content. In addition, several of the industrial processes use the latex which has concentration higher than 33%.

There are several concentration methods, such as evaporation, creaming, centrifugation, and electrodecantation, etc. Typical methods are described below.

2.2.1 Concentration by Creaming

Concentration by creaming is a sedimentation process using creaming agents e.g. ammonium alginate. In NRL, the density of rubber particles is less than that of dispersion medium. After creaming, therefore, the rubber particles tend to rise to the surface of the dispersion medium. The concentrated latex is known as **cream**, while the dilute latex forming the lower layer is known as **skim**. The serum layer is drained off after incubation in the tank for about 40 hours.

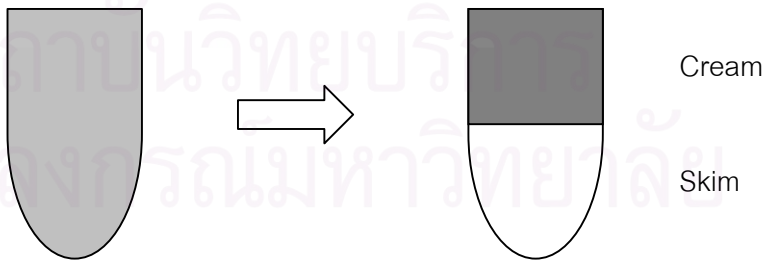


Figure 2.4 Creaming process of natural rubber latex.

2.2.2 Concentration by Centrifugation

Among the methods currently used for the concentration of natural rubber latex, centrifugation is the most important, about 90% of concentrated latex used in industrial is produced by centrifugation. Centrifugation is a type of accelerated creaming process, by which successful concentration can be obtained with a significant difference between with the density of the rubber particles that of the aqueous phase. The centrifuge concentrate is known as cream, and the dilute latex obtained as a by-product is known as skim. The production process of concentrated latex by centrifugation is shown in Figure 2.5.

Concentrated latex consists of approximately 60% DRC with a lesser amount of non-rubber substances. The most of rubber particles in this cream phase are large particles, which show a size distribution ranging from 0.1-0.3 μm [5]. The molecular weight of the rubber from cream phase is a typical bimodal molecular weight distribution, with peaks at MW of 2.0×10^{6} g/mol and 1.2×10^{5} g/mol [5].

The serum or skim latex contains a small amount of rubber particles, 4-10% DRC, with a high amount of non-rubber components, including amino acid, proteins, carbohydrates, organic acid, inorganic salts and nucleotidic materials [3]. The rubber particles in serum phase are small particles and show a size distribution in a range of 0.05-0.3 μm. The molecular weight distribution is a unimodal, with a peak at 1.0×10^{6}g/mol [5]. The $\overline{M}_n$ obtained by osmometry is higher than that of cream phase but no significant difference in the $\overline{M}_w$.

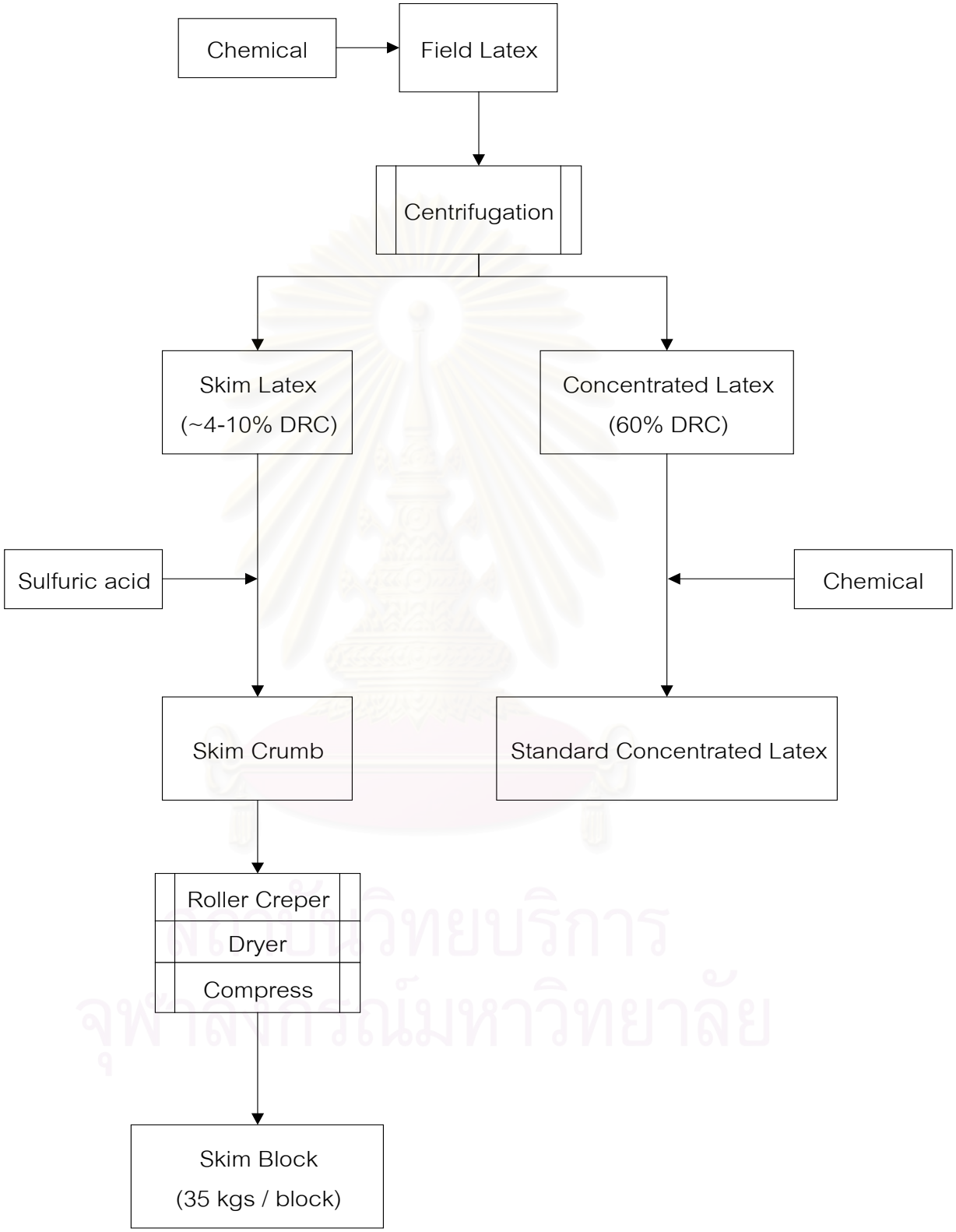


Figure 2.5 Commercial production of concentrated latex, skim latex and skim rubber[10]

2.3 Natural Rubber

2.3.1 Composition and Structure of Natural Rubber

Natural rubber (NR), obtained by coagulation of fresh latex contains over 90% rubber hydrocarbon with about 2% protein, 3% acetone-soluble resin, small amounts of sugars and little amounts of mineral matters. Natural rubber is a high molecular weight polymer composed of isoprene unit (Figure 2.6), C_5H_8, with *cis*-configuration linked each other by 1,4 addition in head-and-tail arrangement.

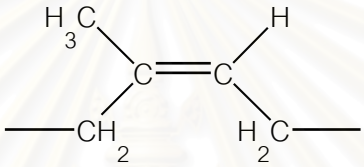
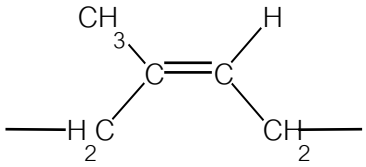
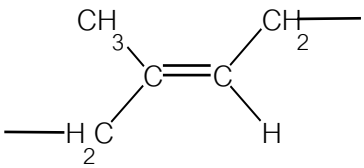


Figure 2.6 Isoprene unit.

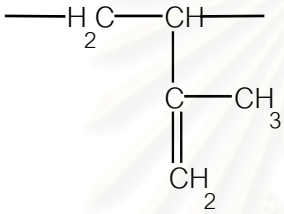
Synthetic polyisoprene was first produced in 1956, with a Ziegler type catalyst composed of titanium tetrachloride and alkylaluminum (Ti-Al) as well as alkyl lithium initiator. The former consists of essentially the same configuration as natural rubber, composed of mainly *cis*-1,4-polyisoprene. Initially, the Ti-Al catalyzed *cis*-polyisoprene was believed to have the same physical properties as natural rubber because of its structural resemblance. However, it was soon found to be a different rubber in terms of its physical properties, mechanical properties and processability. The differences can be described in part to the structural purity of polyisoprene chains and a particular component in natural rubber. By ^{1}H-NMR analysis, synthetic polyisoprene prepared with Ti-Al catalyst was found to be composed of the isoprene units with 99% *cis*-1,4, 0.3-1.0% 3,4 and 0-0.7% *trans*-1,4 unit (Figure 2.7). The later, polymerized by using an anionic polymerization with alkyllithium as an initiator consists of linear molecules with 90% *cis*-1,4, 4-5% 3,4 and 5-6% *trans*-1,4 unit. The 3,4 and *trans*-1,4 units are absent in natural rubber. The difference of properties between natural rubber and two types of synthetic *cis*-1,4 polyisoprene was shown in Table 2.2.



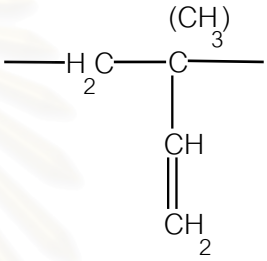
cis-1,4



trans-1,4



3,4



1,2

Figure 2.7 Isomeric units in synthetic polyisoprene.

Chemical and spectroscopic analyses found the presence of non-rubber component in natural rubber, such as protein, lipid, sugar, etc. It has been postulated that these non-rubber constituents are the causes for outstanding properties of natural rubber.

In fact, the natural rubber molecule is not a pure *cis*-1,4 polyisoprene. Besides, it contains very small amounts of functional groups in rubber chain termed as abnormal groups, such as aldehyde groups [22], ester or lactone group [23], and epoxides [24,25]. Structural studies using ^{13}C-NMR spectroscopy disclosed that the rubber molecule contains about two to three *trans* isoprene units [26]. Recently, detailed structure characterization of natural rubber was investigated by means of ^{13}C-NMR and ^{1}H-NMR spectroscopies [26,27]. From the relative intensity of the signal and the degree of polymerization of highly purified natural rubber, the number of *trans* isoprene existing at the initiating terminal of the rubber molecule is estimated to be two. Accordingly, the structure of natural rubber is assumed to be as shown in Figure 2.8.

Table 2.2 Difference of properties between natural rubber, Ti-Al and Li catalyzed synthetic *cis*-1,4 polyisoprene [28].

Properties	NR	Catalyst	
		Ti-Al	Li
Cold flow	Excellent	Excellent	Good
Green strength	Excellent	Poor	Worst
Green tack	Excellent	Excellent	Excellent
Role processability	Excellent	Excellent	Good
Injection processability	Excellent		
Press processability	Excellent	Excellent	Excellent
Calendar processability	Excellent	Excellent	Good
Vulcanization rate	Excellent	Excellent	Good
Tensile strength	~320	~300	~280
Tear strength	Excellent	Good	Good
Resilience	Excellent	Excellent	Excellent
Permanent set	Good	Good	Good
Abrasion	Good	Good	Good
Heat build-up	Excellent	Excellent	Excellent
Chipping	Good~ Excellent	Good	Poor
Cutting	Good~ Excellent	Good	Poor
Heat resistance (maximum, °C)	Poor (120)	Poor (120)	Poor (120)

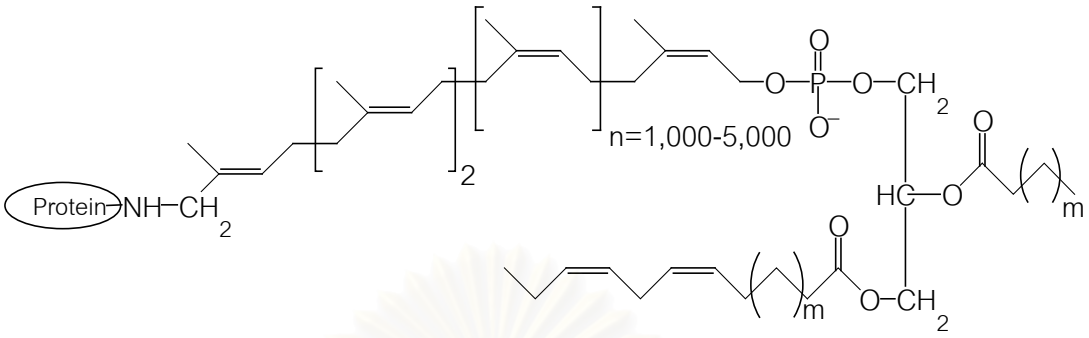


Figure 2.8 Presumed structure of natural rubber.

2.3.2 Molecular Weight and Molecular Weight Distribution

Hevea rubber is a polymer of very high molecular weight with broad molecular weight distribution (MWD). The broad MWD of *Hevea* rubber is presumed to be derived from the branching and crosslinking reaction by certain special functional groups in rubber molecule.

Osmometry, light scattering, solution viscometry and gel permeation chromatography have been commonly used to determine the molecular weight (MW) of rubber. MWD can be obtained by the analysis of fractionated samples, which are usually obtained by solvent fractionation techniques. Among these techniques gel permeation chromatography (GPC) is the most popular method for the determination of the MWD as well as MW of natural rubber.

By using GPC, It was found that the MWD of natural rubber in freshly tapped latex is bimodal [29]. MWD of various clonal rubbers was classified three types as shown in Figure 2.9.

Type A distinctly bimodal distribution with nearly equal peak height.

Type B bimodal distribution with small low molecular weight peak.

Type C skewed unimodal distribution with a shoulder in the low MW region

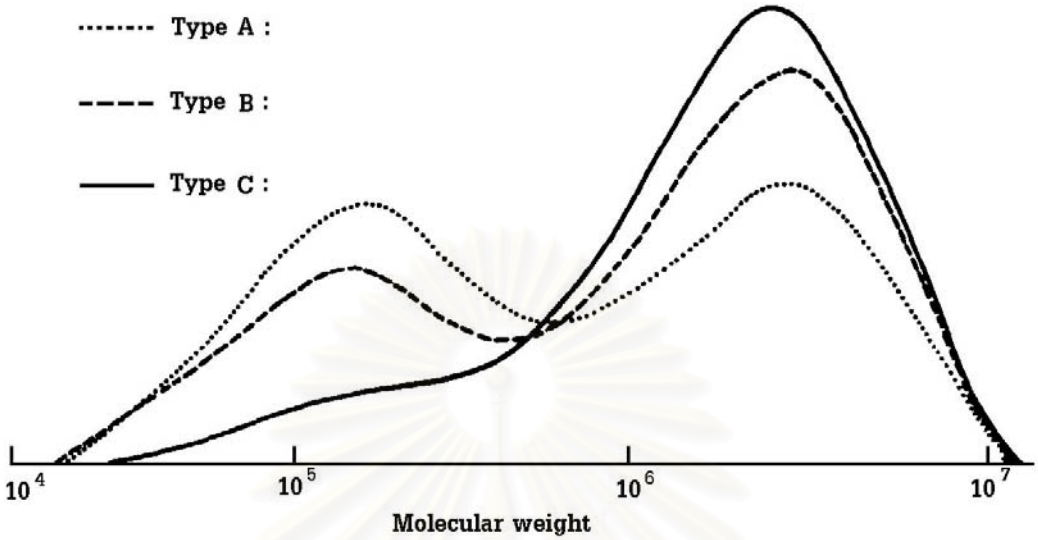


Figure 2.9 Type of molecular weight distribution curves of natural rubber [30].

The high molecular weight peak in MWD is centered at around 1×10^6 and 2.5×10^6, while it is around 1×10^5 - 2×10^5 for the low molecular weight peak. The average molecular weight is $\overline{M}_w$ = 1.6 - 2.3×10^6 and $\overline{M}_h$ = 2.0-5.2 $\times10^5$. The polydispersity index $\left(\overline{M}_w / \overline{M}_n\right)$ is extremely wide ranging from 2.5 to 10.

The molecular weight of natural rubber can be reduced by various factors such as mechanical or chemical mastication, exposure to sunlight and heat treatment. This is due to the degradation of rubber chain.

2.3.3 Branching and Gel Phase

There are two components called “sol” and “gel” in natural rubber. The sol phase is a rubber part that dissolves easily in good solvents such as cyclohexane, toluene, tetrahydrofuran (THF), etc., while the gel phase swells without dissolving.

Commercially available dry *Hevea* rubber contains 5-50% gel phase, depending on the clonal origin of the rubber, processing conditions and the time and temperature of storage. The true gel phase in natural rubber was presumed to consist of small crosslinked latex particles or “microgels” [31]. The microgel are combined into a matrix with the sol fraction and form an apparent gel phase, as shown in Figure 2.10

The gel phase in natural rubber contains nitrogenous and mineral components higher than the sol phase. This can be postulated that the gel phase is linked up with the network of proteins via hydrogen bonding. The gel content of rubber can be decreased by deproteinization and transesterification [32]. These treatments decompose the branching and crosslinks composed of protein and fatty acid ester group, respectively. This can be attributed to the fact that the branching and crosslinks are composed of two types of branch-points. One is presumed to be formed by the intermolecular interaction of proteins and another by phosphoric ester group and long-chain fatty acid ester group as illustrated in Figure 2.11.

In addition, the gel phase in *Hevea* rubber is sometimes classified to “loose gel” or “soft gel” and “tight gel” or “hard gel”. The soft gel is derived from various non-rubber components, which can be decomposed by chemical reaction such as enzymatic deproteinization, transesterification and saponification [32]. On the other hand, the hard gel is formed by crosslinking of unsaturated rubber chain, which can not be decomposed by chemical reaction.

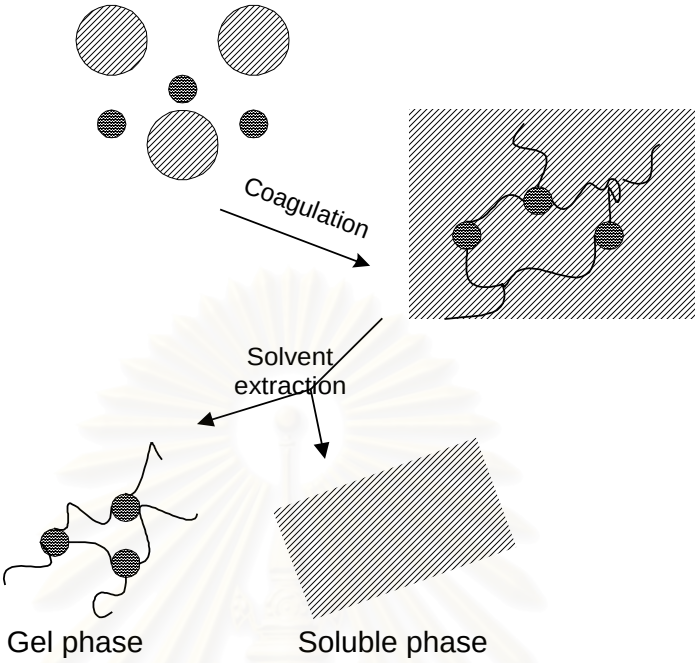


Figure 2.10 Schematic representation of gel phase in rubber [31].

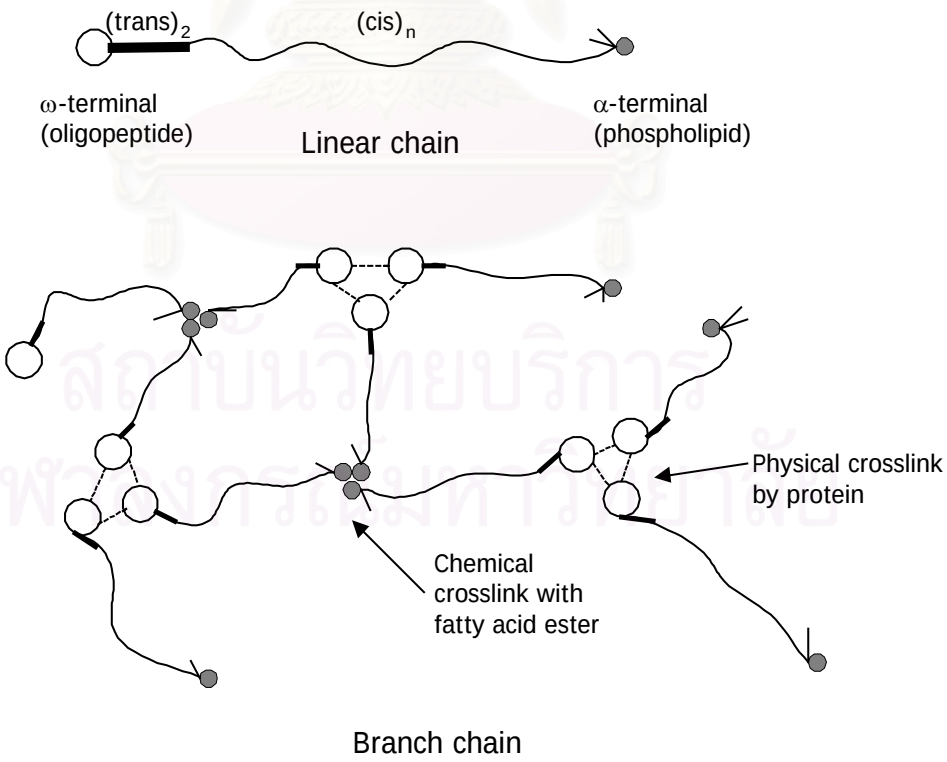


Figure 2.11 Presumed structure of braching and crosslinking in NR [32].

2.4 Skim Rubber

Skim rubber obtained from skim latex contains 75-85% rubber hydrocarbon with 9-18% proteins and 5-10% acetone-soluble material. Skim rubber, which has not been purified, contains a much higher proportion of natural non-rubber substances than normal rubber. This is due to the fact that skim rubber is mostly derived from small rubber particles with a high specific surface. Impurities such as protein have a higher specific gravity than rubber and migrate to the serum fraction during centrifuging. Skim rubber is of little value due to the presence of non-rubber components.

Actually, skim rubber composed of linear rubber molecule with no phospholipid groups at the terminal end. Thus, it contains almost no branching and gel [5]. Rubber molecules in skim rubber also show the lower nitrogenous compounds attached to rubber molecules. In addition, skim rubber shows a unimodal molecular weight distribution, which differs from the ordinary rubber as illustrated in Figure 2.12. It also shows low green strength compared to the ordinary NR due to the low fatty acid ester content. Furthermore, the natural antioxidant in skim rubber was assumed to be absent [5].

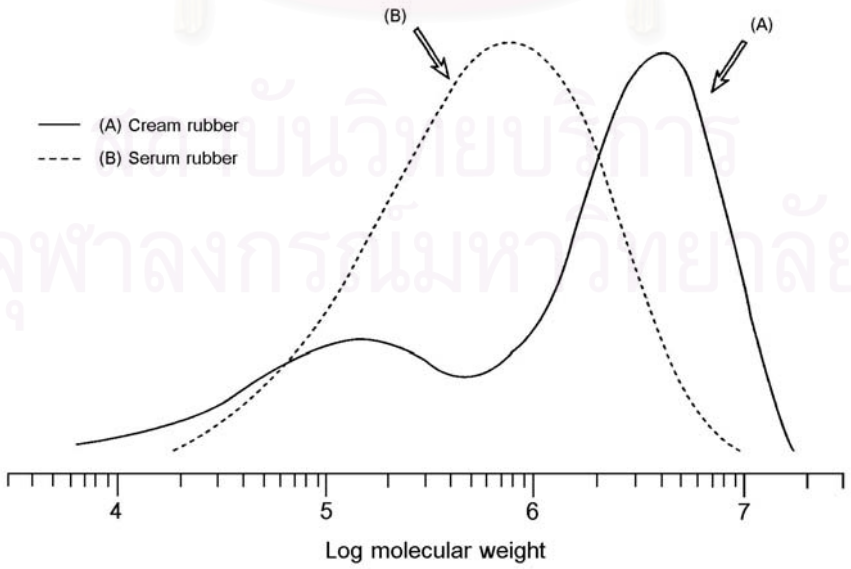


Figure 2.12 Molecular weight distribution of skim rubber and ordinary rubber [5].

2.5 Latex Allergy

Natural rubber latex products have one serious drawback. Many people are allergic to them. The rubbers produced from NRL contain latex protein that makes people itch and others burn with rashes. In 1991, the US Federal Food and Drug Administration (FDA) issued a medical alert regarding to the allergenicity of NRL products [33], suspecting that the natural proteins embedded in latex are the prime antigenic candidates responsible for inducing allergic reaction. The medical alert recommended to remove water-soluble proteins or to protect the surface of latex-products not to elute proteins was announced. These proteins, known as extractable proteins, may be absorbed through skin, carried by lymph or in the blood stream. An immune system response then occurs.

There are two types of allergy caused by NRL products. One is type I or immediate-type. The other is type IV or delayed-type hypersensitivity (DTH). Type I (IgE-mediated immediate) allergic reactions arising from contact from NRL products have been attributed to latex proteins leached from their surface [34]. This type occurs within an hour of exposure to NRL products. The contact urticaria syndrome includes localized urticaria, angiedema, ashma, and sometimes resulted in an anaphylactic shock. Type IV allergic reactions occurred as a result from some chemicals added to NRL during manufacturing such as 1,2-benzisothiazolin-3-one [35], epichlorohydrin, and sorbic acid [36].

The allergens, which causes type I allergy, were assumed to be residual water-soluble proteins having molecular weights of 14 kDa to 30 kDa. Hasma [37] found that proteins of HA latex concentrate were fractionated in two main fractions. One contained six serum proteins and the other consisted of two rubber-particle membrane proteins. The serum proteins had MW of 14, 24, 29, 36, and 45 kDa and another protein of MW greater than 100 kDa. It is expected that these proteins, which are present in aqueous extracts of NRL product are the most readily leached out with water. The proteins associated with the rubber particle had MW of 14 and 24 kDa. The 14-kDa

protein was tightly bound to the rubber particle and expected to remain in the leached latex products.

The main problem at present is that the amount of proteins cause type I allergy is not known exactly. Yip et al [38] showed that there is good correlation between total extractable protein (TEP) and allergic reactions. Although it has not been possible to determine a lowest level of TEP, which can cause an allergic reaction, their results demonstrate that at TEP levels of 400 μg/g, only 60% of the 59 latex sensitized subjects showed an allergic reaction. They concluded that for higher negative responses, TEP content should be less than 100 μg/g.

2.6 Literature Reviews

J. T. Sakdapipanich et al. [5] has studied the structural characteristic of the small rubber particles in the serum phase in fresh Hevea latex compared with the large rubber particles in the cream phase. The fresh latex was centrifuged into the serum and cream phases. The rubbers from both fractions were recovered and characterized for the particle size distribution, nitrogen, fatty acid ester and gel contents, MW and MWD. The rubber particle in serum phase had a mean diameter of 0.13 μm. The small rubber particles in serum phase consisted of the rubber molecules showing a unimodal MWD and was presumed to be linear molecules due to the absence of phospholipid in the terminal end.

G. M. Bristow [39] has studied the composition and cure behaviour of skim block rubber. Eight skim block rubber with various properties, such as nitrogen content, ash content, dirt content were investigated for cure behavior and aging resistance. The results revealed that the high levels of protein presented in skim rubber could be responsible for cure behaviour. The modulus of skim rubber was higher than that of DPNR, SMR 20, and SMR L due to the high nitrogen content of skim rubber. While, fatty acid adding to skim rubber had no effect to cure behaviour. The high modulus of skim rubber took an advantage

in blends of skim rubber with ordinary rubber. Skim rubber contained much higher copper content than normal rubber but this did not result in low PRI or aging resistance of skim rubber. Skim rubber can be added to normal rubber up to 20 phr without addition of further curative: with retaining of a high level of vulcanisate properties.

B. Nithi-Uthai et al. [40] has studied the change of stability of skim latex during storage. It was found that the aged skim latex sometimes was not coagulated by the addition of acid. To solve this problem, the addition of some chemicals or some surfactants to skim latex prior to the addition of acid was made. In the case of the old skim latex, the addition of polyelectrolytes is required. The process of deproteinized skim latex by the addition of enzyme could coagulate the old skim latex.

Dunlop Rubber Co, Ltd. [41] invented a method for recovering rubber from skim latex. The method involved the digestion of skim latex with 0.01% trypsin for 12 hours at 28 $^{\circ}$C with bactericides. Coagulation of skim rubber was made by adding formic acid. The coagulum was separated, macerated on rolls with water, crepe, and dried in the normal way. The purified skim rubber was similar in color to normal crepe rubber and had nitrogen content of 0.42. The recovery of 99% of skim rubber was achieved.

The Firestone Tire & Rubber Company [42] patented a method for producing a high-grade rubber from skim latex, which was obtained from creaming process. Skim latex was allowed to spontaneously coagulation. The coagulum was milled to the fine coagulum and treated with 2.75% aqueous sodium hydroxide solution for 20 hours. The resulting treated skim crumb rubber was washed, dried and baled. The nitrogen content of rubber was reduced from 1.98% to 0.24%. The water extractable fraction was also decreased from 2.34% to 0.63%. The physical properties of compounded purified skim rubber were similar to a high-grade commercial rubber.

A. Rungvichaniwat et al. [43] has studied the color and nitrogen content of purified skim rubber, prepared by saponification of skim latex or skim rubber crumbs

with sodium hydroxide. The wet skim crumbs soaking technique was more effective in the reduction of the nitrogen content than the saponification in latex at the same condition. Sodium hydroxide concentration of 3% was the appropriate condition for saponification time of 24 hours at room temperature. The higher sodium hydroxide concentration for treated skim latex gave the less nitrogen content of skim rubber and with no change on the color of purified skim rubber, which was around 3-5 Lovibond unit.

K. Jayachandran et al. [44] has studied the coagulation skim rubber from skim latex by inoculation of skim latex with an *Acinetobhacter sp*, which was isolated from latex centrifugation effluent. Skim latex diluted with water of 1:10(v/v) was incubated with an inoculum concentration of 6.4 mg dry cell/ml at 28°C. After coagulation, dry rubber content (DRC) of skim rubber was 8%(w/v) and the COD of the residual solution was 0.4 μg/L. On the other hand, chemical coagulation at the same dilution resulted in 7%(w/v) yield of dry rubber content and 2.2g COD/L.

Y. Tanaka et al. [45] patented the method of producing particulate natural rubber from skim latex. An inorganic salt was added to skim latex obtained by centrifugation of natural rubber latex in an amount enough to cause phase separation of the particulate natural rubber component in the serum. Inorganic salt used in this work comprised of sodium carbonate, diammonium hydrogenphosphate, sodium sulfate, potassium carbonate, sodium chloride, ammonium sulfate etc. The separation as cream phase was investigated in three cases: 1) phase separation of skim rubber in the form of latex by adding an inorganic salt to the serum of high ammonia latex. 2) phase separation of deproteinized skim rubber in form of latex by adding an inorganic salt and 0.04% protease. 3) phase separation of deproteinized skim rubber in the form of latex by adding an inorganic salt to the serum of a deproteinized natural rubber latex. The average particle diameter of recovered particulate skim rubber was increased after the incubation for 24 hours. The nitrogen content in case of deproteinized skim rubber was reduced to about 0.017-0.019%.

J. Tangpakdee et al. [32] has studied the component and structural changes in natural rubber after purification by enzymatic deproteinisation, transesterification with sodium methoxide and saponification with potassium hydroxide. Enzymatic deproteinization decomposed the protein linkages, while transesterification decomposed the phospholipid ester linkages, and resulted in the linear molecules. Furthermore, both of the protein and phospholipid linkages were removed by saponification to form the linear molecules. The branch-points were assumed to be composed of phospholipid due to the decrease of gel content and molecular weight by means of transesterification and saponification.

P. Klinpituksa et al. [46] has studied the preparation and properties of DPNR (Deproteinized natural rubber). Fresh NRL of RRIM 600 was digested with 0.2 phr alcalase enzyme in the presence of 0.4% sodium dodecyl sulfate at pH 7.5 and 37°C for 20 hours. The centrifugation speed of treated latex was studied. The results showed that at speed 10,000 rpm for 3 times and a period 30-min was the appropriate condition for removing the nitrogen content. The nitrogen content of that purified rubber decreased from 0.415% to 0.024%. The particle size distribution of deproteinized natural latex with average particle size 0.5 μm was the same as the original latex. The DPNR from purified latex showed the lower ash content, viscosity, plasticity and water absorption than those of original rubber or STR 5L. The vulcanisates of DPNR, however, showed higher cure time and elongation at break, while tensile strength, tear strength, modulus and hardness were lower than those of both rubbers.

CHAPTER 3

EXPERIMENTAL

3.1 Materials and Chemicals

3.1.1 Raw Materials

Skim latex and solid skim rubber were obtained from N.Y. Rubber Company in Chonburi, Thailand. Skim rubber from skim latex was obtained by coagulation of skim latex with 2% (v/v) formic acid, washed with water and purified by precipitation from toluene solution into methanol.

Solid skim rubber was of commercial grade, which was obtained from skim latex with 7% (v/v) sulfuric acid and dried up in oven at 100°C. The solid skim rubber was used as received.

3.1.2 Chemicals

No.	Chemicals	Supplier	Grade
1.	Acetic acid (CH_3COOH)	BDH	Analytical
2.	Acetone (C_3H_6O)	SR Lab	Commercial
3.	Boric acid, ortho (H_3BO_3)	BDH	Analytical
4.	Chloroform ($CHCl_3$)	BDH	Analytical
5.	Chloroform, deturated ($CDCl_3$)	Aldrich	Analytical
6.	Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$)	BDH	Analytical
7.	Formic acid, 99% (HCOOH)	APS Ajax Fine chem	Analytical
8.	Methanol (CH_3OH)	SR lab	Commercial
9.	Molecular sieves type 4A (2.5-5.5 mm)	APS Ajax Finechem	Laboratory

No.	Chemicals	Supplier	Grade
10.	Methyl stearate, specially pure ($CH_3(CH_2)_{18}COOCH_3$)	BDH	Laboratory
11.	Proteolytic enzyme (KP-3939)	Kao	
12.	Dry yeast	Fleischmann	Food
13.	Phosphoric acid, 85% (H_3PO_4)	APS Ajax Finechem	Analytical
14.	Potassium sulphate (K_2SO_4)	APS Ajax Finechem	Analytical
15.	Potassium hydrogen phthalate ($COOH{\cdot}C_6H_4{\cdot}COOK$)	BDH	Analytical
16.	Sodium chloride (NaCl)	BDH	Analytical
17.	Sodium hydroxide (NaOH)	BDH	Analytical
18.	Selenium powder (Se)	Fluka	Analytical
19.	Sodium dodecyl sulfate (SDS) ($CH_3(CH_2)_nOSO_3Na$)	APS Ajax Finechem	Analytical
20.	Synthetic cis-1,4-polyisoprene		
21.	Tetrahydrofuran, (THF)	APS Ajax Finechem	Analytical
22.	Sodium carbonate, anhydrous (Na_2CO_3)	BDH	Analytical
23.	2,6-di-tert-butyl-p-cresol (BHT) $[(CH_3)_3C]_2C_6H_2(CH_3)OH$	BDH	GPR

3.2 Equipment and Instruments

1.) 500-cm^3 reactor flask
2.) Mechanical stirrer motor : HEIDON 1200G
3.) Magnetic stirrer hotplate : Ika Werker RW20, Germany
4.) General glasswares and equipments
5.) Centrifuge : Backman model J2-21, USA
6.) Mastersizer-S analyzer : Malvern, England

7.) Total nitrogen analyzer : Gerhalt, Germany

8.) FTIR spectrophotometer : Nicolet (Impact 410), England

9.) Gel-permeation chromatograph

Column : styrene-divinylbenzene copolymers (exclusion limits of 2.0×10^7 and 5.0×10^4)

Detector : refractive index detector (TOSOH LS-8000)

10.) Wallace test equipment : Rapid Plastimeter MK.11

11.) Mooney viscometer : TECHPRO

12.) Tensile tester : LLOYD (LR 5K), England

3.3 Recovery and Purification Method

3.3.1 Enzymatic Deproteinization of Skim Latex in the presence of Sodium Chloride

3.3.1.1 Effect of Sodium Chloride Concentration

200 cm^3 of skim latex in 250-cm^3 glass beaker was incubated with 0.04% (w/v) proteolytic enzyme (KP-3939, Kao) at 30°C. The latex was gently stirred with magnetic stirrer. Sodium chloride was slowly added to the latex to make 0, 1, 3, 5, 7, and 9% (w/v) concentration. The reaction was allowed to proceed for 3 h. The deproteinized latex was allowed to stand at 30°C for 48 h. After phase separation occurred, the separation ratio was evaluated from the following expression.

$$\text{Separation ratio} = \frac{\text{The height of original skim latex}}{\text{The height of cream phase}} \qquad (3.1)$$

The resulting cream phase was separated from the water phase and the particle size distribution was determined. The cream phase was coagulated with methanol and dried under vacuum. The dried rubber was analyzed for ash content.

Before other testing, the rubber was precipitated from toluene solution into the mixture of acetone/methanol (50/50). The precipitated rubber was then dried under vacuum. The nitrogen, ester contents, and molecular weight and molecular weight distribution of the purified skim rubber were investigated.

3.3.1.2 Effect of Washing by Centrifugation

200 cm^3 of skim latex in 250-cm^3 glass beaker was incubated with 0.04% (w/v) proteolytic enzyme (KP-3939, Kao) at 30°C. The latex was gently stirred with magnetic stirrer. Sodium chloride was slowly added to the latex to make 0, 1, 3, 5, 7, and 9% (w/v) concentration. The reaction was allowed to proceed for 3 h. The deproteinized latex was allowed to stand at 30°C for 48 h. After phase separation occurred, the separation ratio was determined according to the equation 3.1.

After the occurrence of phase separation, The resulting cream phase was separated from the water phase and diluted with water to the original volume. Sodium dodecyl sulphate was added to the diluted cream phase to make 1% (w/v) solution. The purified latex was centrifuged at 10,000 rpm for 30 minutes. The cream phase was separated and diluted with water, coagulated with methanol and dried under vacuum. The dried rubber was analyzed for ash and gel contents. Before other testing, the rubber was precipitated from toluene solution into the mixture of acetone/methanol (50/50). The precipitated rubber was then dried under vacuum. The nitrogen, ester contents, and molecular weight and molecular weight distribution of the purified skim rubber were investigated.

3.3.2 Deproteinization of Skim Latex by Saponification with NaOH

3.3.2.1 Effect of Sodium Hydroxide Concentration

200 cm^3 of skim latex in 250-cm^3 glass beaker was saponified with 1, 2, 3, 4, and 5% (w/v) sodium hydroxide. Saponification was carried out at 50°C for 5 h slowly stirring with magnetic stirrer. The purified latex was incubated at room temperature for 24 h without stirring. After phase separation occurred, the separation ratio was determined. A part of the resulting cream phase was subjected to the determination of particle size. The residual cream phase was coagulated with methanol, dried under vacuum and subjected to the analysis of ash content. The rubber was precipitated from toluene solution into the mixture of acetone/methanol (50/50) and dried under vacuum. The nitrogen and ester contents of purified skim rubber were analyzed.

3.3.2.2 Effect of Washing by Centrifugation

200 cm^3 of skim latex in 250-cm^3 glass beaker was saponified with 1, 2, 3, 4, and 5% (w/v) sodium hydroxide. After saponification at 50°C for 1 hour, sodium chloride was added to give a solution of 1% (w/v) concentration. The saponification was further done for 4 h. The purified latex was incubated at room temperature for 24 hr. Phase separation occurred after 24 h. The resulting cream phase was subjected for the determination of the particle size distribution. After washing by centrifugation in the presence of 1% (w/v) SDS at 10,000 rpm for 30 minutes, the washed-cream phase was coagulated with methanol and dried under vacuum. The ash and gel contents of purified rubber was determined. The rubber was precipitated from toluene solution into the mixture of acetone/methanol (50/50) and dried under vacuum. The nitrogen, ester contents and MW and MWD of purified skim rubber were investigated.

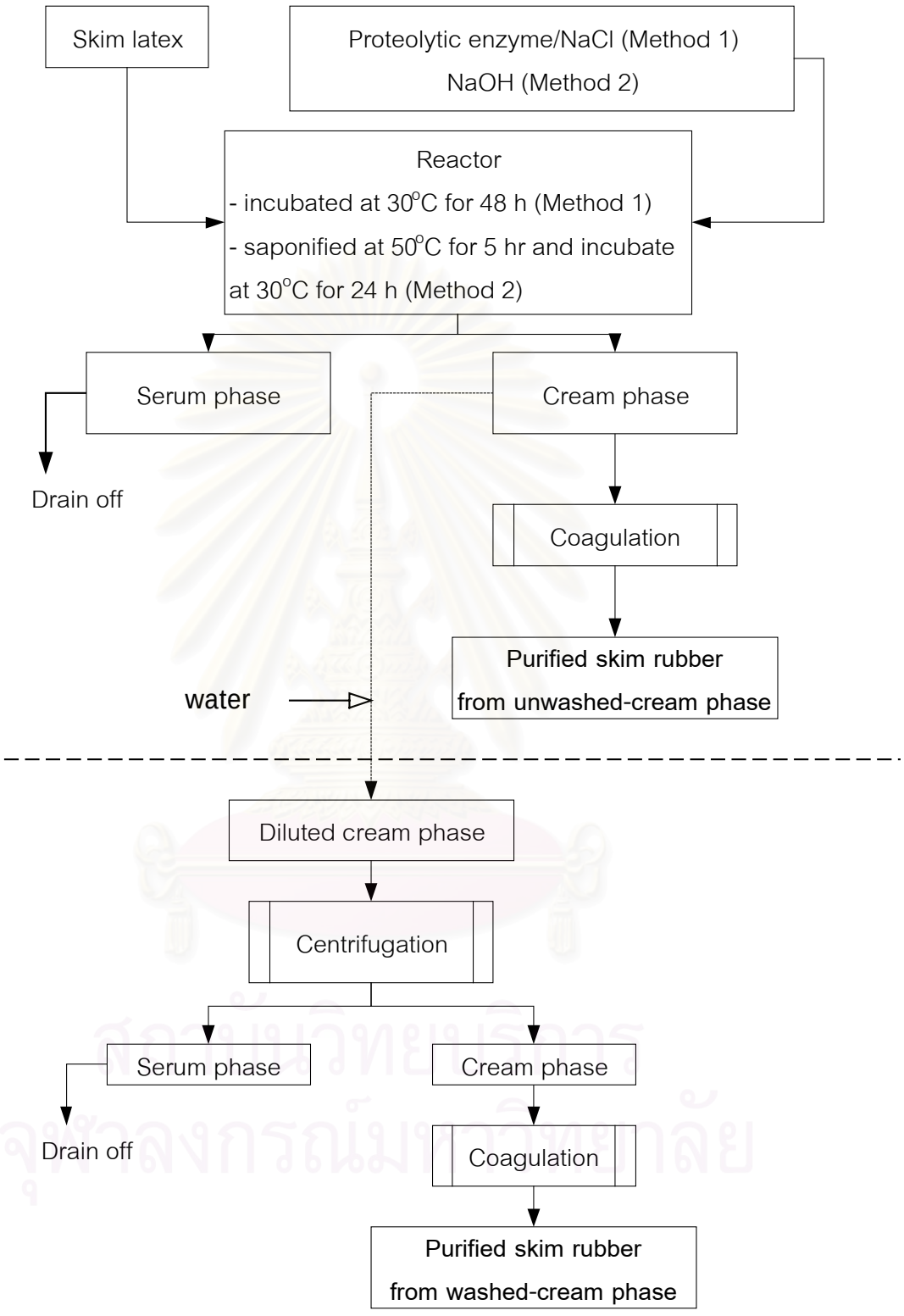


Figure 3.1 Diagram of DPNR and SAP skim rubber productions.

3.3.3 Incubation of Skim Latex with Baker's Yeast

3.3.3.1 Effect of pH

Skim latex (3.5%DRC, pH 10.2) of 200 cm^3 was adjusted to pH 7, 8, and 9 with 10% phosphoric acid in a 400 cm^3 glass beaker. The skim latex of 200 cm^3 was incubated by the addition of a Baker's yeast of 1 g at room temperature (30 °C) for 48 h. The control latex of 3 cm^3 was prepared for each pH sample into a small glass vial and kept at room temperature (30 °C) for 48 h.

After incubation, the particle size distribution was determined for both control and the yeast-treated skim latex. The yeast-treated skim latex was centrifuged at 10,000 rpm for 30 minutes.

3.3.3.2 Effect of Yeast Concentration

Sodium dodecyl sulphate solution (20%, w/v) was added to the skim latex of 200 cm^3 before adjusting pH of skim latex to 7 with 10% phosphoric acid. Control latex of 3 cm^3 was prepared into a small glass vial and kept at room temperature (30 °C) for 48 h. The Beaker's yeast was slowly added to a 200 cm^3 latex to give the concentration of 0.10, 0.25, 0.5, and 1.0% (w/v) without stirring. After soaking the yeast with latex for 5 minutes, the latex was slowly stirred with magnetic stirrer for 3 h and incubated without stirring at room temperature. After 48 h, the particle size distribution of the control and yeast-treated latex was determined.

The yeast-treated skim latex was centrifuged at 10,000 rpm for 30 minutes. The resulting cream phase was separated, coagulated with methanol and dried under vacuum. The nitrogen content of yeast-treated rubber was determined. The resulting serum was determined for dry rubber content and recovery percent was calculated by the following equation:

$$\%\text{recovery} = \left[1 - \frac{\text{DRC of serum phase}}{\text{DRC of original latex}}\right] \times 100 \qquad (3.2)$$

3.3.3.3 Combination of Incubation of Skim Latex with Yeast and Saponification

The skim latex of 200 cm^3 was adjusted pH to 7 with 10% phosphoric acid. The Beaker's yeast was slowly added to a 200 cm^3 latex to give the concentration of 0.5% (w/v) without stirring. After soaking the yeast with latex for 5 minutes, the latex was slowly stirred with magnetic stirrer for 3 h and incubated without stirring at room temperature. After 48 h, the cream phase was separated from the serum phase and saponified with 1, 2 and 4% (w/v) NaOH at 50°C for 3 h. The saponified-cream phase was coagulated with formic acid, dried under vacuum and subjected to the analysis of ash content. The rubber was precipitated from toluene solution into the mixture of acetone/methanol (50/50) and dried under vacuum. The nitrogen content of purified skim rubber was analyzed.

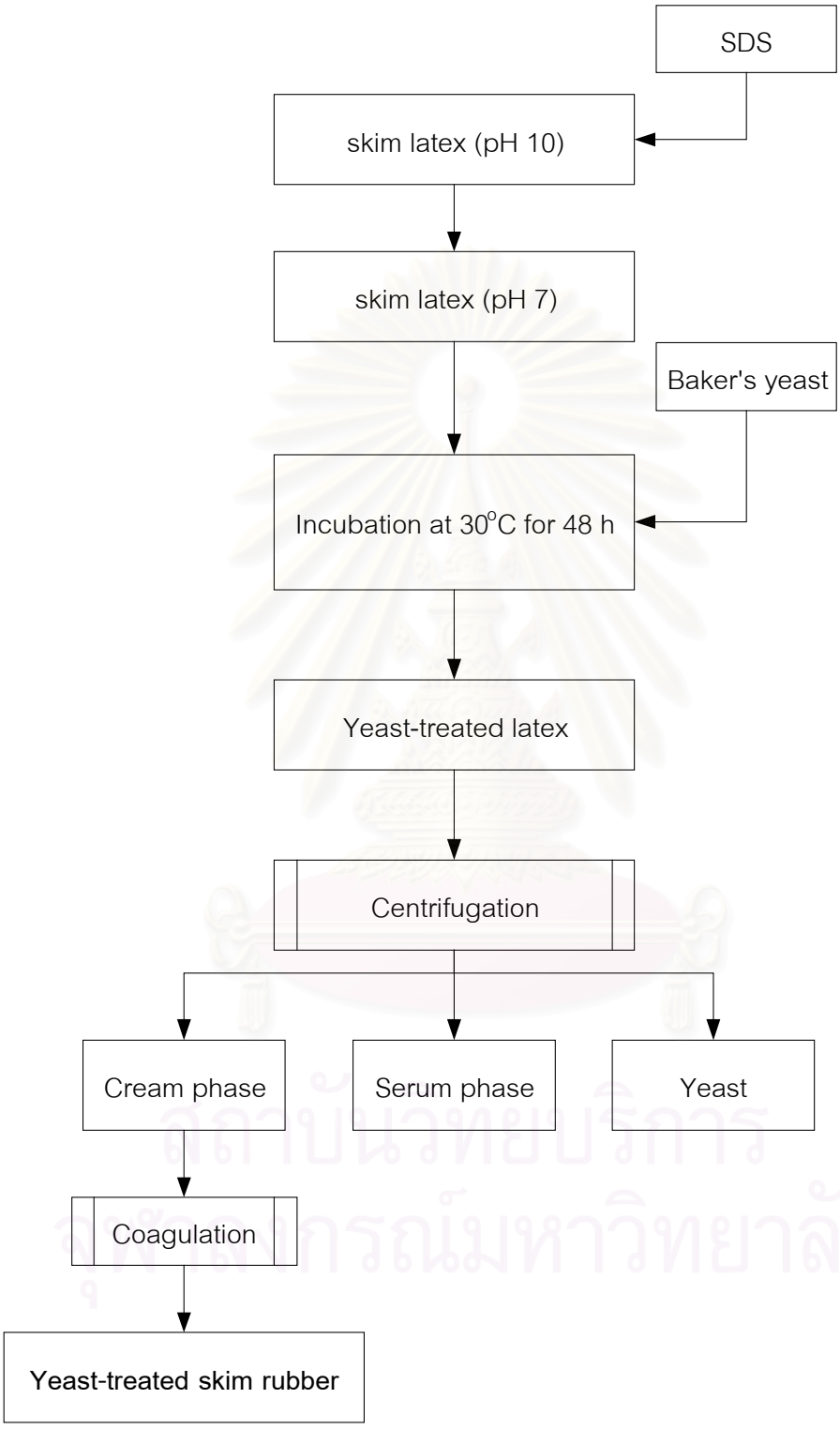


Figure 3.2 Diagram of the recovery of skim rubber by the incubation with yeast.

3.3.4 Saponification of Solid Skim Rubber in Toluene Solution

3.3.4.1 Effect of Rubber Concentration in Solution

Solid skim rubber was dissolved in 250 cm^3 toluene to make 6, 10, 12, and 15% (w/v) rubber solution in 500-cm^3 reactor flask with the mechanical stirrer. 100 cm^3 of 5% (w/v) sodium hydroxide solution was added to the rubber solution, which was continually stirred at 70°C for 3 h. The saponified rubber solution was washed with 100 cm^3 distillated water at 60°C for 6 h 3 times with stirring and the salts involved in the saponified rubber was washed. The resulting rubber solution was coagulated with methanol and dried under vacuum.

3.3.4.2 Effect of Sodium Hydroxide Concentration

Skim rubber solution of 10% (w/v) rubber concentration was saponified with various amounts of 5% (w/v) sodium hydroxide as follows:

Volume of NaOH (ml)	Concentration in solution (%, weight / volume)
100	1.43
80	1.21
60	0.97
40	0.69
20	0.34
5	0.10
0	0.00

Saponification was done at 70°C for 3 h. Washing and coagulation processes were the same as the method that mentioned above.

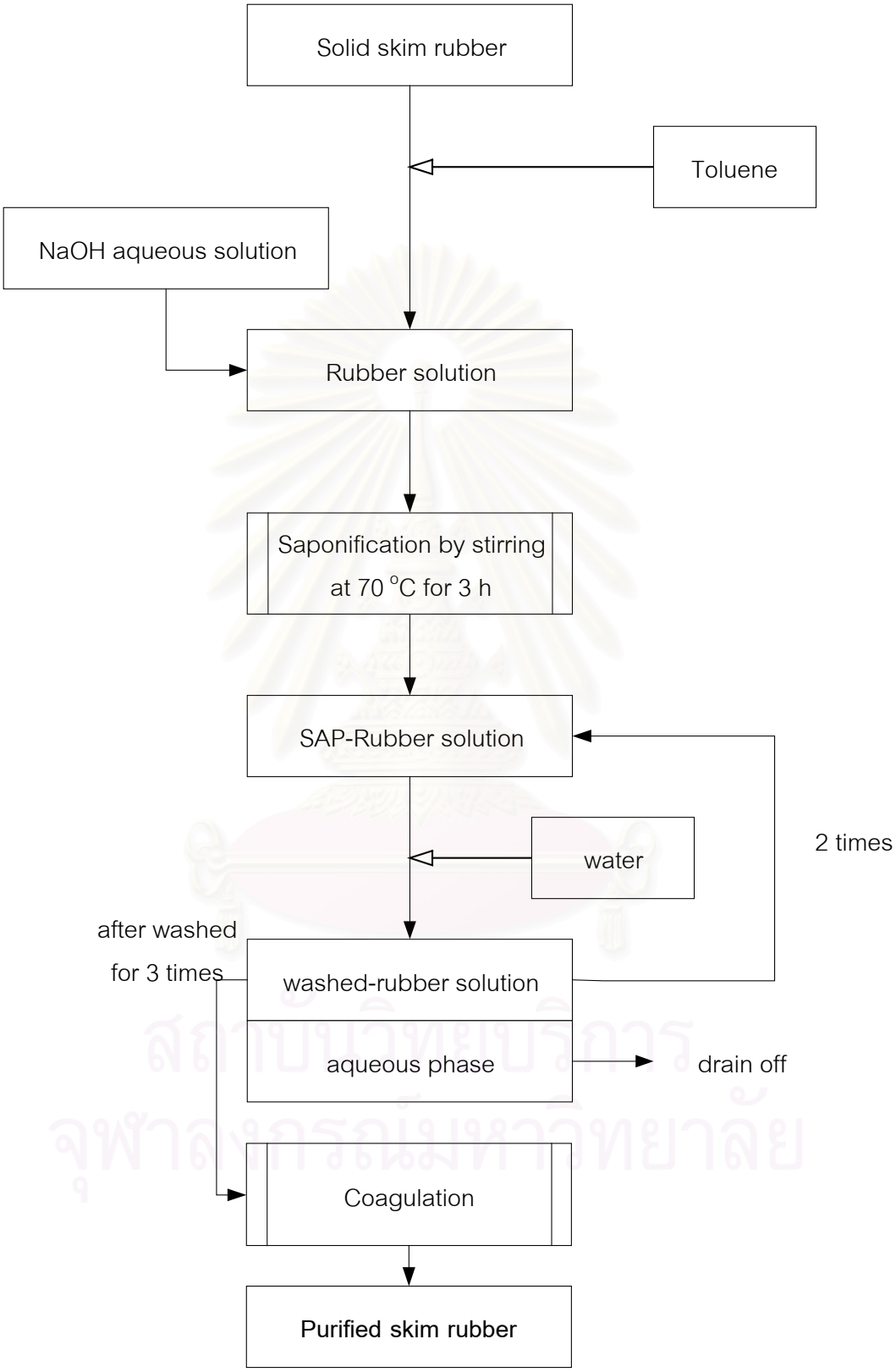


Figure 3.3 Diagram for production of purified skim rubber from solid skim rubber.

3.4 Characterization of Skim Latex and Skim Rubber

3.4.1 Determination of Total Solids Content (TSC), Dry Rubber Content (DRC) and pH

Total solids content and dry rubber content of skim latex were determined according to ASTM D-1076 test method [47] as described in Appendix A.

The pH of skim latex was measured with a pH meter, by calibration with pH buffer 7.0 and 4.01.

3.4.2 Measurement of Particle Size and Particle Size Distribution

The particle size and particle size distribution of rubber particle in skim latex was measured with a Mastersizer-S analyzer using Laser-scattering technique.

Rubber particles in the latex were dispersed with deionized water in 100-cm^3 sample chamber (Small Volume Mode). Laser source, He-Ne laser, generates the beam at wavelength 632.8 nm. The particle size and particle size distribution can be measured in the range of 0.05-380 μm with the uncertainty less than 5%.

3.4.3 Determination of Nitrogen Content

The nitrogen content of rubbers was analyzed using the modified Kjeldahl method, according to RRIM Test Method B7 [48]. The measurement was made with an automatic Kjeldahl analyzer (TT215 Gerhalt, Germany). The principle and procedure of this measurement was described in Appendix B.

3.4.4 Determination of Ash Content

The ash content of rubber samples was analyzed according to RRIM Test Method B6 [48], as described in Appendix B.

3.4.5 Determination of Ester Content

Methyl stearate was used as a model compound for the FTIR analysis of ester groups in natural rubber. The absorbance of the carbonyl groups was measured based on a calibration curve obtained by using a series of mixtures of methyl stearate and synthetic *cis*-1,4-polyisoprene (Carliflex-305). The FTIR analysis was performed using a Nicolet FTIR spectrometer Model Impact 410.

The rubber samples for FTIR analysis were prepared by casting 1% (w/v) rubber solutions in chloroform on a NaCl disk placed on silica gel and dried under a stream of nitrogen gas. The resulting round transparent film of about 1-1.5 cm in diameter on NaCl plate was subjected to FTIR analysis. The spectrum was obtained by 300 scans at a resolution of 4 cm^{-1} with auto gain from 4000-500 cm^{-1}.

The intensity ratio of peaks at 1738 cm^{-1} (C=O) and 1664 cm^{-1} (C=C), (A_{1738}/A_{1664}), was plotted against the concentration of total ester groups in the rubber to get a calibration curve. The data for calibration curve was shown in Appendix C.

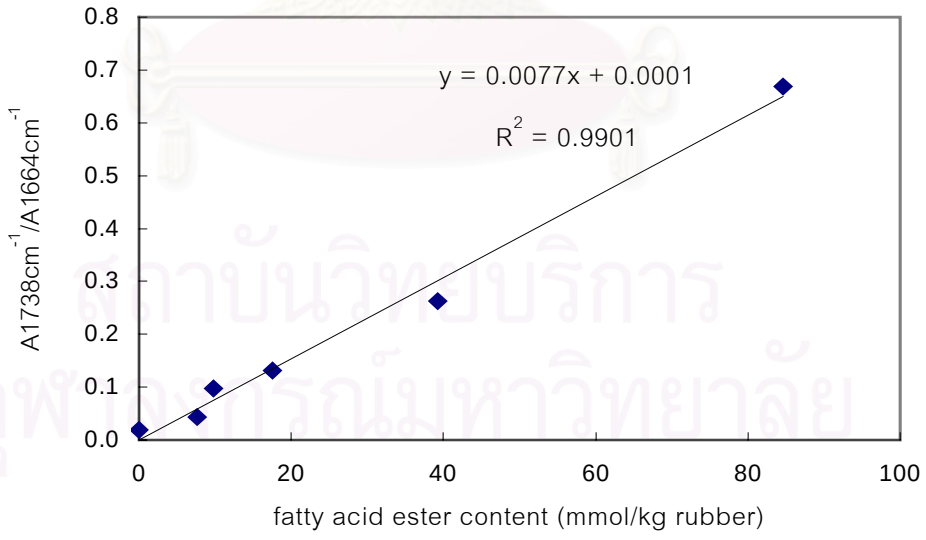


Figure 3.4 Calibration curve for ester content determination

The rubber samples, containing unknown amounts of ester groups, were casted according to the procedure mentioned above for the measurement of FTIR spectrum. The ester content of rubber samples was obtained the following expression:

$$\text{Ester content (mmol/kg rubber)} = \frac{\left(A_{1738} / A_{1664}\right) - 0.0001}{0.0077} \qquad (3.3)$$

3.4.6 Determination of Gel Content and Toluene-Soluble Fraction

Toluene of 10 cm^3 dried on activated 4A molecular sieves, was added to rubber sample cut into small pieces to make 0.2% (w/v) rubber solution and kept in the dark at room temperature without shaking or stirring for one week. The rubber solution was separated from the gel fraction by centrifugation at 3,000 rpm for 45 minutes and filtration through 300-mesh stainless steal gauze. The gel was washed with methanol and dried under vacuum at room temperature. The gel content and toluene-soluble fraction were calculated from the following expression:

$$\text{Gel content, \%} = \frac{\text{weight of gel}}{\text{weight of original rubber}} \times 100 \qquad (3.4)$$

$$\text{Toluene - soluble fraction, \%} = 100 - \left(\text{\%, gel content}\right) \qquad (3.5)$$

3.4.7 Determination of Molecular Weight and Molecular Weight Distribution

The molecular weight distribution of rubber samples was determined by gel-permeation chromatography (GPC) using two columns in series, packed with styrene-divinylbenzene copolymers, having exclusion limits of 2.0×10^{7} and 5.0×10^{4}. Measurements were made using THF as eluent with a flow rate of 0.5 ml/min at 35°C, monitored with a TOSOH LS-8000 refractive index detectors. Commercially obtained standard polystyrenes were used for the calibration. Purified and original rubbers, at a concentration of 0.1-0.3 mg/cm^3 in THF after filtering through a Millipore LS prefilter and 0.22-micron membrane filter, were subjected to GPC analysis.

A calibration curve is obtained by plotting the molecular weight against the elution volume. Since narrow fraction natural weight *cis*-1,4 polyisoprene standards are not available, the narrow fraction polystyrene standards was used instead. When tetrahydrofuran was used as an eluent, the following empirical equation was used to convert the polystyrene molecular weights into natural rubber molecular weights of equivalent coil size [30]:

$$\log M_{PI} = 0.185 + 0.950 \log M_{PS} \qquad (3.6)$$

3.4.8 NMR measurement

The ^{13}C-NMR measurements were carried out on $CDCl_3$ solutions of rubber with tetramethylsilane (TMS) as an internal standard using an Avance DPX-400 NMR spectrometer, operating at 250 MHz. The spectra were obtained with sweep width 25,000 Hz at 50°C.

3.4.9 Determination of Raw Rubber Properties of Rubber

3.4.9.1 Measurement of Stress-Strain Curve

Sample for the measurement of the green strength was prepared by dissolving the dried rubber in toluene to make a 5%(w/v) solution. The rubber solution was cast into film of approximately 0.5-mm thickness and dried at room temperature for at least 48 h. The cast films were stamped with a compress air sample cutter (Model SDAP-100-N) using a dumbbell die “A” (ASTM D412). The stress-strain curve of the unvalcanized rubber samples was measured with a LLOYD LR5K tensile tester at the following conditions:

Test speed	:	500 mm/min
Gauge length	:	25 mm
Load cell	:	500 N

3.4.9.2 Measurement of Wallace Plasticity (P_o), Plasticity Retention Index (PRI), Mooney viscosity, and Color

Rubber samples for the measurement of Mooney viscosity, P_o, PRI, and color were prepare by passing a rubber piece through the gap between the rolls of a 150 × 300 mm laboratory mill for six times. The rolls were cooled with running water at room temperature and the gap was set at 1.65 ± 0.16 mm. The homogenized rubber piece was cut into the approximate weights for each test.

A) Wallace Plasticity (P_o) and Plasticity Retention Index (PRI)

Test portion of 20 ± 5 g was twice passed through the rolls of a cool mill with nip setting adjusted such that the final sheet thickness was 1.6 mm to 1.8 mm. The final sheet was immediately doubled and the two halves pressed lightly together by hand. The resulting piece was cut into six test pellets, which were a round disc of thickness between 3.2 mm to 3.6 mm and approximately 13 mm in diameter. The test

pellets were divided into two sets of three samples and applied to the plasticity determination before and after oven aging at 140°C for 30 min.

Determination of initial rapid plasticity (P_o) and plasticity of aged sample (P_{30}) were done using a Rapid Plastimeter MK.11. The plasticity retention index (PRI) was calculated by the following equation:

$$PRI = \frac{P_{30}}{P_o} \times 100 \qquad (3.7)$$

B) Mooney viscosity

The homogenized rubber of 25 g was divided into two equal portions and placed between the rotor in the die cavity, which heated up to 100 ± 1°C. The Mooney viscosity was measured with a Mooney Viscometer (TECHPRO).

C) Determination of color

The color of raw rubber was compared and matched as closely as possible with that of the standard color slides by using the standard method from RRIM Test Method B10.

CHAPTER 4

RESULTS AND DISCUSSION

In this work, the recovery and purification of skim rubber was carried out by using four different methods: 1) Enzymatic deproteinization of skim latex in the presence of sodium chloride, 2) Saponification of skim latex with sodium hydroxide, 3) Incubation of skim latex with baker's yeast, and 4) Saponification of solid skim rubber in toluene with sodium hydroxide. In the methods 1) and 2), the recovery of skim rubber from skim latex was carried out by phase separation after deproteinization. The separation ratio and particle size distribution of cream phase was measured. The purity of resulting skim rubber was evaluated for the content of nitrogen and ash. In the method 3 the recovery of skim rubber from skim latex was achieved as a result of the increase in the particle size by the incubation of skim latex with baker's yeast. The particle size distribution of yeast-treated latex and the recovery percent were determined. In the method 4 the solid skim rubber in toluene solution was saponified with sodium hydroxide and the nitrogen, ash and ester contents as well as toluene-soluble fraction of purified skim rubber were analysed. The green strength, P_o, PRI and Mooney viscosity of purified skim rubber of method 4 were evaluated.

4.1 Enzymatic Deproteinization of Skim Latex in the Presence of Sodium Chloride

The effect of sodium chloride concentration on the separation ratio, particle size distribution, nitrogen, ash and ester contents were investigated for NaCl concentration of 0, 1, 3, 5, 7 and 9% (w/v). The deproteinization of skim latex was carried out with proteolytic enzyme of 0.04% (w/v) at the typical condition reported in the work of Tanaka et al. [45].

4.1.1 Effect of NaCl Concentration on Separation Ratio and Particle Size Distribution

Table 4.1 shows the effects of NaCl concentration on the separation ratio and particle size distribution of rubber particles in the cream phase. The phase separation was observed at NaCl concentration higher than 3% after the incubation at room temperature (30°C) for 48 hours (Figure 4.1). The separation efficiency was described in the term of separation ratio, defined as ratio of the height of original latex to the height of cream phase. Thus, the high separation ratio indicated the high concentration of rubber in cream phase. It was found that the separation ratio increased with an increase in NaCl concentration and reached a maximum value of 2.8 at NaCl concentration of 7% (Figure 4.2).

One possible explanation for the occurrence of phase separation of small rubber particles as cream phase is that the increase in the density of serum by the addition of NaCl. The rubber particles (density = 0.92 g/cm^3) surfaced to the upper phase, and it will be accelerated by the increase of the density difference between rubber and serum phases. However, it was found that this effect was not predominant, because some salts such as sodium carbonate, diammonium sulfate, magnesium chloride etc. did not cause the phase separation for fresh skim latex [45].

The addition of NaCl caused the increase in rubber particle size, as shown in Figure 4.2. Rubber particles of the separated cream phase showed a substantial increase in the average particle size. The average rubber particle size of skim latex and deproteinized skim latex was 0.11 μm and 3-4 μm, respectively. Proteolytic enzyme decomposed the proteinic materials. Thus, the proteins on the surface of rubber particles were hydrolyzed to oligopeptides or polypeptide, which was soluble in water. Accordingly, the colloidal stability of rubber particles decreased because of the loss of the negative charge from surface-protein and this resulted in the aggregation of rubber particles. In addition, sodium ion neutralized the negative charges of residual polypeptide on the surface of rubber particles. This caused in the reduction of electrostatic repulsion, which caused the rubber particles remained

suspension in aqueous serum. The increase in rubber particle size presumed to occur the synergistically with the sodium chloride ion and the destabilized rubber particles. Similar results were observed for other inorganic salts such as sodium carbonate, diammonium hydrogen phosphate, potassium carbonate, sodium sulphate etc. These inorganic salts were added to the skim latex from the deproteinized natural rubber latex, which was the aged serum latex, and resulted in phase separation as cream phase as well as the increase in average particle size [45].

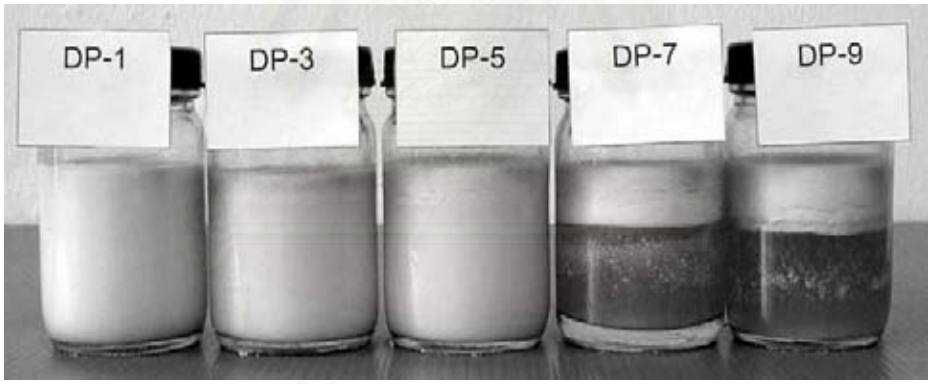


Figure 4.1 Phase separation of skim latex with NaCl

Table 4.1 Effect of NaCl concentration on separation ratio, average particle size of skim latex and nitrogen, ash and ester contents of purified skim rubber from unwashed-cream phase treated with 0.04% (w/v) enzyme at 30°C for 48 h.

Sample	NaCl (%, w/v)	Separation ratio	Recovery (%)	Average particle size (μm)	%N (%, w/w)	%Ash (%, w/w)	Ester content (mmol/kg rubber)
Control	0	1.0	-	0.11	2.97	0.82	5.8
DP-0	0	1.0	0	0.24	0.50	0.51	1.4
DP-1	1	1.0	0	0.88	0.44	0.63	1.1
DP-3	3	2.3	65	3.31	0.55	1.03	3.7
DP-5	5	2.6	92	4.48	0.67	1.07	0.2
DP-7	7	2.8	100	4.21	0.71	1.56	4.9
DP-9	9	2.7	100	3.39	0.67	1.43	3.0

4.1.2 Effect of NaCl Concentration on Nitrogen, Ash and Ester contents

The nitrogen content is related to the proteinic materials in rubber. The nitrogen content can be converted to the protein content using the following equation: %protein = %N × 6.25. Table 4.1 also shows the nitrogen, ash and ester contents of purified skim rubber. It was found that the nitrogen content of the rubber from cream phase decreased from 2.97% (control) to about 0.5-0.7%, independent of the concentration of NaCl. The proteolytic enzyme cleaves the peptide linkages selectively and deproteinization results in the shorter polypeptides or oligopeptides. These decomposed peptides have the higher density than the rubber particles and should tend to move to the serum phase. The residual nitrogen compounds of 0.5-0.7% were both the dissociated polypeptide and the residual polypeptide associated with the rubber molecule. If it was only the dissociated polypeptide in the serum, it would be removed by washing with centrifugation.

Figure 4.4 shows the FTIR spectra of the original skim rubber and the purified skim rubber after deproteinization (0.04% enzyme in the presence of 7% NaCl). For the original skim rubber, the infrared band was at 3270 cm^{-1}, which is assignable to N-H stretching [49]. For treated rubber, the intensity of this band decreased and shifted to 3290 cm^{-1} as the nitrogen content of the deproteinized skim rubber decreased to 0.71%. The characteristic bands of amide and amine bonds at 1628 and 1540 cm^{-1} also decreased in intensity. This suggests that the nitrogen in the purified skim rubber is the decomposed-nitrogenous compound that attached to the coagulated rubber during coagulation. It was desired to remove these nitrogenous compounds in serum by washing with centrifugation. Since the particle size increased to a level higher than 1.0 μm, the washing by centrifugation could be applied. The nitrogen content of the purified skim rubber after washing by centrifugation was expected to be decreased, this results will be discussed in Section 4.1.3.

In addition to the organic compounds, natural rubber contains the other substances such as metal ions, which was evaluated in term of ash content. Ash consists of potassium, magnesium, iron, sodium and copper. In Table 4.1, the original

skim rubber (control) contained 0.82% ash, while the ash content of rubber from cream phase was increased with the increase of NaCl concentration. The skim rubber from cream phase, which was deproteinized in the presence of 1% NaCl concentration contained lower ash content than that in the control. However, the skim rubber from cream phase that contained NaCl concentration higher than 3% showed the higher ash content than that in the control. This indicated that sodium ion was included in the rubber during the phase separation and coagulation step. Therefore, it is also necessary to remove these inorganic salts by the washing process.

From Table 4.1, the purified skim rubber contained the ester groups from 0.2-4.9 mmol/kg rubber, independent of NaCl concentration. *Hevea* rubber contains two types of long chain fatty acids. The first are fatty acids linked to rubber chain at the terminal end. Another is the mixed fatty acid [51]. In this experiment, the purified skim rubber was precipitated from toluene solution with a mixture of acetone and methanol before the FTIR measurement to remove the mixed fatty acids from the sample. It was reported by Sakdapipanich [5] that the purified serum rubbers, in which the free fatty acids were removed by acetone extraction for 24 h showed the ester content of 0.1 mmol/kg rubber. The ester content of control skim rubber and purified skim rubber in this experiment was higher than that reported by Sakdapipanich [5]. These values are expected to be the result of the free fatty acids, which remained in the rubber even precipitation for one time. The lowest value of 0.2 mmol/kg rubber shows the residual ester groups attached to rubber molecule in the purified skim rubber. It is estimated to be about 0.03 molecules per rubber chain, based on the degree of polymerization of 2118 for one rubber chain. This is similar to the result reported by Sakdapipanich [5].

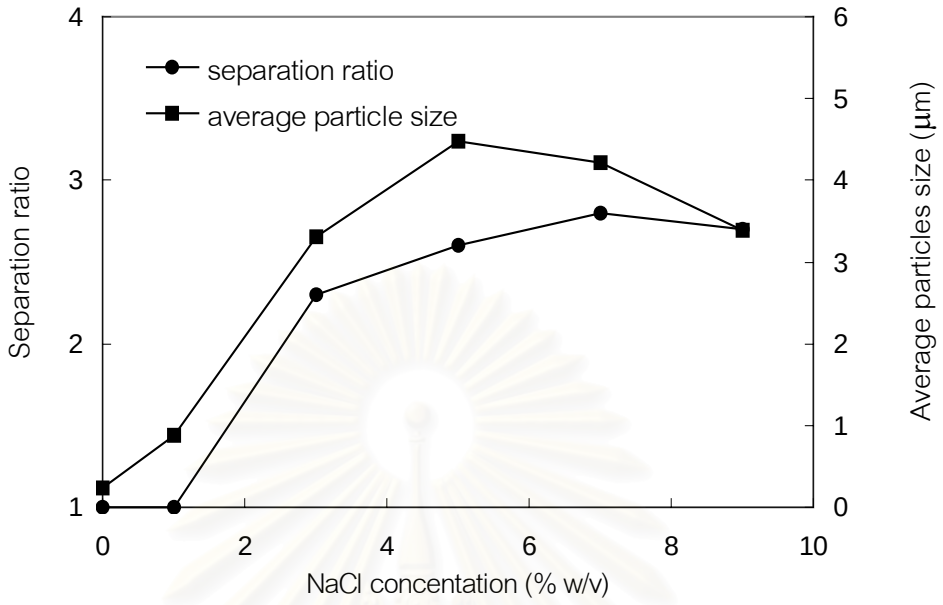


Figure 4.2 Separation ratio and average particle size of cream phase treated with 0.04% (w/v) Enzyme and NaCl at 30°C for 48 h.

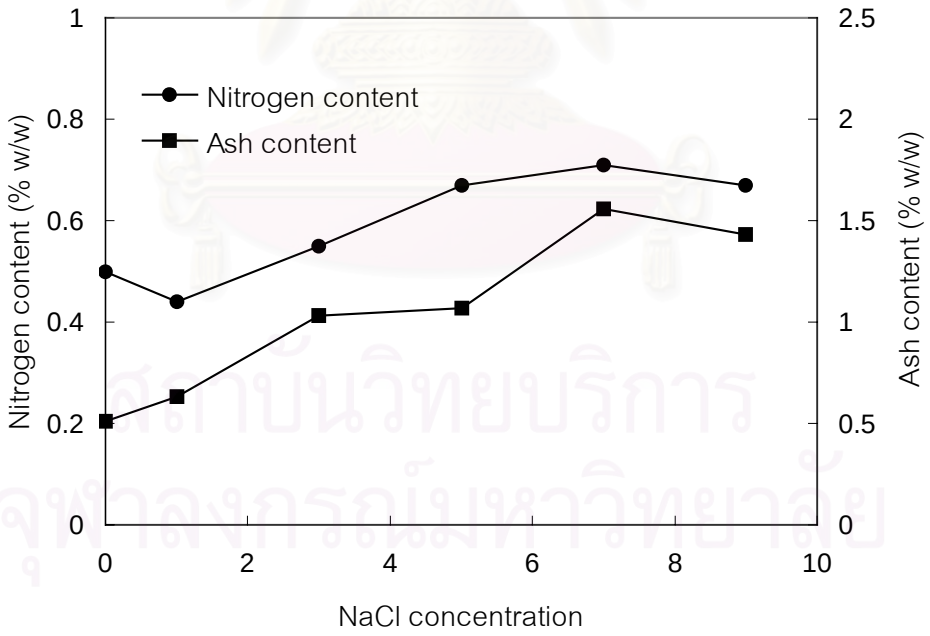


Figure 4.3 Nitrogen and ash contents of purified skim rubber from unwashed-cream phase treated with 0.04% (w/v) enzyme and NaCl at 30°C for 48 h.

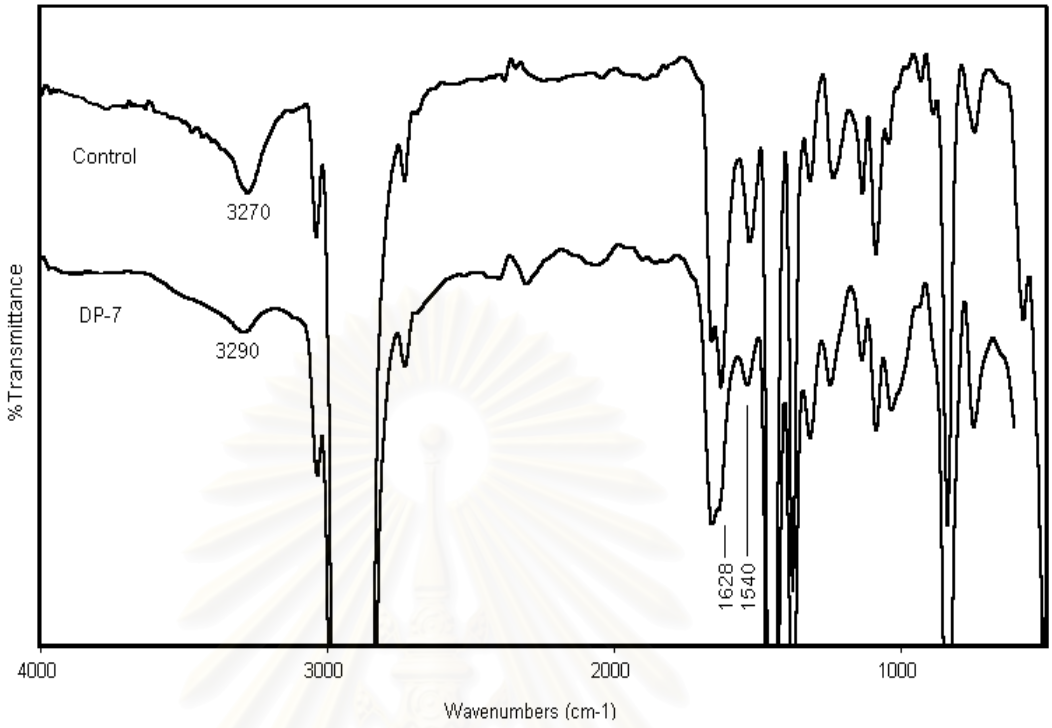


Figure 4.4 FTIR spectra of original skim rubber (control) and purified skim rubber from unwashed-cream phase treated with 0.04% (w/v) enzyme and 7% (w/v) NaCl at 30°C for 48 h (DR-7).

4.1.3 Effect of Washing by Centrifugation

As mentioned above, further purification of cream phase is required to obtain highly purified skim rubber, which contains very low nitrogen and ash contents. The nitrogen, ash, ester and gel contents of the washed–purified skim rubber are shown in Table 4.2. The nitrogen content of washed–rubber decreases dramatically from 0.5–0.7% (value from unwashed rubber) to 0.04% for NaCl concentration of 3, 7 and 9% as shown in Figure 4.5. In the case of NaCl concentration of 0 and 1% (w/v), the nitrogen content was about 0.1%. This may be due to the average particle size of rubber particles was slightly increased without sodium chloride (DP-C0) and with only 1% NaCl (DP-C1). The washing by centrifugation of these purified skim latices showed no phase separation as cream phase, thus the residual nitrogenous materials are presumed to remain in rubber latices.

Figure 4.6 illustrated FTIR spectra of the purified skim rubber coagulated from washed-cream phase (DP-C7) compared with the control sample. It is clear that the intensity of band at 3270 cm^{-1} markedly reduced in intensity and shifted to 3317 cm^{-1} as the nitrogen content of DP-C7 decreased. The characteristic bands of the amide and amine bonds at 1628 cm^{-1} and 1540 cm^{-1} also disappeared in the DP-C7. The bands at 3270 cm^{-1}, 1628 cm^{-1}, 1540 cm^{-1} are characteristic of proteins. This implies that the residual nitrogenous substances in DP-7, which can be washed off by centrifugation, are dissociated polypeptides hydrolyzed by enzyme. The nitrogen content of DP-C7 was 0.04%, which was estimated to be about 4-5 mole-atom per rubber chain (based on the number average molecular weight of 1.68×10^5 as shown in Table 4.3). These amide groups, which remained in the rubber molecule and could not be removed by washing with centrifugation, may be an associated polypeptide linked to the rubber chain.

From Table 4.2, it was also found that ash content of rubber from washed–cream phase varied from 0.2-0.6%, which was lower than that of unwashed–cream phase. The ash content increased with increasing the NaCl concentration. Therefore the washing by centrifugation could be a useful technique to purify the cream phase.

The ester content of control skim rubber was 5.8 mmol/kg rubber. After the deproteinization and washing by centrifugation, the ester content of the purified skim rubber slightly decreased from 5.8 to about 2-4 mmol/kg rubber. The sample DP-C7 contained 3.5 mmol/kg rubber, corresponding to 0.6 mole per rubber chain, based on the degree of polymerization of 2470. This result was confirmed by ^{13}C-NMR, as shown in Figure F-1. The purified skim rubber show almost no ^{13}C-NMR signals at 174.2, 34.4, 29.7 and 14.0 ppm, which are due to the carboxylic-carbon (-CH_2-$\underline{C}O_2$-), terminal methylene (-$\underline{C}H_2$-CO_2-), methylene (-($\underline{C}H_2$)-) and methyl carbon ($\underline{C}H_3$-) atoms in the long chain fatty acid groups, respectively. This result implies that the rubber molecules in purified skim rubber are not terminated by phospholipid groups.

Table 4.2 Effect of NaCl concentration on nitrogen, ash, ester and gel contents of the purified skim rubber from washed-cream phase treated with 0.04% (w/v) enzyme and NaCl at 30°C for 48 h.

Sample	NaCl (%, w/v)	%N (%, w/w)	%Ash (%, w/w)	Ester content (mmol/kg rubber)	Gel content (%, w/w)
Control	0	2.97	0.82	5.8	3.4
DP-C0	0	0.15	0.18	1.5	2.6
DP-C1	1	0.13	0.29	2.8	2.4
DP-C3	3	0.04	0.31	3.2	2.9
DP-C5	5	0.04	0.50	5.2	2.2
DP-C7	7	0.04	0.57	3.5	3.1
DP-C9	9	0.04	0.62	4.9	3.3

Table 4.3 MW and MWD of the purified skim rubber from wash-cream phase treated with 0.04% (w/v) enzyme and 7% (w/v) NaCl at 30°C for 48 h.

sample	$\overline{M}_n$ (x 10^{-5})	$\overline{M}_w$ (x 10^{-5})	$\overline{M}_w / \overline{M}_n$
Control skim latex	1.44	4.80	3.33
DP-C7	1.68	4.72	2.80

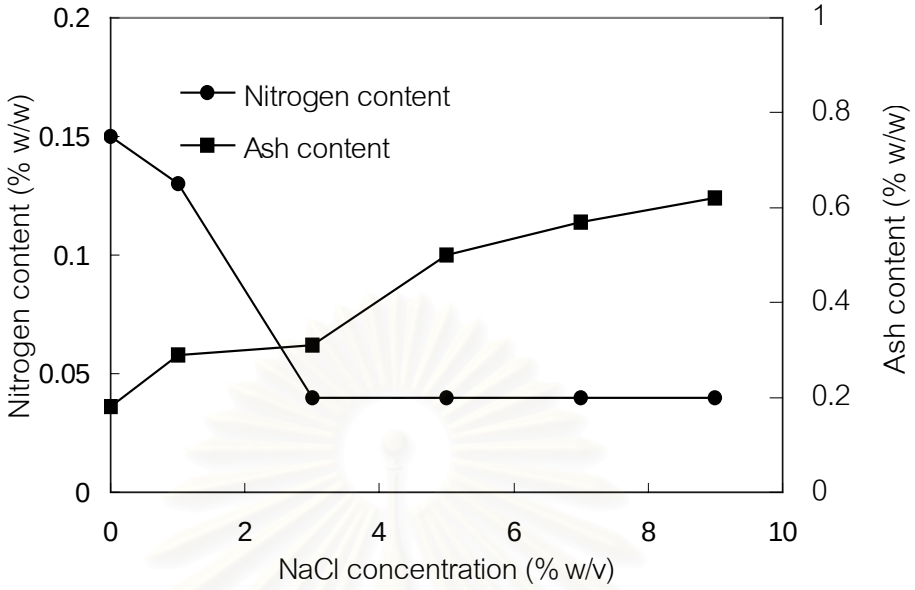


Figure 4.5 Nitrogen and ash contents of the purified skim rubber from washed-cream phase treated with 0.04% (w/v) enzyme and NaCl at 30°C for 48 h.

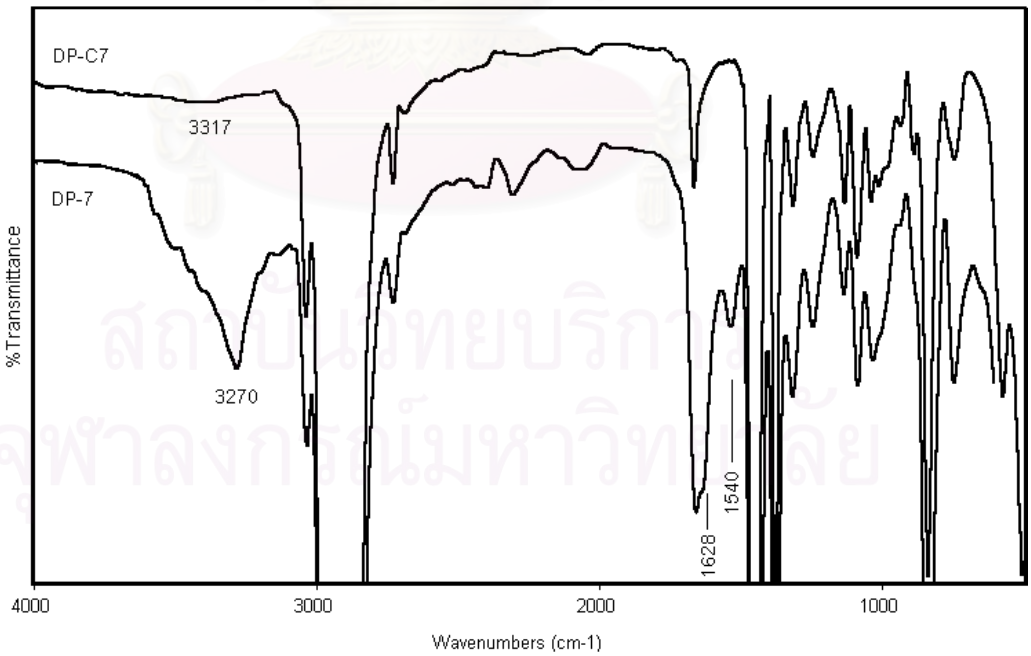


Figure 4.6 FTIR spectra of the purified skim rubber from washed-cream phase (DP-C7) and unwashed-cream phase (DP-7) treated with 0.04% (w/v) enzyme and 7% (w/v) NaCl at 30°C for 48 h.

The gel phase in natural rubber is reported to be the swelling components consisted of small crosslinking particles [31]. On the other hand, the branching and crosslinking points are composed of two types of branch-point [32]. One is presumed to be form by protein *via* hydrogen bonding and another by phospholipid linkage. The gel content of purified skim rubber was insignificantly reduced after deproteinization. Since enzymatic deproteinization decomposes only the peptide linkages and no effect on the phospholipid linkages. This indicated that the gel phase in deproteinized skim rubber was derived from phospholipids linkage. However, it was disclosed recently that rubber molecule from small rubber particles consists of almost no phosholipids at the terminal end [5]. Thus, this gel phase was expected to be the hard gel, which was formed by crosslinking of unsaturated rubber chain and/or abnormal group on the rubber. The hard gel could not be decomposed by chemical reaction.

Based on the results mentioned above, the presumed structural change after deproteinization of skim rubber is assumed as shown in Figure 4.7.

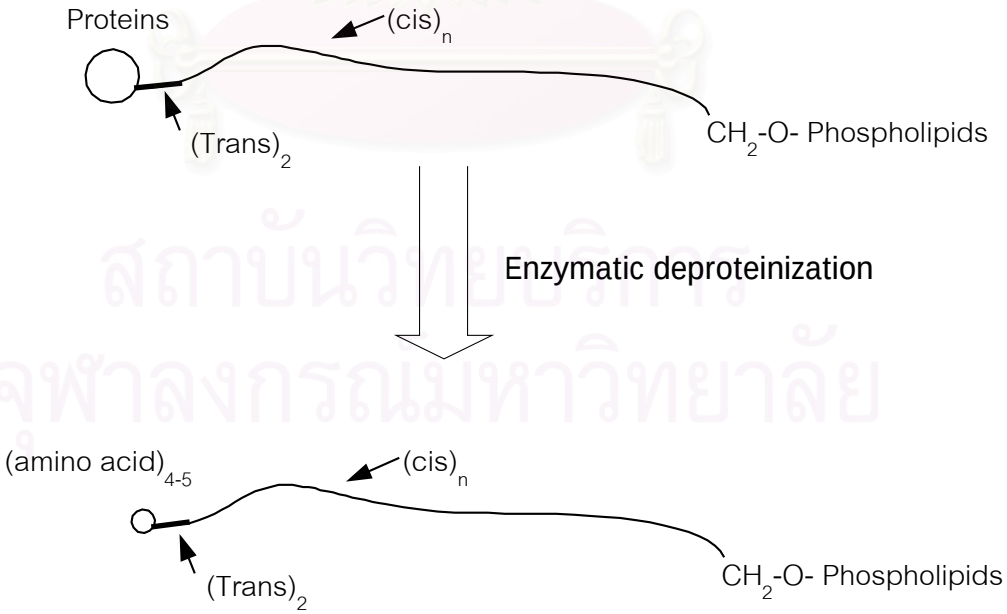


Figure 4.7 Schematic representation of structural change after deproteinization reaction

4.2 Saponification of Skim Latex with Sodium Hydroxide

The effect of NaOH on separation ratio, particle size distribution, nitrogen, ash, ester and gel contents were investigated by varying NaOH concentration of 1, 2, 3, 4, and 5% (w/v). The deproteinization by saponification of skim latex was carried out at 50°C for 5 h and incubated at 30°C for 24 h.

4.2.1 Effect of NaOH Concentration on Separation Ratio and Particle Size Distribution

The phase separation was firstly observed for the saponification with NaOH concentration higher than 4 % and incubation at room temperature (30°C) for 2 h. After incubation for 24 h, a clear phase separation as cream phase was observed at NaOH concentration higher than 3% as illustrated in Figure 4.8.

Table 4.4 shows the separation ratio, recovery percent and average particle size of the rubber in cream phase. In Table 4.4 and Figure 4.9, the separation ratio was in the range of 2.1–3.1 and slightly increased with increasing NaOH concentration. The separation ratio reached a maximum value of 3.1 at NaOH concentration of 5%. At NaOH concentration of 3%, the separation ratio was 2.2, but the separation was not perfect and some residual rubber particles were observed in the serum phase. The recovery percent of rubber in that case was 97% while it was 100% in case of 4 and 5% NaOH concentration. It can be concluded that there are two factors to be the driving force for the phase separation. The first is the effect of inorganic salt. The addition of inorganic salts may cause the higher density of serum phase. The second is the destabilization of rubber particles due to the decomposition of protein on the rubber surface by the saponification with NaOH. Furthermore, sodium ion neutralizes the negative charges of residual polypeptide on the surface of rubber particles. This causes the reduction of electrostatic repulsion, which helps rubber particles remained suspension in aqueous serum. It should result in the increase in particle size of rubber particle due to the partial aggregation from the lesser stability of rubber particles.

From Figure 4.9, the phase separation concentrated the skim latex to about 2–3 times and the average particle size of 0.1 μm was unchanged. Thus, This is another effect that tends to increase the resistance of latex to aggregate. Deproteinization by saponification could not increase the rubber particle size as enzymatic deproteinization in the presence of NaCl. The unchange in particle size of rubber particles in saponified skim latex is expected to be the presence of fatty acid soaps in the latices. Saponification hydrolyzes the amide linked to rubber molecule in latex, moreover it changes the fatty acid ester groups to the fatty acids that accumulate as sodium soaps at the rubber-water interface. Eventhough, there are very low amount of fatty acid ester groups linked to rubber molecules, skim latex contains a high level of free fatty acid content [39, 50]. These free fatty acid is expected to be saponified by sodium hydroxide result in the formation of sodium soaps, which will stabilize the rubber particles and prevent aggregation.

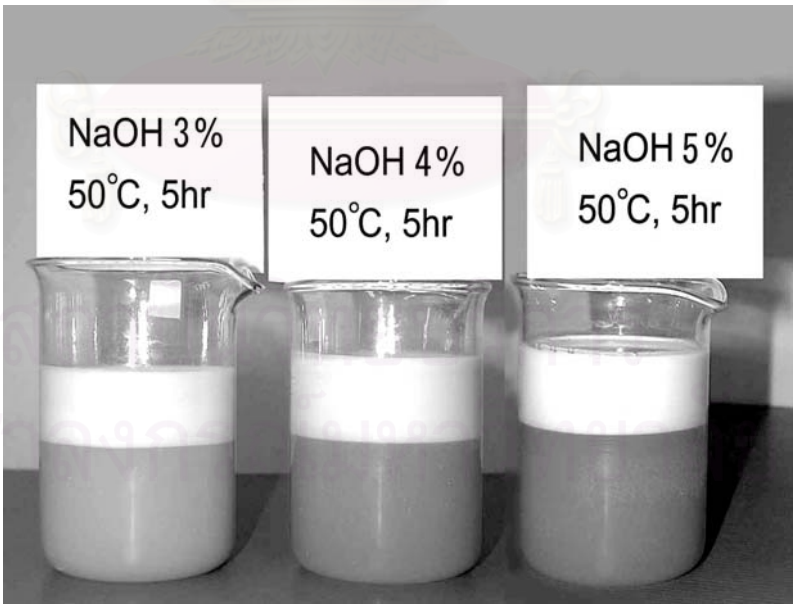


Figure 4.8 Phase separation of skim latex by saponification with NaOH

4.2.2 Effect of NaOH Concentration on Nitrogen, Ash, and Ester Contents

Table 4.4 also shows the nitrogen, ash and ester contents of the purified skim rubber from unwashed-cream phase. The nitrogen content of purified skim rubber decreased from 2.70% (control) to the range of 0.33-2.26% after saponification with NaOH 1-5%. The nitrogen content decreased with increasing NaOH concentration as shown in Figure 4.10. The nitrogenous compounds were decomposed by saponification of skim latex with high NaOH concentration. The decomposed proteins are expected to move into the aqueous phase, which has higher density than the rubber or cream phase. The high value in residue nitrogen in case of NaOH concentration of 1 and 2% shows that the phase separation as cream phase is an important factor, which will encourage the migration of decomposed-nitrogenous compounds to the serum phase.

Figure 4.11 shows FTIR spectra of control and purified skim rubber with 1% and 5% NaOH. These spectra showed that the bands at 3270 cm^{-1} , 1628 cm^{-1} and 1540 cm^{-1}, which are the characteristic vibrations of N-H stretching, amide and amine, respectively [49], decreased in intensity after saponification. Furthermore, the intensity of these bands was reduced with increasing NaOH concentration. It was presumed that the residual nitrogenous compounds in purified rubber were the decomposed polypeptides, present in a coagulum. In this case, it is necessary to remove these compounds by washing before coagulation. Due to the fact that the particle size of purified–rubber particles in skim latex did not increase after saponification, it is desired to increase the rubber particle size by adding NaCl for facilitating the washing by centrifugation.

Table 4.4 and Figure 4.10 indicates that the ash content of the purified rubber from cream phase is high. The ash content increased with increasing NaOH concentration. Because the sodium ion acts as the counter ions for the negative charge on residual polypeptide and for fatty acid soaps that stabilized the rubber particles, sodium ion is included in the rubber during the coagulation of cream phase.

From Table 4.4, it can be seen that the ester content of the purified rubber after saponification decreased with increasing NaOH concentration. The removal of ester groups was also confirmed by FTIR analysis. The characteristic band of C=O, which indicates the ester groups in rubber, is 1738 cm^{-1}. The band intensity of the purified skim rubber was decreased after saponification. This indicates that saponification of skim latex with NaOH decomposed both protein and fatty acid ester linked to rubber chain and those present as a mixture. However, there were the fatty acid soaps, which assisted the stabilization of rubber particles including in rubber while coagulation. Thus, it presented as the residual ester content in saponified skim rubber.

Table 4.4 Effect of NaOH concentration on separation ratio, average particle size of skim latex and nitrogen, ash and ester contents of the purified skim rubber from unwashed-cream phase saponified with NaOH at 50°C for 5 h and incubated at 30 °C for 24 h.

Sample	NaOH (%, w/v)	Separation ratio	Recovery (%)	Average particle size (μm)	%N (%w/w)	%Ash (%w/w)	Ester content (mmol/kg rubber)
Control	0	-	-	0.11	2.70	0.55	8.5
SAP-1	1	1.0	0	0.11	2.26	0.37	6.3
SAP-2	2	1.0	0	0.11	1.08	0.40	3.2
SAP-3	3	2.2	97	0.12	0.57	2.25	2.4
SAP-4	4	2.7	100	0.12	0.37	2.45	2.1
SAP-5	5	3.1	100	0.13	0.33	2.64	3.6

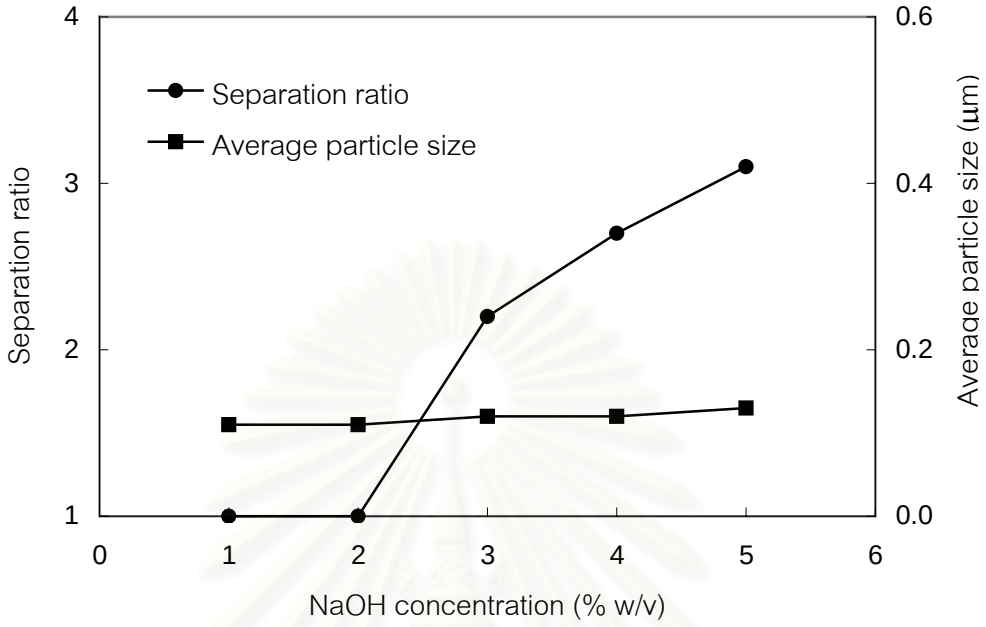


Figure 4.9 Separation ratio and average particle size of cream phase saponified by NaOH at 50°C for 5 h and incubated at 30°C for 24 h.

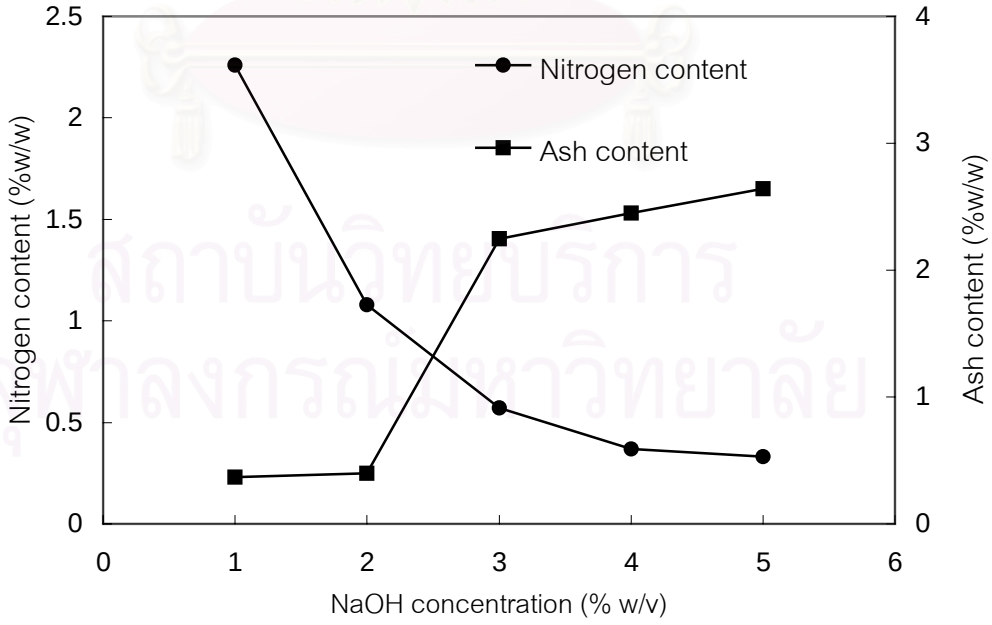


Figure 4.10 Nitrogen and ash contents of purified skim rubber from unwashed-cream phase saponified by NaOH at at 50°C for 5 h and incubated at 30°C for 24 h.

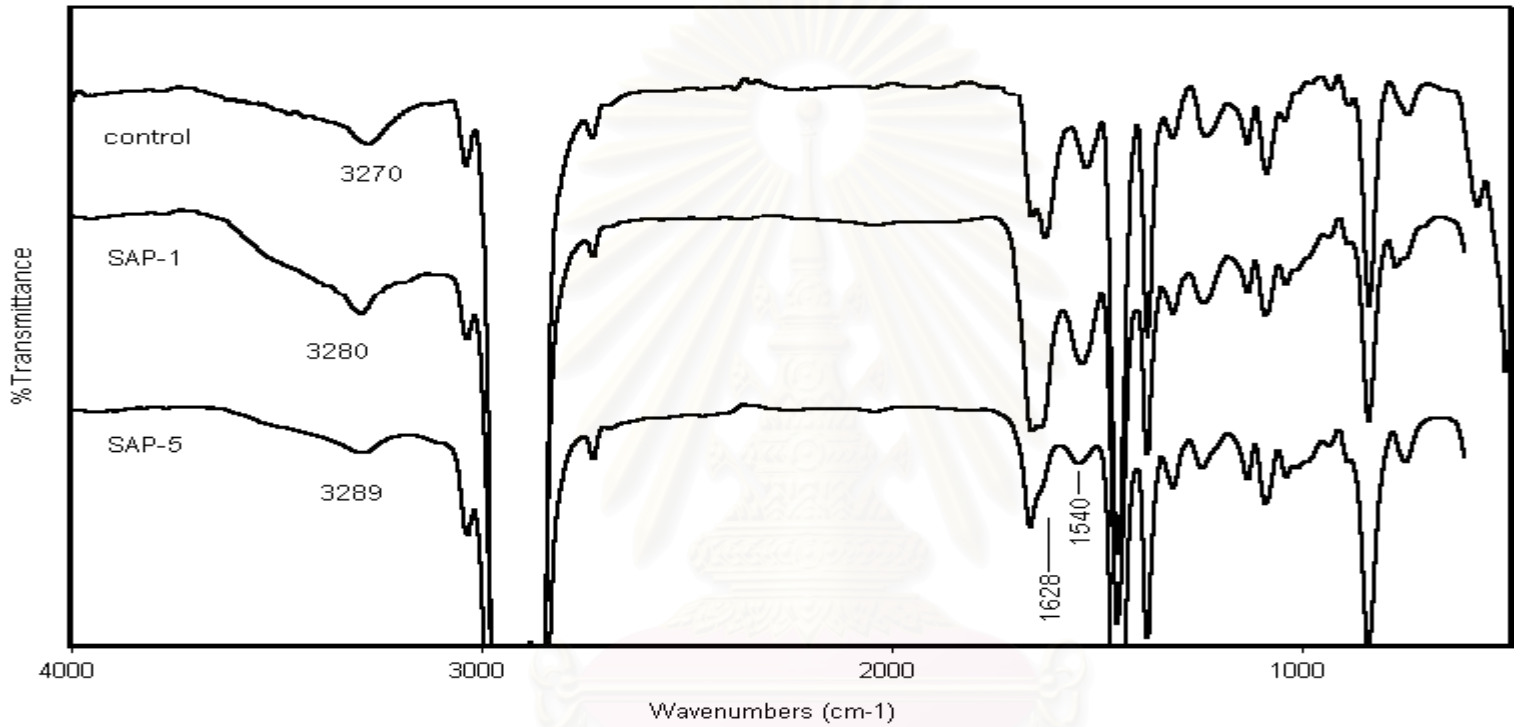


Figure 4.11 FTIR spectra of original skim rubber and purified skim rubber from unwashed-cream phase by saponification of skim latex with NaOH at 50°C for 5 h and incubated at 30°C for 24 h

Control = original skim rubber from skim latex

SAP-1 = Saponified skim rubber with 1%NaOH

SAP-5 = Saponified skim rubber with 5%NaOH

4.2.3 Effect of Washing by Centrifugation

To facilitate the washing by centrifugation, NaCl of 1%(w/v) was added to increase the rubber particle size. Table 4.5 shows the nitrogen, ash, ester and gel contents of the saponified skim rubber. From Figure 4.12, The separation ratio was slightly increased from 2–3 time to 3–4 times by the addition of NaCl. This may be due to the addition of NaCl cause the higher density of serum phase. The average particle size of rubber in cream phase was increased from 0.11 μm to approximately 0.6 μm after incubation for 24 h. It also implied that sodium chloride play a role in increasing the particle size of rubber particles. However, the mechanism is unclear.

After saponification and incubation, the cream phase was separated from the serum phase and 1%(w/v) SDS was added to stabilize the rubber particles. The cream phase was washed by centrifugation. The rubber from washed-cream phase showed a dramatic decrease in nitrogen and ash content as shown in Figure 4.13. The nitrogen content of the control rubber was 2.70%. The nitrogen content of the rubber from washed-cream phase decreased to 0.09-0.02% for NaOH concentration of 1-5% (Table 4.5). The nitrogen content decreased with increasing NaOH concentration. This nitrogen content is very low, comparing with that of 2.26 – 0.33% in purified rubber from unwashed-cream phase. This result implies that the washing by centrifugation cause the migration of the residual nitrogeneous compound from rubber phase to serum phase.

Figure 4.14 shows FTIR spectra of the purified skim rubber from washed-cream phase saponified with 4% NaOH at 50°C for 5 h and incubated at 30°C for 24 h. It was found that the rubber from the washed-cream phase contained no characteristic bands of amide and amine at 1628 cm^{-1} and 1540 cm^{-1}. In addition, the band at 3280 cm^{-1} decreased and shifted to 3324 cm^{-1}. The residual nitrogen content in the samples SAP-C1 and SAP-C4 was estimated to be about 9 and 2 mol-atom/rubber chain, based on the degree of polymerization of 2132 and 1162, respectively (see the number average molecular weight in Table 4.6).

Table 4.5 and Figure 4.13 show the ash content of the purified skim rubber from washed-cream phase saponified with various concentration of NaOH. The ash content of purified skim rubber was 0.25-0.55%, which are lower than that of the rubber from unwashed-cream phase (0.37-2.64%). The ash content in the purified skim rubber saponified with 1% and 2% NaOH (SAP-C1, SAP-C2) in the presence of 1% NaCl was similar to that in the saponified rubber under the same condition without NaCl. On the other hand, the others saponified skim rubber from washed-cream phase showed dramatically the decrease in ash content as compared to that from unwashed-cream phase. The ash content decreased from about 2% to 0.2–0.7% for saponification with NaOH of 3-5%.

The ester content of rubber from washed-cream phase is presented in Table 4.5. The ester content of the saponified rubber was in the range of 1.6-3.9 mmol/kg rubber for NaOH concentration of 2-5%. While the control skim rubber contained the fatty acid ester group of 8.5 mmol/kg rubber. The ester content in purified rubber was dramatically reduced from that in the control rubber, and it was slightly lower than that of rubber from unwashed-cream phase. The sample SAP-C4 contained the fatty acid ester of 1.8 mmol/kg rubber, corresponding to 0.1 mole per rubber chain (based on the degree of polymerization of 1162). This result was confirmed by ^{13}C-NMR, as shown in Figure F-2. The saponified skim rubber show no ^{13}C-NMR signals at 174.2, 34.4, 29.7 and 14.0 ppm, which are due to the carboxylic-carbon (-CH_2-$\underline{C}O_2$-), terminal methylene (-$\underline{C}H_2$-CO_2-), methylene (-($\underline{C}H_2$)-) and methyl carbon ($\underline{C}H_3$-) atoms in the long chain fatty acid groups, respectively.

Gel content of rubber from skim latex after saponification is presented in Table 4.5. The gel content of 3.3% in the rubber from skim latex reduced to about 1.5% by saponification. In general, skim rubber is linear molecule, which contains no gel [5]. The gel fraction observed here is assumed to be formed by certain chemical cross-linking by the reaction of abnormal group. This gel is preferred to be a hard gel, which can not be disintegrated by saponification [32].

Table 4.5 Effect of NaOH concentration and washing on separation ratio, average particle size of skim latex and nitrogen, ash, ester and gel contents of the purified skim rubber from washed-cream phase saponified with NaOH at 50°C for 5 h and incubated at 30 °C for 24 h.

Sample	NaOH (%, w/v)	Separation ratio	Recovery (%)	Average particle size (μm)	%N (%, w/w)	%Ash (%, w/w)	Ester content (mmol/kg rubber)	Gel content (%w/w)
Control	0	-	-	0.11	2.70	0.55	8.5	3.3
SAP-C1	1	1.0	0	0.64	0.09	0.55	-	1.5
SAP-C2	2	1.0	0	0.64	0.08	0.40	1.6	1.2
SAP-C3	3	3.0	98	0.55	0.03	0.25	3.0	1.6
SAP-C4	4	3.2	100	0.60	0.03	0.68	1.8	1.7
SAP-C5	5	3.9	100	0.60	0.02	0.38	3.9	1.2

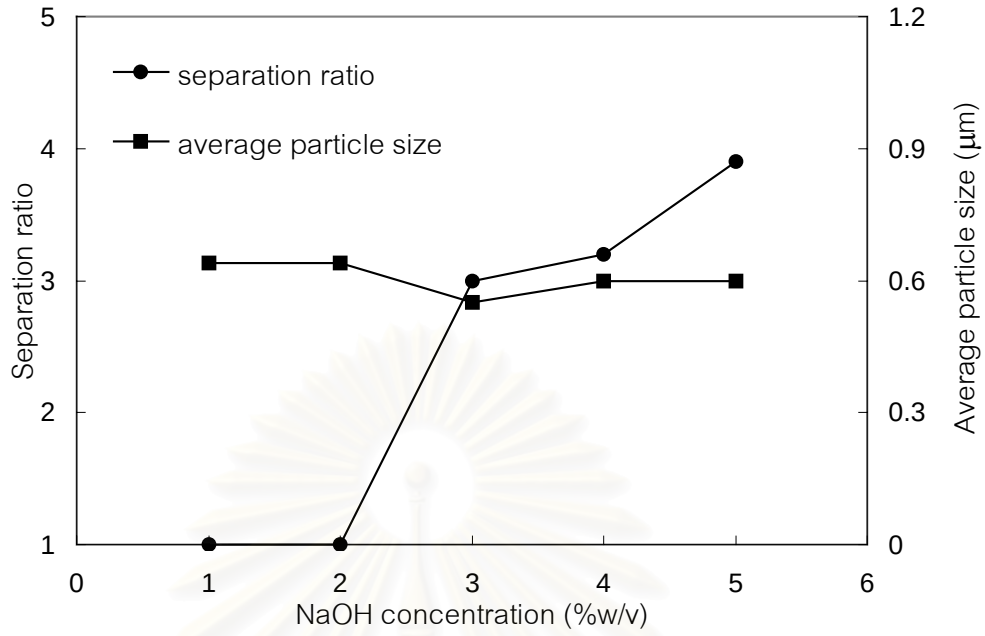


Figure 4.12 Separation ratio and average particle size of the purified skim rubber in cream phase saponified with NaOH in the presence of 1% NaCl at 50°C for 5 h and incubated at 30°C for 24 h.

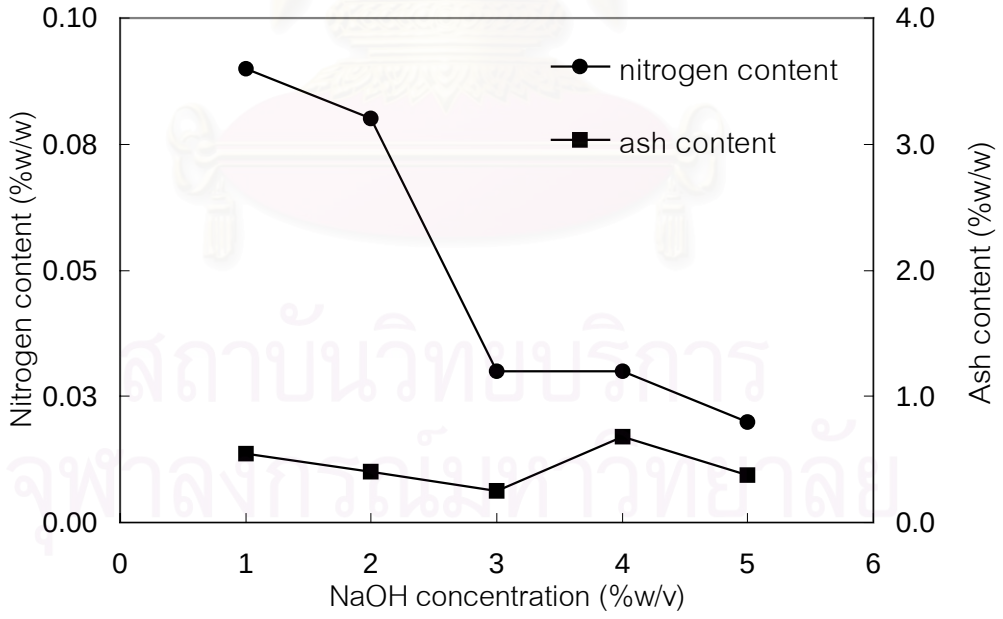


Figure 4.13 Nitrogen and ash contents of the purified skim rubber from wash-cream phase saponified with NaOH in the presence of 1% NaCl at 50°C for 5 h and incubated at 30°C for 24 h.

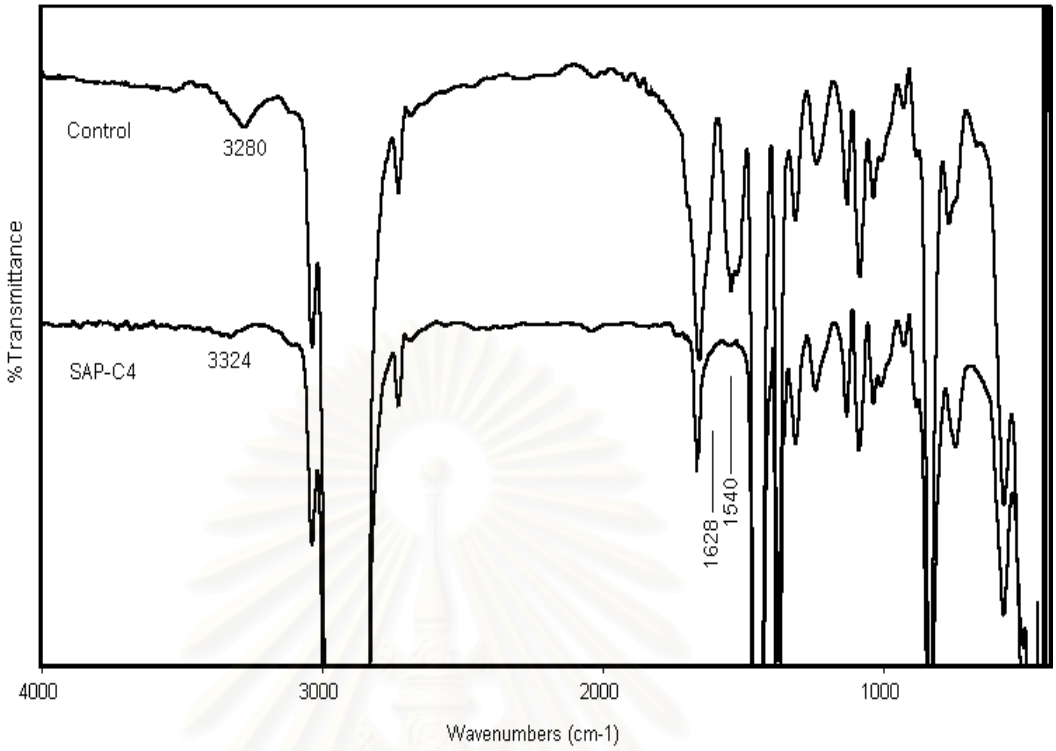


Figure 4.14 FTIR spectra of the purified skim rubber from washed-cream phase saponified with 4%(w/v) NaOH at 50°C for 5 h and incubate at 30°C for 24 h.

Table 4.6 MW and MWD of the purified skim rubber for wash cream phase saponified with NaOH of 1% and 4% at 50°C for 5 h and incubated at 30°C for 24 h.

sample	$\overline{M}_n$ (x 10^{-5})	$\overline{M}_w$ (x 10^{-5})	$\overline{M}_w / \overline{M}_n$
Control skim latex	1.44	4.80	3.33
SAP-C1	1.45	5.80	4.00
SAP-C4	0.79	2.72	3.44

4.3 Phase Separation of Skim Latex by Incubation with Dry Yeast

4.3.1 Effect of pH

The optimal pH, which was suitable for the best activity of yeast, was investigated by adjusting pH of skim latex from 10.2 to 7, 8, and 9 with 10% (v/v) phosphoric acid. The skim latex was incubated with 0.5% (w/v) baker's yeast at 30 °C for 48 h. The adjustment of pH lower than 6.5 could not be done due to the occurrence of coagulation even though it is the appropriate condition for the growth of yeast [52]. The particle size distribution of rubber particles in the resulting skim latex was determined after the incubation for 48 h. A bimodal particle size distribution was observed as shown in Figures 4.15 A, B, and C. The controlled latex without the addition of yeast at pH 8 and 9 showed a slight increase in the particle size from 0.18 μm of the original latex to 0.3 μm. At pH 7, however, the relative amount of large particle fraction was much higher than that of pH 8 and 9 and the average particle size increased to 1.8 μm. This may be due to partial agglomeration of rubber particles at lower pH.

Table 4.7 shows the average particle size of the yeast-treated rubbers at various pH. It is clear that yeast causes the agglomeration of rubber particles in skim latex, especially at pH 7. Rubber particles in the skim latex at pH 7 contained no small particle fraction and the large particle fraction was predominant compared with those in latex at pH 8 and 9 as shown in Figure 4.16.

The increase in particle size of rubber particles may be due to the presence of carbon dioxide (CO_2), which was generated by the fermentation with yeast using non-rubber substrates such as glucose and fructose in the skim latex as starting materials. The evolution of CO_2 also results in a lowering of the pH of skim latex and causes partial agglomeration of rubber particles.

These yeast-treated latices were subjected to the centrifugation after the incubation for 48 h. The yeast-treated latices of pH 8 and 9 showed no phase separation of rubber particles and serum because it contained very low amount of large particles. A

clear phase separation was observed in the case of skim latex at pH 7, as three phases, coagulated-cream phase, serum phase and yeast phase at the bottom. The agglomerated rubber particles were coagulated because of the mechanical force applied during the centrifugation. The skim latex at pH 7 was the appropriate condition for the recovery of the small rubber particles in the skim latex although it causes the coagulation of rubber. Thus, the addition of small amount surfactant should be done in order to stabilize the rubber particles before the centrifugation. 0.2% (w/v) tritron-x100 and sodium dodecyl sulphate (SDS) was used in order to stabilize the yeast-treated skim latex. The color of yeast in the skim latex in the presence of tritron-x100 changed to the darker color. The small bubbles of CO_2 due to the fermentation were not observed. While the latex stabilized with SDS showed a significant increase in the large particle fraction due to the presence of CO_2. The occurrence of coagulum was not observed. Consequently, the latex condition of pH 7 with 0.2%(w/v) of SDS was chosen to examine the suitable amount of yeast for the increase in the particle size of small rubber particles in skim latex.

Table 4.7 Average particle size of yeast-treated rubber at different pH.

pH	Average particle size (μm)		
	All	Small	Large
Control pH7	1.8	0.3	3.3
Control pH8	0.3	0.3	2.1
Control pH9	0.3	0.3	2.1
0.5% yeast at pH 7	17.5	-	17.5
0.5% yeast at pH 8	9.7	0.6	20.3
0.5% yeast at pH 9	3.3	0.3	12.8

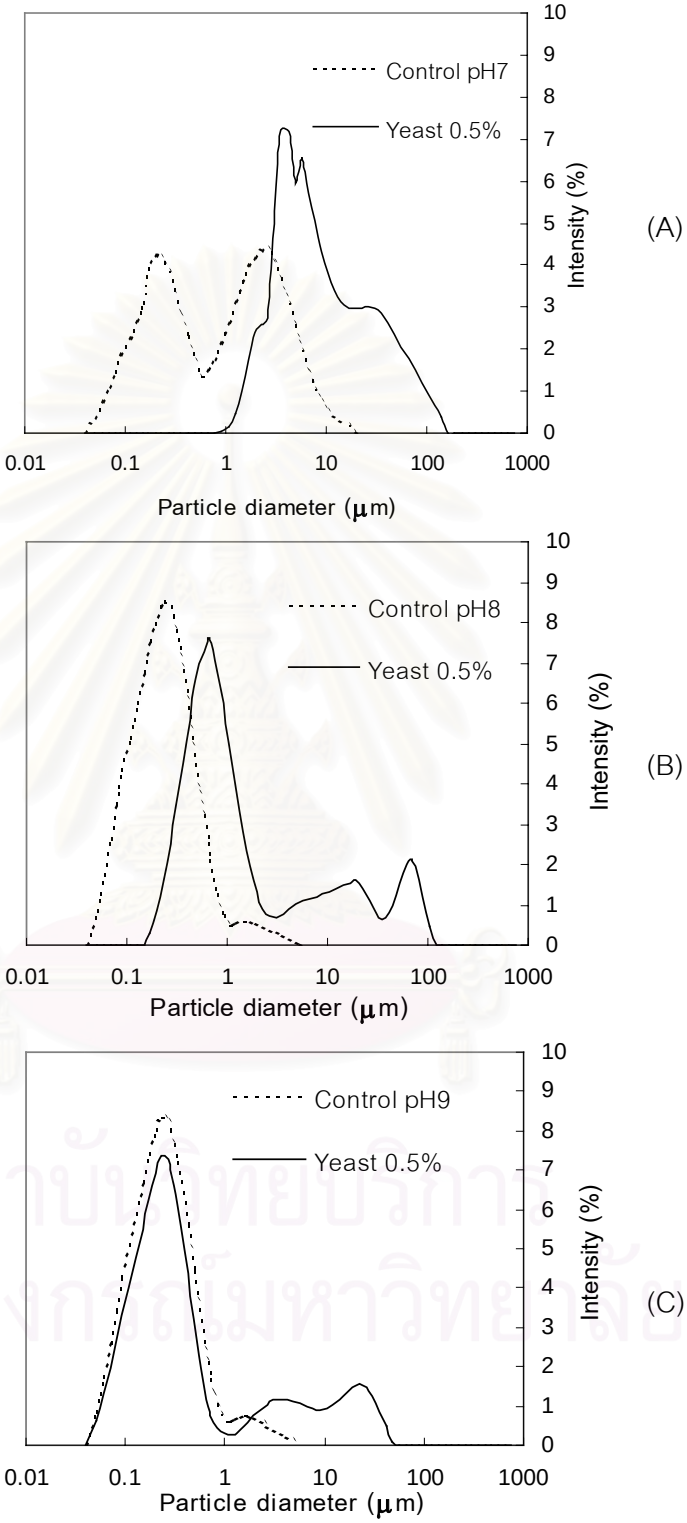


Figure 4.15 Particle size distribution of rubber particles in skim latex at (A) pH 7, (B) pH 8, and (C) pH 9.

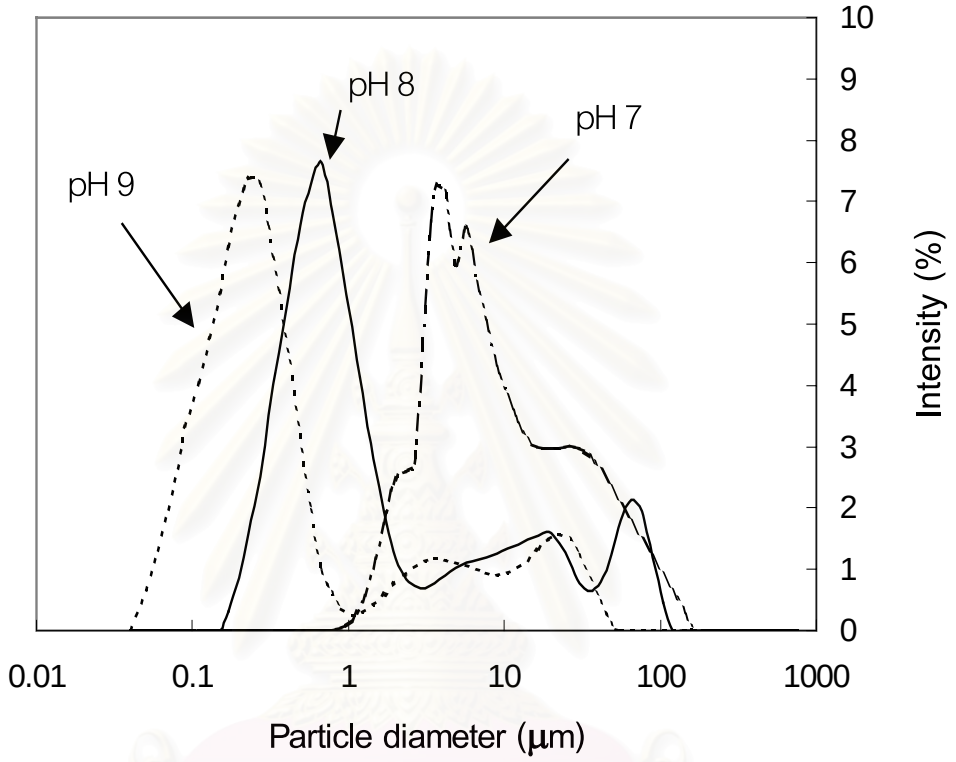


Figure 4.16 Particle size distribution of yeast-treated latex at different pH.

4.3.2 Effect of Yeast Concentration

Incubation of skim latex with yeast was carried out by varying the amount of yeast of 0.1, 0.25, 0.5, and 1%(w/v) with other parameters as follow:

pH of skim latex	: 7
SDS concentration	: 0.2%(w/v)
Temperature	: 30 °C
Time	: 48 h

Table 4.8 shows that the average particle size of yeast-treated skim latex increased from 0.9 μm to about 1-5 μm after incubation for 48 h. A bimodal particle size distribution was also observed after incubation. The large particle fraction increased with increasing the amount of yeast as shown in Figure 4.10. From Figure 4.10, at the amount of yeast higher than 0.5%(w/v) there was no small particle fraction. However, after centrifugation, the recovery percent was 44-45% can be seen in Table 4.9. This indicates that there were residual small rubber particles in the serum phase even after centrifugation. In fact, the dry rubber content of serum phase after determination was 1.9%DRC. Consequently, The absence of the small particle fraction that observe by the particle size analyzer may be due to the comparatively lower intensity of small rubber particles in latex compared to the large rubber particles. Consequently, a peak at small particles fraction could not be seen in Figure 4.17.

The nitrogen content of rubber in the cream phase was 2.7% (w/w) which was the same as the control. This indicates that the treatment of skim latex with yeast causes the partial agglomeration of small rubber particles resulting in the phase separation of skim latex, but has no effect on the protein reduction in skim latex. This means that the treatment with baker's yeast can be applied to concentrate the skim latex. Accordingly, the combination of increasing the particle size with yeast and saponification of resulting large particles will be an appropriate method to decomposed proteins.

Table 4.8 Average particle size of yeast-treated rubber at the different yeast concentration.

Sample	Yeast (%, w/v)	SDS (%, w/v)	Time (h)	Average particle size (μm)		
				All	Small	Large
Control pH10	0	0	48	0.1	0.1	-
Control pH7	0	0.2	48	0.9	0.1	5.5
F01	0.10	0.2	48	1.2	0.2	3.2
F02	0.25	0.2	48	1.8	0.2	4.6
F03	0.50	0.2	48	3.2	-	3.2
F04	1.00	0.2	48	5.6	-	5.6

Table 4.9 Recovery (%) and nitrogen content of rubber of yeast-treated cream phase after centrifugation.

Sample	Yeast (% w/v)	DRC of serum phase	Recovery (%)	%N (%, w/w)
Control pH10	0	-	0	2.70
Control pH7	0	-	0	2.70
F01	0.10	-	~0	2.62
F02	0.25	-	~0	2.67
F03	0.50	1.97	44	2.66
F04	1.00	1.95	45	-

DRC of the original skim latex = 3.52%

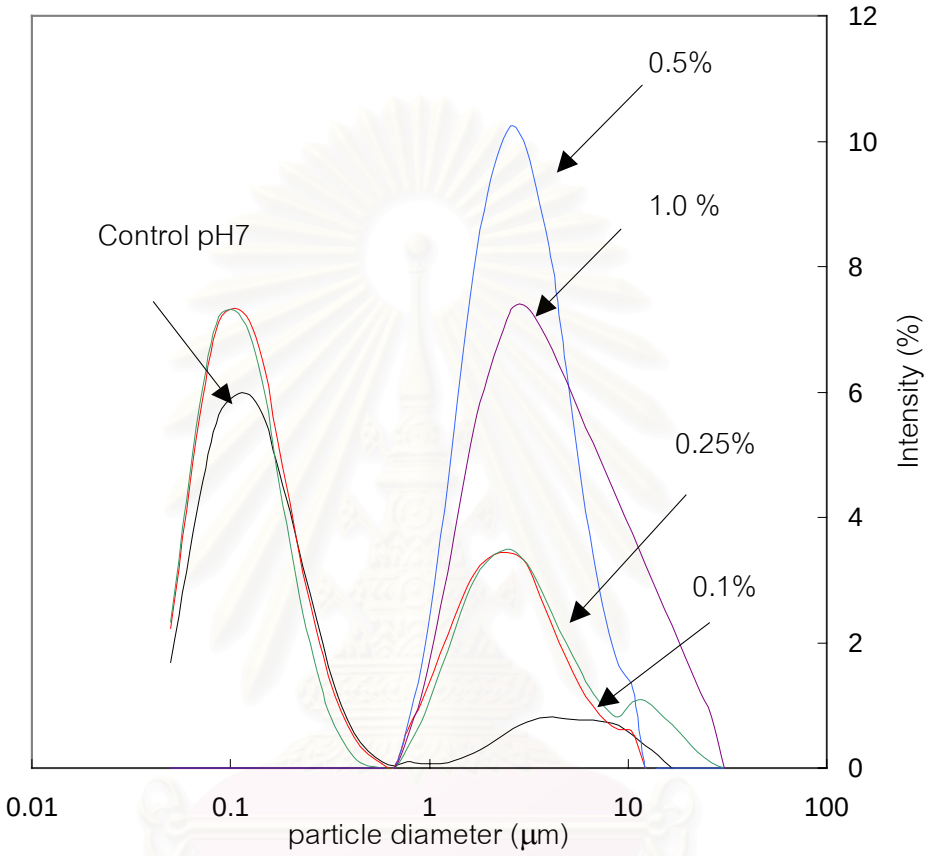


Figure 4.17 Particle size distribution of skim latices after incubation with the different dry yeast concentration for 48 h.

4.3.3 Combination of Incubation of Skim Latex with Yeast and Saponification

The cream phase obtained by incubation of skim latex with yeast was saponified with NaOH. The effect of NaOH on nitrogen and ash contents was investigated by varying NaOH concentration of 1-4% (w/v). The saponification was carried out at 50°C for 5 h and incubated at 30°C for 24 h. The phase separation was observed again after the saponification of the cream phase with 4% NaOH, while it did not occur after the saponification with NaOH concentration lower than 4%.

The nitrogen and ash contents of the saponified yeast-treated skim rubber were shown in Table 4.10. The nitrogen content of the saponified yeast-treated skim rubber decreased from 2.72% (yeast-treated rubber) to the range of 0.71-2.30% after saponification with NaOH 1-4%. The nitrogen content decreased with increasing NaOH concentration. The nitrogen content of the saponified yeast-treated rubber was slightly higher than that of the saponified skim rubber (Section 4.2.2). This may be due to the cream phase obtained by incubation of skim latex with yeast contained the higher DRC than the original skim latex. Thus, the ratio of NaOH to the rubber in latex was decreased and the decomposition of nitrogenous compounds was less effective. The ash content of the saponified yeast-treated skim rubber increased from 0.74% (yeast-treated rubber) to the range of 0.95-1.69%, increased with increasing NaOH concentration. This indicated that sodium ion was included in the rubber during the coagulation of cream phase.

The raw rubber properties (green properties) of saponified yeast-treated skim rubber were characterized for the saponified yeast-treated skim rubber prepared by incubating the skim latex with 0.5% yeast and using saponification condition of 4% NaOH 50°C for 3 h and incubated at room temperature for 24 h. The green properties of the control skim rubber and the saponified yeast-treated skim rubber are presented in Table 4.11. The green strength of the saponified yeast-treated skim rubber of 0.92 MPa was almost the same as the control skim rubber (0.88 MPa). The elongation at break of the saponified yeast-treated skim rubber slightly decreased from 610 (control) to 516%.

These results suggested that the ability of the saponified yeast-treated skim rubber to crystallize during straining did not change. The Wallace plasticity (P_o) of the saponified yeast-treated skim rubber of 55.5 was higher than 46 of the control. This indicated that after the saponification, the rubber was harder than the original rubber. The Plasticity Retention Index (PRI) refers to the resistance of rubber to the oxidative degradation. Both the original and saponified yeast-treated skim rubbers showed very low value of PRI, i.e., 8.3 and 14.4, respectively. This means that the heat resistance of both rubbers was low. The resistance of rubber to the thermal degradation could be improved by adding the antioxidant.

Table 4.10 Nitrogen and ash contents of yeast-treated rubber and saponified yeast-treated rubber

Sample	NaOH conc. (%, w/v)	%N (%, w/w)	%Ash (%, w/w)
Yeast-treated rubber	0	2.72	0.74
SAP-Y01	1	2.30	0.95
SAP-Y02	2	1.90	1.01
SAP-Y04	4	0.71	1.69

Table 4.11 Raw rubber properties of saponified yeast-treated rubber

Parameters	Rubber sample	
	Original skim rubber	Saponified yeast-treated rubber
Ash content, %	0.74	1.69
Nitrogen content, %	2.72	0.71
Green strength, MPa	0.88	0.92
Elongation at break, %	610	516
Wallace plasticity, P_o	46	55.5
Plasticity retention index, PRI	8.3	14.4

4.4 Saponification of Solid Skim Rubber with Sodium Hydroxide in Toluene Solution

4.4.1 Characteristic of Solid Skim Rubber

The fractionation steps of solid skim rubber by toluene are shown in Figure 4.18. The toluene-soluble and toluene-insoluble fraction of skim rubber was 68% and 32%, respectively. The insoluble fraction was swelled in toluene, the FTIR spectrum was obtained by swelling technique. Figures 4.19 (A), (B) show FTIR spectra of the soluble and insoluble fractions of solid skim rubber, respectively. The characteristic bands of protein at 3280 cm^{-1}, 1630 cm^{-1} and 1540 cm^{-1} were observed in both spectra. In the insoluble fraction these bands showed the significant higher in intensity than that of the soluble fraction. The FTIR spectra of the insoluble fraction also showed the characteristic bands of polyisoprene chain at 1664 cm^{-1}, 1450 cm^{-1}, 1378 cm^{-1}, and 837cm^{-1}. This indicates that the insoluble fraction consist of both rubber and non-rubber components. It is generally accepted that the amount of non-rubber materials in the gel fraction is much higher than that of the sol fraction. This data revealed that the insoluble fraction contained both gel phase, composed of the branch and crosslinking rubber chain, and the non-rubber substances.

The nitrogen content of the soluble and insoluble fraction is shown in Table 4.12. The nitrogen content of the insoluble fraction was 7.33%, which was higher than that of the soluble fraction (0.20%). This result also confirms that the insoluble fraction contains higher amount of the nitrogenous substances than the soluble fraction.

The toluene-insoluble fraction was saponified with 5% NaOH at 70°C for 3 h followed by washing with water 3 times, the saponified-insoluble fraction (SAP-INSOL) was solubilized to toluene and showed the presence of only 2.4% insoluble fraction. The nitrogen content of both soluble and insoluble fraction after saponification is shown in Table 4.12. The nitrogen content was reduced to 0.15% for both the saponified-soluble and saponified-insoluble fraction. This indicates that most of the nitrogenous substances in toluene-insoluble fraction are decomposed and removed.

Saponification is a reaction that decomposes both amide and the ester linkages, so the branch-points originated from hydrogen bonding and ester bonding are

broken-down. The soluble fraction of SAP-INSOL (SOL-3) from the gel phase corresponds to "soft gel" (97.6%) that can be easily broken-down to form the linear rubber chains [32]. On the other hand, the residual insoluble fraction (INSOL-3) is considered to be "hard gel" that formed by crosslinking of unsaturated rubber chain and/or the residual non-rubber substances.

Figure 4.20 shows the FTIR spectra of saponified-insoluble fraction (SAP-INSOL) and saponified-soluble fraction (SAP-SOL). The spectrum of SAP-SOL showed a decrease in a intensity of a band at 1540 cm^{-1}, and a shift of a band at 3278 cm^{-1} to 3213 cm^{-1}, while the spectrum of SAP-INSOL shows a significant decrease in a intensity of bands at 1540 cm^{-1} and 3279 cm^{-1}. The characteristic band of protein at 3279 cm^{-1} of SAP-INSOL also shifts to 3302 cm^{-1}. This confirmed that the saponification effectively decomposed the proteins contained in both fractions.

Table 4.13 shows molecular weight (MW) and molecular weight distribution (MWD) of solid skim rubber, soluble and solubilized gel fractions. The solid skim rubber and the soluble fraction (SOL-1) had the same number-average molecular weight ($\overline{M}_n$) as well as the weight-average molecular weight ($\overline{M}_w$). It is only natural, because the GPC measurement was done using only the solubilized fraction. After saponification, $\overline{M}_n$ and $\overline{M}_w$ of the soluble fraction (SOL-2) slightly decreased to 0.84×10^5 and 2.69×10^5, respectively. It is noteworthy that after saponification the solubilized insoluble-fraction had the same $\overline{M}_n$ and $\overline{M}_w$ as the soluble fraction before saponification. This suggests that the gel fraction referred to soft gel is composed of loose crosslinked linear rubber chains, which are decomposed by saponification.

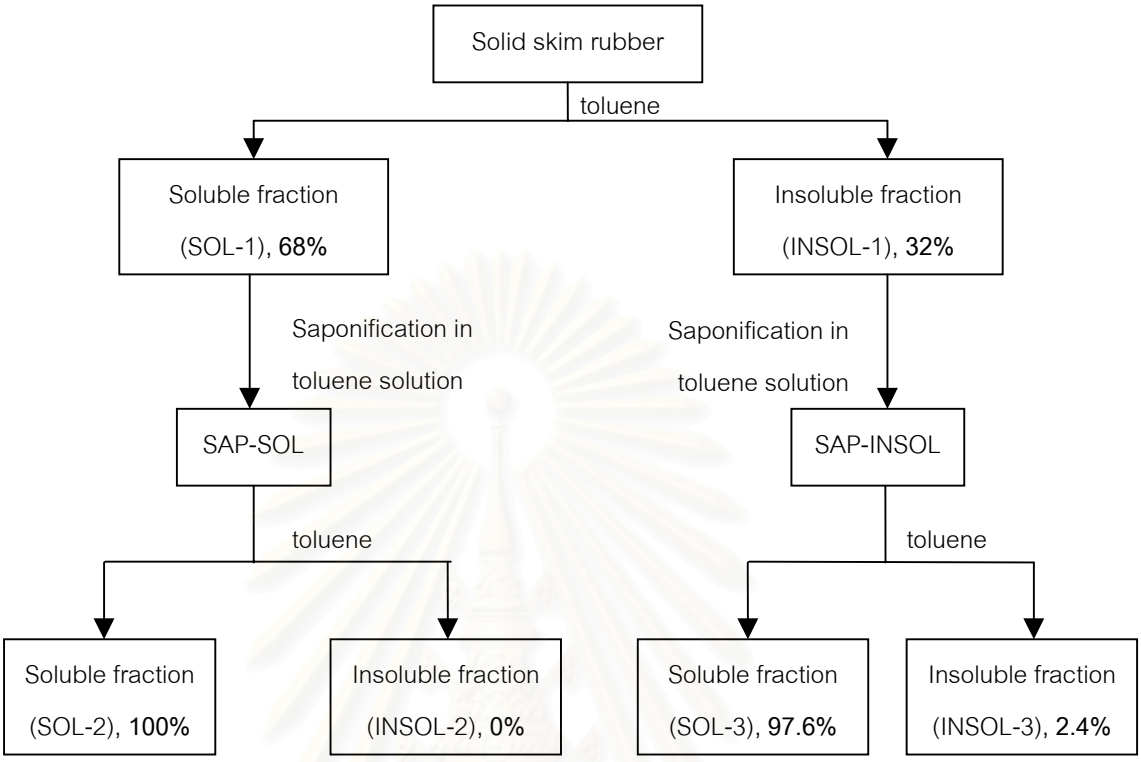


Figure 4.18 Fractionation of solid skim rubber by toluene.

Table 4.12 Nitrogen content of fractions of skim rubber.

Fractions	%N (%, w/w)
Solid skim rubber	2.57
SOL-1	0.20
INSOL-1	7.33
SAP-SOL	0.15
SAP-INSOL	0.15

Table 4.13 MW and MWD of solid skim rubber; soluble and solubilized gel fractions

Fraction	$\overline{M}_n$ ($\times 10^{-5}$)	$\overline{M}_w$ ($\times 10^{-5}$)	$\overline{M}_w / \overline{M}_n$
Solid skim rubber	1.10	3.14	2.85
SOL-1	1.34	3.86	2.88
SOL-2	0.84	2.69	3.20
SOL-3	1.14	4.00	3.49

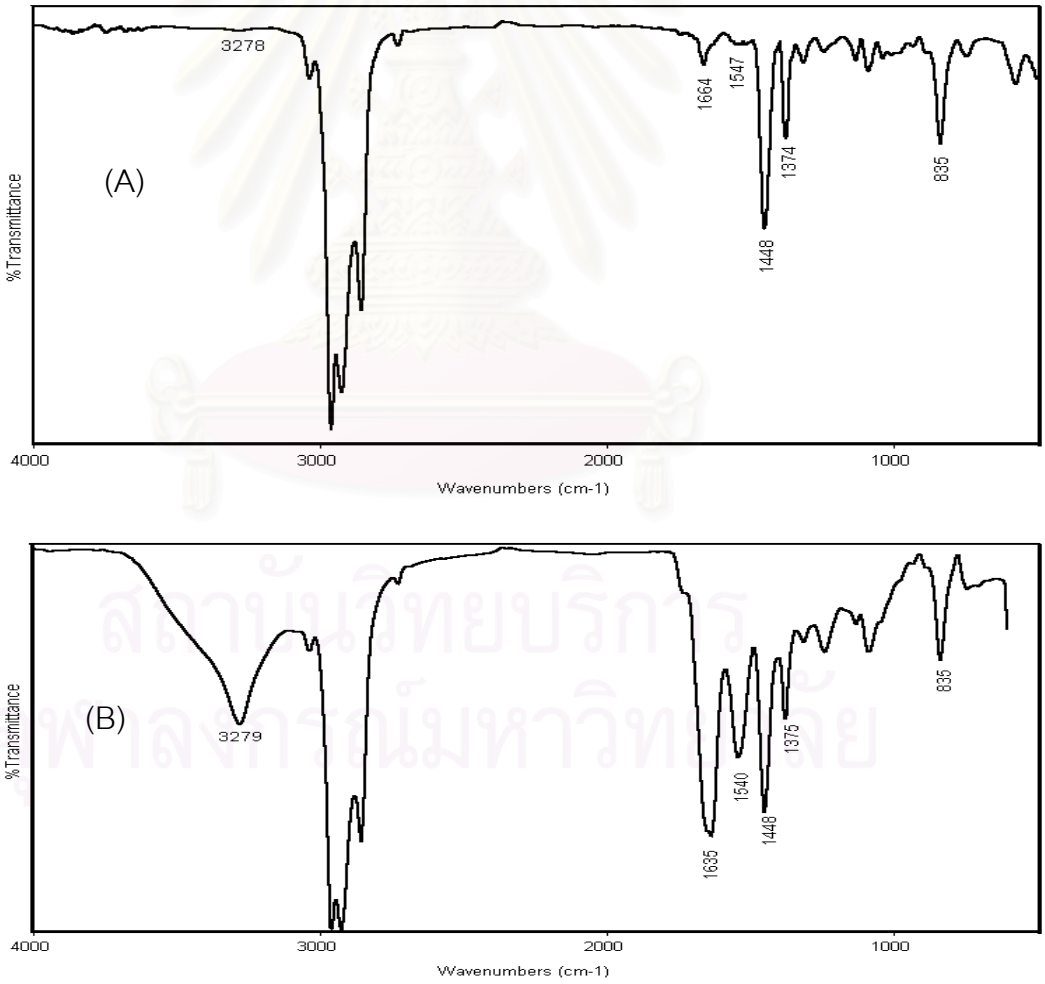


Figure 4.19 FTIR spectra of (A) soluble fraction (SOL-1) (B) insoluble fraction (INSOL-1) from solid skim rubber.

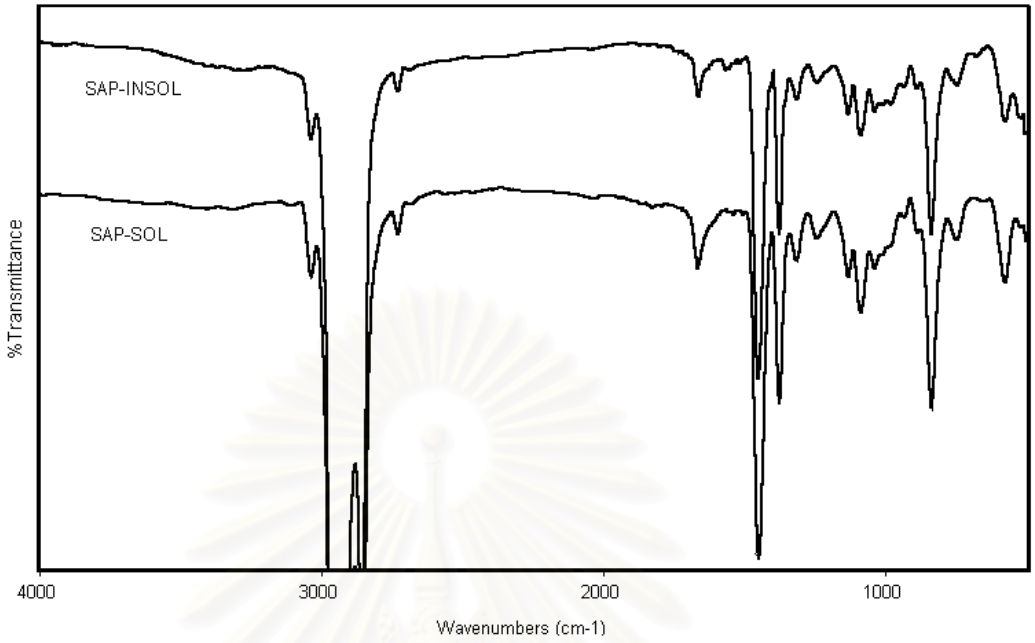


Figure 4.20 FTIR spectra of saponified-insoluble fraction (SAP-INSOL) and saponified-soluble fraction (SAP-SOL) after treatment with aqueous NaOH at 70°C for 3 h

4.4.2 Effect of Rubber Concentration in Toluene Solution

It is necessary to find the highest rubber concentration in toluene solution in order to carry out the saponification reaction effectively. The effect of rubber concentration was investigated by saponification of solid skim rubber in toluene solution with varying the rubber concentration of 6, 10, 12 and 15 % (w/v). Conventional natural rubber in organic solvent with concentration higher than 1% gives the highly viscous solution due to the presence of branched molecules and gel. On the other hand, skim rubber can dissolve in toluene to make a high concentration easily because it contained less branched molecules. Saponification was carried out with 100 cm^3 of 5% NaOH aqueous solution at 70°C for 3 h, followed by washing with 100 cm^3 of water for three times. In this experiment, convention solid rubber, 2% (w/v) STR-5L was used as a control sample for saponification in toluene solution.

The nitrogen, ash, ester contents and toluene soluble fraction of control rubber, solid skim rubber and purified skim rubber are presented in Table 4.14. The saponified control sample, SAP-STR-5L, showed a decrease in the nitrogen content from 0.31% to 0.018%. The nitrogen content of the saponified skim rubber with 6% and 15% rubber concentration in toluene, decreased from 2.57% to 0.02% and 0.12%, respectively. 15% rubber concentration, the solution showed very high viscosity, thus the reaction between NaOH and non-rubber components will not proceed effectively to give the high residual nitrogen content.

Figure 4.21 shows the FTIR spectra of solid skim rubber and purified skim rubber at the rubber concentration of 6% and 15%. The solid skim rubber (%N = 2.57) showed dominant bands at 3270 cm^{-1} and 1540 cm^{-1}, which are characteristic vibration of N-H stretching and bending of protein, respectively. After saponification of 15% rubber concentration with aqueous NaOH, the relative intensity of these bands reduced significantly. Concurrently, a band around 3309 cm^{-1} appeared. On the other hand, in the saponified skim rubber at 6% rubber, the bands at 3270 cm^{-1} and 1540 cm^{-1} of protein disappeared and a band at 3318 cm^{-1} appeared clearly. The band at 3318 cm^{-1} in the purified skim rubber at 6% rubber concentration was presumed to be band characteristic of N-H stretching in the amino acid of di-peptide and tri-peptide [49]. It was reported that, the number of repeating units of peptide increases, the intensity of band at 3270 cm^{-1} due to the inner N-H units of the peptide linkages increased in intensity [49]. In addition, in the associate (bonded) and dissociate proteins, the N-H peaks appeared at about 3313 cm^{-1} and 3390 cm^{-1}, respectively [49]. Therefore, the bands at 3309 cm^{-1} of purified rubber at 15% rubber concentration was postulated to be the band due to tetra-peptide and penta-peptide which are associated to rubber chain.

As can be seen in Table 4.14 and Figure 4.22, the ash content of purified skim rubber at 6%, 10%, 12% and 15% rubber concentration decreased from 0.94% to 0.26, 0.36, 0.44, 0.6%(w/w), respectively. Ash content decreased with decreasing rubber concentration. This indicates that the rubber concentration affected the washing efficiency.

The higher concentration of rubber solution resulted in the lower efficiency of the removal of the alkaline involved in the reaction due to viscosity of the solution.

From Table 4.14, the ester content of purified skim rubber decreased from 3.8 to about 2 mmol/kg rubber. This indicated that saponification in toluene solution liberated the fatty acid groups from the ester groups in rubber chain and free fatty acid esters. In addition, the saponification increased the soluble fraction of skim rubber in toluene from 73.2% to 77.8-95.7% for the rubber concentration of 6-15%, respectively. This implied that the non-rubber component was removed by saponification and washing with water.

On the basis of these findings, saponification in 10% rubber solution was presumed to be the appropriate condition. Eventhough 12% rubber in solution, the reduction of the nitrogen content was almost the same as 10% rubber concentration, the mixing of 12% rubber concentration solution was much more difficult than that of 10%.

Table 4.14 Nitrogen, ash and ester contents and toluene-soluble fraction in purified solid skim rubber saponified in toluene at 70°C for 3 h and washed with water.

Sample	Rubber concentration (%, w/v)	Nitrogen content (%, w/w)	Ash content (%, w/v)	Ester content (mmol/ kg rubber)	Toluene-soluble fraction (%, w/w)
STR-5L	-	0.310	0.16	20.23	91.0
SAP-STR-5L	2	0.018	0.13	19.50	100.0
Original skim rubber	-	2.570	0.94	3.76	73.2
SS-6	6	0.024	0.26	2.22	96.8
SS-10	10	0.037	0.36	1.94	94.4
SS-12	12	0.042	0.44	1.74	92.4
SS-15	15	0.119	0.60	3.16	77.8

SS-6 = Saponified skim rubber at 6% rubber concentration

SS-10 = Saponified skim rubber at 10% rubber concentration

SS-12 = Saponified skim rubber at 12% rubber concentration

SS-15 = Saponified skim rubber at 15% rubber concentration

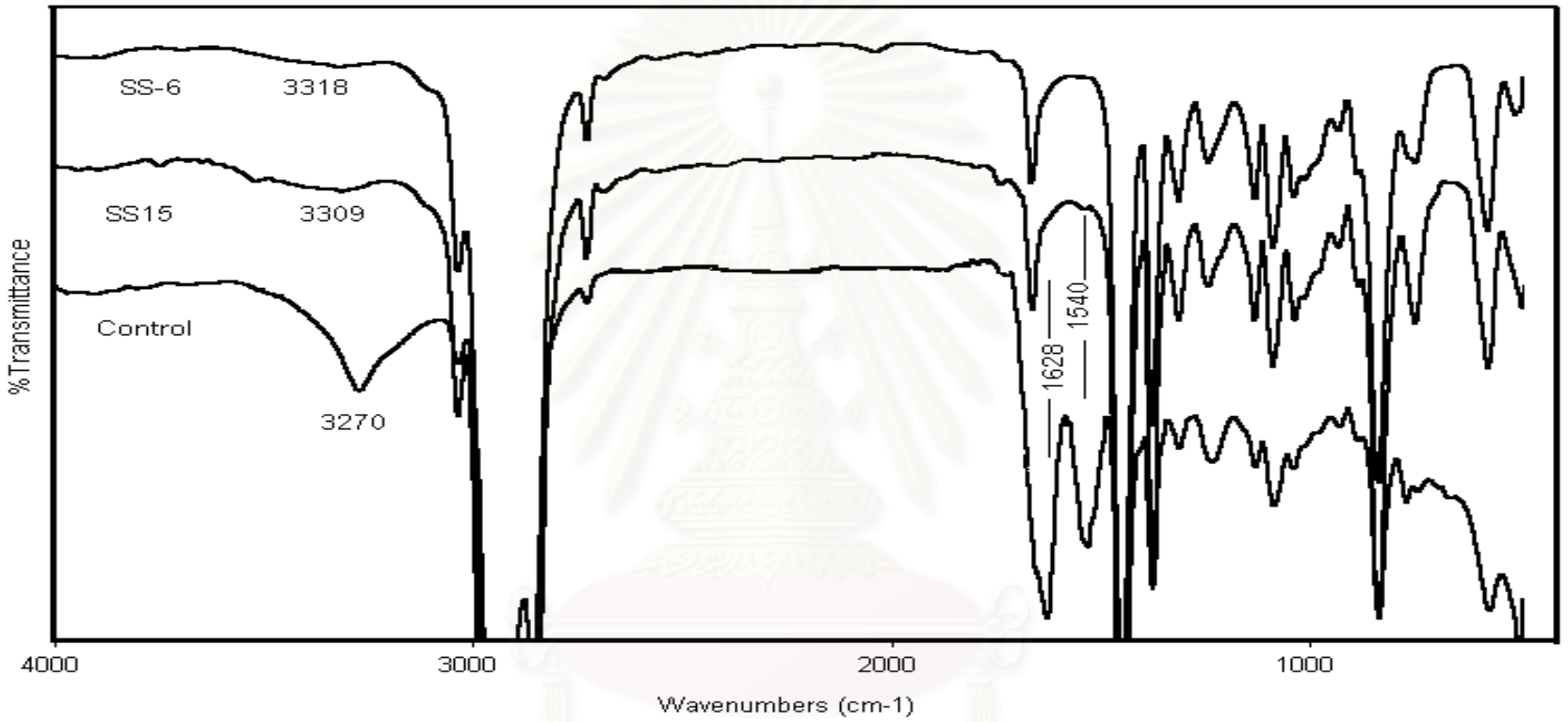


Figure 4.21 FTIR spectra of purified skim rubber by saponification in toluene solution at 70 °C for 3 h and washing with water

SS-6 = Saponified skim rubber at 6% rubber concentration

SS-15 = Saponified skim rubber at 15% rubber concentration

Control = solid skim rubber

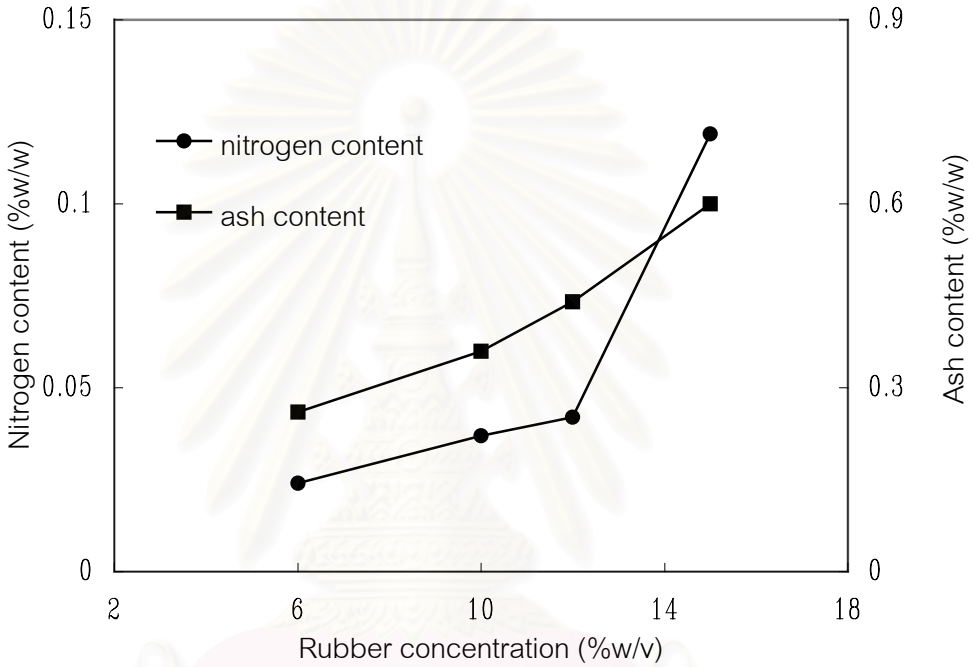


Figure 4.22 Nitrogen and ash contents of the purified skim rubber with different rubber concentrations by saponified in toluene solution at 70°C for 3 h, followed by washing with water 3 times

4.4.3 Effect of Sodium Hydroxide Concentration

Effect of NaOH concentration on nitrogen, ash, ester contents and toluene-soluble fraction was investigated by varying the amount of sodium hydroxide from 0 to 100 cm^3 (0 -1.43% w/total volume). The rubber concentration in toluene solution was kept at 10% (w/v). Table 4.15 shows the nitrogen, ash, ester contents and toluene-soluble fraction of the solid skim rubber and purified skim rubber.

From Table 4.15 and Figure 4.23, The nitrogen content was reduced with increasing NaOH concentration to 0.34%, while NaOH concentration higher than 0.34% has no significant influence on the reduction of nitrogen content. The nitrogen content of 0.03% was obtained independent of the NaOH concentration. Thus, the concentration of NaOH of 0.34 %(w/ total volume) is appropriate for the decomposition of proteinaeous materials in skim rubber. The ash content in the resulting rubber decreased with increasing NaOH to 0.34%, and slightly increased with increasing NaOH concentration from 0.34 to 1.43%. The saponification using NaOH concentration lower than 0.34% didn't effect to the reduction of ash content because the phase separation between rubber in toluene phase and water phase didn't clear. Thus, the washing efficiency of these solutions was low. This indicates that the optimum NaOH concentration that reduced the alkaline present in rubber was 0.34%.

The ester content in control skim rubber and saponified skim rubber is presented in Table 4.15. The control skim rubber contained ester content of 3.46 mmol/kg rubber. The purified skim rubber contained a slightly lower ester content (1.84-3.48 mmol/kg rubber) than the control sample and showed slightly decreased with increasing NaOH concentration. It is natural to assume that, the hydrolysis of an ester with a base proceeds more effective at the higher concentration of a base. Thus, the lower NaOH concentration can give the purified skim rubber of the higher ester content. The sample SS-20 was analyzed for ^{13}C-NMR, the spectrum is shown in Figure F-3. The purified skim rubber show almost no ^{13}C-NMR signals at 174.2, 34.4, 29.7 and 14.0 ppm, which are due to the carboxylic-carbon (-CH_2-$\underline{C}O_2$-), terminal methylene (-$\underline{C}H_2$-CO_2-), methylene (-($\underline{C}H_2$)-) and methyl carbon ($\underline{C}H_3$-) atoms in the long chain fatty acid groups, respectively.

Table 4.15 Nitrogen, ash, ester contents and toluene-soluble fraction in the purified solid skim rubber with the different NaOH concentrations.

Sample	NaOH concentration (%, w/total volume)	Nitrogen content (%, w/w)	Ash content (%, w/v)	Ester content (mmol/ kg rubber)	Toluene-soluble fraction (%, w/v)
Solid skim rubber	-	2.570	0.94	3.76	68.0
SS-00	0.00	1.944	0.72	1.84	70.8
SS-05	0.10	1.106	0.55	2.74	79.3
SS-20	0.34	0.032	0.15	3.49	94.8
SS-40	0.69	0.032	0.30	2.34	95.9
SS-60	0.97	0.031	0.25	2.34	96.5
SS-80	1.21	0.036	0.33	2.74	96.1
SS-100	1.43	0.037	0.36	1.94	94.4

The toluene-soluble fraction of control skim rubber and saponified skim rubber are presented in Table 4.15. The toluene-soluble fraction of saponified skim rubber was 71-96% for NaOH concentration ranging from 0 to 1.43 % (w/ total volume). Saponification by varying the amount of NaOH in the range of 0.34-1.43% did not show any effect on the amount of the toluene-soluble fraction of purified-skim rubber (95-96%). Thus, The appropriate amount of sodium hydroxide was 0.34% (w/total volume).

Rungvichaniwat et at [43] reported that the NaOH concentration of 3%(w/v) is necessary for the saponification of wet skim crumb by soaking the skim crumb at 70°C for 24 h to reach the nitrogen content of 0.23%. Therefore, the saponification of skim rubber in solution is more effective than the soaking technique due to the very low nitrogen content in the resulting rubber. As mentioned in the Section 4.2, the saponification was done in the skim latex, with 3%(w/v) NaOH at 50°C for 5 h and incubated at 30°C for 24 h followed by centrifugation. In this case, the purified skim rubber contained 0.03% N, and 0.25% ash. By comparison of the amount of NaOH necessary for saponification, it is clear that the saponification in toluene solution can reduce the usage of the NaOH by 9 times.

Table 4.16 shows the average molecular weights and MWD of the control solid skim rubber and purified solid skim rubber by saponification in toluene solution at NaOH concentration of 0.97 and 1.43%. The molecular characteristics of saponified rubber prepared in toluene solution show similar $\overline{M}_n$ and $\overline{M}_w$ to those of the original skim rubber. The saponification of skim rubber resulted in an insignificant change in molecular characteristics. It was reported that the saponification of ordinary natural rubber reduced the $\overline{M}_w$ and $\overline{M}_n$ to about 2/3 of the control sample [27]. This also confirms that skim rubber contains most linear molecules while the ordinary NR consists of branched molecules.

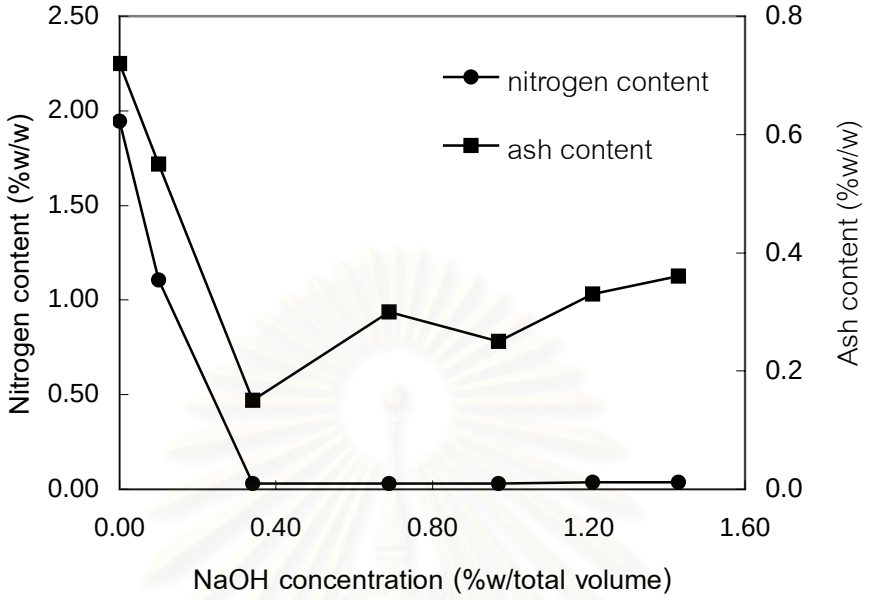


Figure 4.23 Nitrogen and ash contents of the purified skim rubber saponified in toluene with the different NaOH concentration at 70°C for 3 h, followed by washing with water 3 times.

Table 4.16 MW and MWD of the purified solid skim rubber saponified with aqueous sodium hydroxide in toluene at 70°C for 3 h and washed with water.

Sample	$\overline{M}_n$ ($\times 10^{-5}$)	$\overline{M}_w$ ($\times 10^{-5}$)	$\overline{M}_w / \overline{M}_n$
Control solid skim rubber	1.10	3.14	2.85
SS-100	1.17	3.88	3.32
SS-60	1.01	3.57	3.50
SS-20	1.01	3.94	3.90

SS-100 = Saponified skim rubber at 10% rubber concentration with 5% (w/v) NaOH of 100 cm^3

SS-60 = Saponified skim rubber at 10% rubber concentration with 5% (w/v) NaOH of 60 cm^3

SS-20 = Saponified skim rubber at 10% rubber concentration with 5% (w/v) NaOH of 20 cm^3

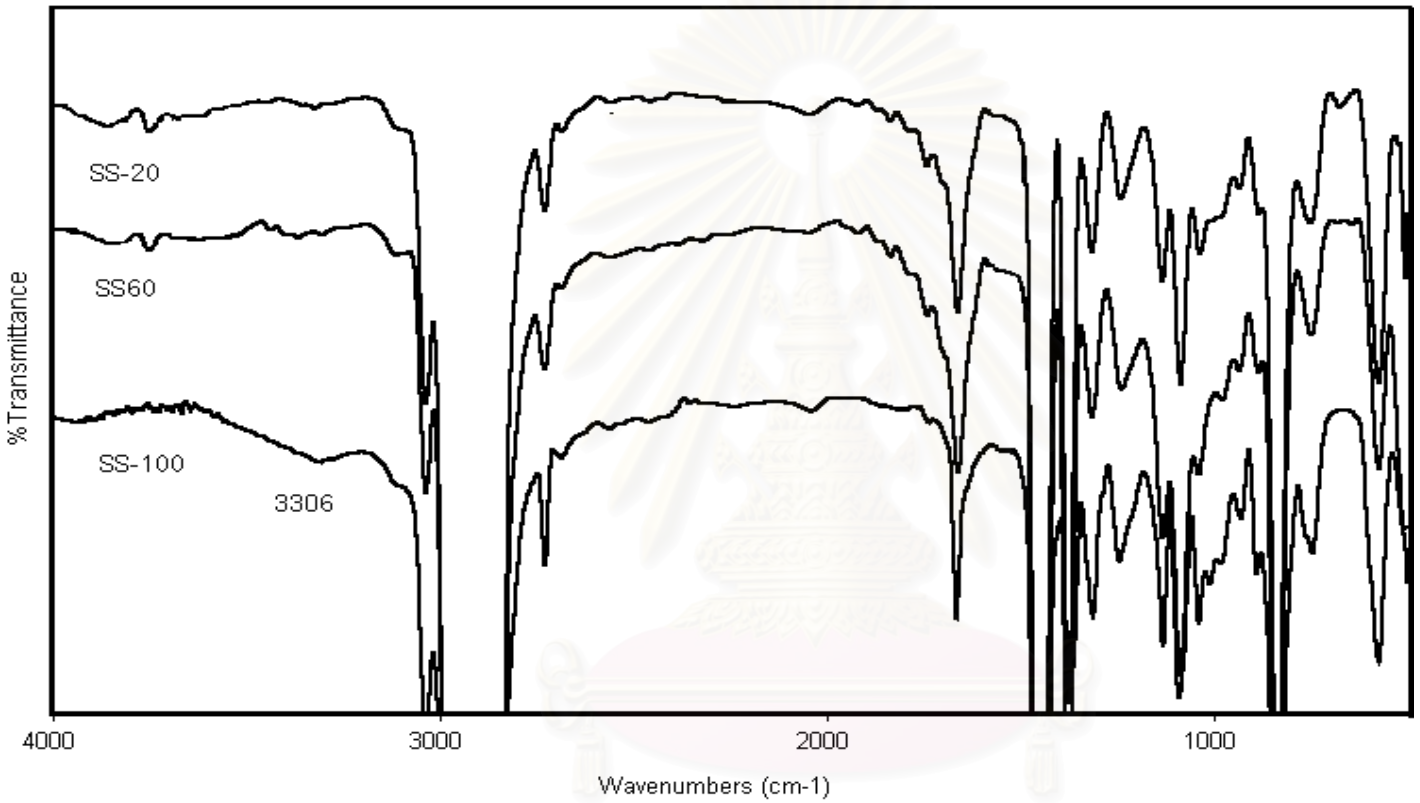


Figure 4.24 FTIR spectra of the purified skim rubber saponified in toluene at 10% rubber concentration and 70°C for 3 h and washed with water.

SS-100 = Saponified skim rubber at 10% rubber concentration with 5% (w/v) NaOH of 100 cm^3

SS-60 = Saponified skim rubber at 10% rubber concentration with 5% (w/v) NaOH of 60 cm^3

SS-20 = Saponified skim rubber at 10% rubber concentration with 5% (w/v) NaOH of 20 cm^3

4.4.4 Raw Rubber Properties of Purified Skim Rubber

The raw rubber properties (green properties) of purified skim rubber were characterized for the saponified skim rubber prepared by using saponification condition of 10% rubber in toluene and 100 cm^3 of 5% NaOH aqueous solution at 70°C for 3 h, followed by washing with water for three times. The green properties of solid skim rubber and purified skim rubber are presented in Table 4.17.

The nitrogen and ash contents of the purified skim rubber were much lower than that of original skim rubber. The color of the purified skim rubber that saponified in toluene solution was 4.5 Lovibond unit, which was lower than that of the original skim rubber (9.0 Lovibond). Thus, the color of purified skim rubber was improved after treatment with aqueous NaOH in toluene solution. The dark color of original skim rubber was due to the non-rubber components included in the course of coagulation. The color is one of the important properties in the production of light-color rubber, such as the SMR-L grade, required a color limit of six units or less on the Lovibond color scale. Thus, the color of purified skim rubber lower than the limitation of STR-5L will be good enough as skim rubber.

Green strength shows the ability of rubber to crystallize rapidly during straining. The stress-strain curves at a given rate of strain are determined for the measurement of green strength. The shape of the stress-strain curve shows the important criterion in determining whether the rubber had good or bad green strength [6]. If the curve shows a positive slope after the yield point, the rubber is evaluated to have good green strength. The more positive the slope, the greater the green strength. The green strength is used as and index of processability.

Figure 4.24 shows the stress-strain curve for untreated skim rubber and purified rubber at a strain rate of 500 mm/min at room temperature, by comparing with conventional natural rubber from field latex (FL). The green strength of skim rubber of 0.86 MPa was lower than that of FL of 4.23 MPa. The rubber from field latex showed the better green strength than skim rubber. The field natural rubber contained fatty acid ester content of 28 mmol/kg rubber and gel content of 7% (w/w). Since the field rubber was not extracted with

acetone, the ester content of field NR is a resulted from mixed fatty acids and linked fatty acids. The effect of linked fatty acid ester groups and gel content on the green strength was reported by Kawahara [4]. It is clear that the higher gel and fatty acid ester contents in the rubber from fresh latex will bring about the larger green strength.

It is noteworthy that the green strength of the purified skim rubber of 0.21 MPa was lower than that of 0.86 MPa of the original skim rubber. By considering the fact that their molecular weights are the same, the decrease in green strength was due to the decrease of the fatty acid ester groups and/or toluene-insoluble fraction that had been removed after saponification. The elongation at break of the purified skim rubber increased double from 880 (original value) to 1683%. This may be due to the decrease of crystallization of the purified skim rubber during stretching. These findings suggest that the toluene-insoluble fraction of 5.6% in the skim rubber induces the crystallization during stretching via the entanglement of branched and crosslinked rubber chains.

The processability of rubber was also measured in term of Mooney viscosity, Wallace plasticity (P_o) and Plasticity retention index (PRI). Mooney viscosity is measured as the torque required to rotate a rotor at constant speed in a sample of the rubber at constant temperature. It is used to study the change in the flow characteristics of rubber during milling or mastication. Mooney viscosity of the original skim rubber and purified skim rubber are the same value, as presented in Table 4.14. Because of their high Mooney viscosity (see Appendix G), both of the original skim rubber and purified skim rubber were considered to be the hard rubber [11]. Thus, both rubbers required long premastication times to obtain the product of a low and consistent viscosity. It is interesting that the Mooney viscosity of purified skim rubber was almost the same as that of the original skim rubber but the toluene-soluble fraction of both rubbers was quite difference. This indicates that the toluene-insoluble fraction had no effect on the Mooney viscosity. In addition, the antioxidant, BHT was added to the purified skim rubber to protect the polyisoprene chain from autoxidation [50]. Thus, the Mooney viscosity of purified skim rubber that added BHT was nearly the original. In fact, without the additional antioxidant the purified skim rubber showed the lower value of Mooney viscosity.

Besides the Mooney viscosity, Wallace plasticity (P_o) and Plasticity retention index are one of the important properties for rubber. These values refer to the resistance of raw rubber to the oxidative degradation before and after aging. P_o of 30 units is required for rubber. If P_o is lower than 30 units, the rubber is considered to be a soft rubber. From Table 4.17, the plasticity (P_o) values of the original and purified skim rubbers were in the range of 41-43, which are acceptable as the STR-5L requirement. Plasticity retention index (PRI) is an index to evaluate the resistance of rubber to molecular breakdown by heat. It is expressed as a percentage of the aged plasticity against the initial plasticity. Without the addition of antioxidant, both the original skim rubber and purified skim rubber showed very low values of PRI, i.e., 11.1 and 15, respectively. This means that the heat resistance of both rubbers was low without the addition of antioxidant. On the other hand, both skim rubber and purified skim rubber contained no naturally occurring antioxidants or some metal ions to accelerate degradation. Some inorganic constituents such as copper, manganese and iron present in NR were considered to be a pro-oxidation [53]. The absence of natural antioxidants in the original skim rubber will be the cause of low PRI value, and the saponification also reduced the ash content significantly (cf. Table 4.12). Eventhough, the purified skim rubber was very prone to thermal oxidative degradation, the addition of the antioxidant to the purified skim rubber can increase the resistance of rubber to oxidation. Consequently, PRI was improved from 15 to 38. This indicates that the antioxidant can improve PRI similar to the results that reported by Silvabalasundevam [54].

Table 4.17 Raw rubber properties of the solid skim rubber and purified skim rubber.

Parameters	Rubber sample		
	STR-5L requirement	Original skim rubber	Purified skim rubber
Ash content, %	<0.40	0.94	0.36
Nitrogen content, %	<0.60	2.57	0.04
Green strength, MPa	-	0.86	0.21
Elongation at break, %	-	880	1683
Wallace plasticity, P_o	35	41	44 (33)
Plasticity retention index, PRI	60	11	38 (15)
Mooney viscosity, ML(1′+4′)100°C	-	79	77 (62)
Color (Lovibond Unit)	<6	9.0	4.5

Note: BHT was added into purified skim rubber as an antioxidant.

Values in the parenthesis are from purified skim rubber without antioxidant.

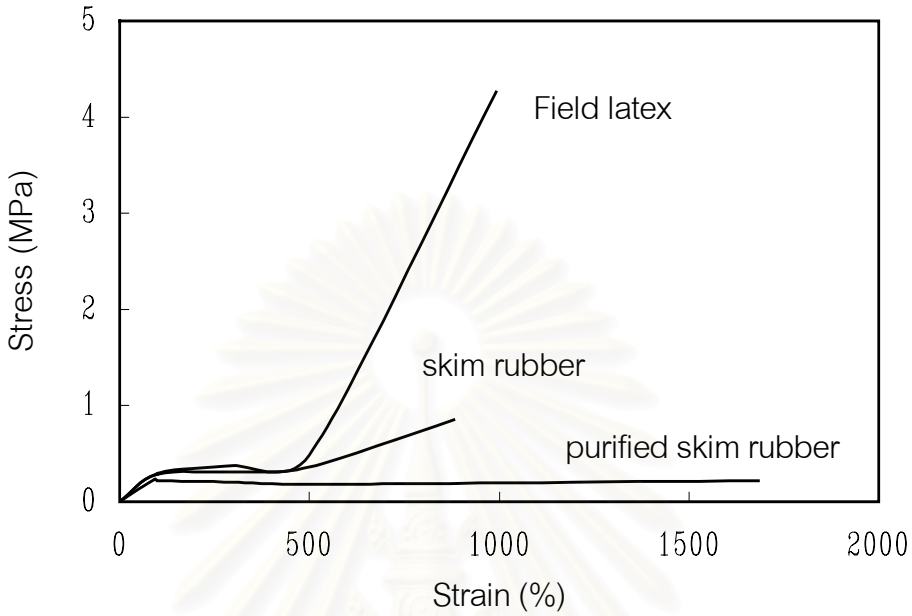


Figure 4.25 Stress-strain curves of unvulcanized rubber.

CHAPTER 5

CONCLUSION AND SUGGESTION

5.1 Conclusions

An attempt was made to get highly purified skim rubber by using four methods. 1) Enzymatic deproteinization in the presence of NaCl. 2) Saponification of skim latex with phase separation. 3) Phase separation of rubber by incubation of skim latex with dry yeast followed by saponification. 4) Deproteinization of solid skim rubber by saponification in toluene solution.

The optimal conditions for enzymatic deproteinization are the treatment of skim latex with 0.04% (w/v) proteolytic enzyme and 7% (w/v) NaCl, at 30°C for 48 h followed by washing with centrifugation for 1 times. The recovery of rubber from skim latex was 100%. The nitrogen and ash contents were 0.04 and 0.57%, respectively.

The appropriate conditions for deproteinization by saponification are the treatment of skim latex with 4% (w/v) NaOH and 1% (w/v) NaCl, at 50°C for 5 h and incubation at 30°C for 24 h followed by washing with centrifugation for 1 times. The recovery of rubber from skim latex was 100%. The nitrogen and ash contents were 0.03 and 0.68%, respectively.

The optimal conditions for the treatment with yeast were incubation at pH 7 in the presence of 0.2% SDS 30°C for 48 hours. The treatment with yeast increased the particle size to facilitate centrifugation. However, the nitrogen content was almost the same before and after the treatment. The recovery of rubber from skim latex was about 45%. The recovery of small rubber particles by incubation with yeast is friendly to the environment. The purified skim rubber by saponification of the yeast-treated cream phase with 4% (w/v) NaOH contained the nitrogen and ash contents of 0.71 and 1.69%, respectively.

The appropriate conditions for saponification of solid skim rubber in toluene solution are the treatment of 10% skim rubber (w/volume of toluene) with 0.34% (w/total volume) NaOH at 70°C for 3 h followed by washing of toluene solution 3 times with water. The nitrogen and ash contents were 0.032 and 0.15%, respectively. The purified skim rubber contained less non-rubber components than the ordinary skim rubber and had the low green strength and high processability. This purified skim rubber was appropriate material for the production of rubber products such as adhesive base.

The incubation of skim latex with dry yeast was the new recovery method for skim rubber. The saponification in toluene solution, involving the organic solvent, produces the highly purified skim rubber by using the small amount of sodium hydroxide. The nitrogen content of the purified rubber was as low as 0.03% by saponification in toluene. The color of purified skim rubber was under the limit of high grad natural rubber.

5.2 Suggestions for Further Work

It is necessary to carry out the following studies for the recovery and purification of small rubber particles.

a) Using other inorganic salts and various concentrations in order to obtain the higher phase separation with lower ash content.

b) Using other type of yeast, which may produce the higher recovery ratio with no effect to the color of serum.

c) Using other kinds of solvents for dissolving rubber in order to get the higher concentration of rubber.

REFERENCES

1. Muller, E. Current Rubber Industries Situation in Thailand and the Need for Structural Changes, *The Rubber International* **2(14)**, 2000: 71-78.
2. Loyen, G. Prospective for Natural Rubber in the Early 21st century, *The Rubber International* **2(13)**, 2000: 71-78.
3. Kageyama, K. Application of Natural Rubber serum. Natural Rubber Current Developments in Product Manufacture and Application. Cetaktama Sdn Bhd: Rubber Research Institute of Malaysia, 1993, pp. 84-97.
4. Kawahara, S.; Isono, Y.; Kakubo, T.; Tanaka, Y.; and Eng, A. H. Crystallization Behavior and Strength of Natural rubber Isolated from Different Hevea Clone, *Rubb. Chem. Technol.* **73**, 1999: 39-45.
5. Sakdapipanich, J. T.; Suksujaritporn, S.; and Tanaka, Y. Structural Characterisation of the Small Rubber Particles in Fresh *Hevea* Latex, *J. Rubb. Res.* **2(2)**, 1999: 160-168.
6. Cameron, A.; and McGill, W. J. A Theory of Green Strength in Polyisoprene, *J. Polym. Sci. Part A-polymer science* **27**, 1989: 1071-1087.
7. Aasa, K. Advances in Natural-Rubber Production, *Rubb. Chem. Technol.* **67(3)**, 1994: 537-548.
8. Goncalves, P. D.; Furtado, E. L.; Bataglia, O. C.; Ortolani, A. A.; May, A.; and Belletti, G. O. Genetics of Anthracnose Panel Canker Disease Resistance and Its Relationship with Yield and Growth Characters in Half-Sib Progenies of Rubber Tree (Hevea brasiliensis), *Genet. Mol. Biol.* **22(4)**, 1999: 583-589.
9. Dunbrook, R. F. Rubber, vol. 20, Colliers Encyclopedia CD-ROM, February 28,1996.
10. Blackley, D. C. Polymer Latices Science and Technology, vol. 2 Types of Latices, 2nd ed., London: Chapman & Hall, 1977, pp. 79.
11. Yip, E. Clonal Characterisation of Latex, and Rubber Properties, *J. nat. Rubb. Res.* **5(1)**, 1990: 52-80.

12. Yew, F. K.; and Pushpapasah, E. Influence of Soil Conditions on Growth of Hevea: Glasshouse Evaluations, *J. nat. Rubb. Res*. **6(1)**, 1991: 62-73.

13. Verhaar, G. Natural Latex as a Colloidal System, *Rubb. Chem. Technol*. **32**, 1959: 1627-1659.

14. Cockbain, E. G.; and Philpott, M. W. Colloidal Properties of Latex. The Chemistry and Physics of Rubber-like Substances, (Bateman, L. ed.), London: Maclaren and Sons, 1963, pp. 73.

15. Johnson, W. Great Britain Patent no. 467 (August 18,1953)

16. Norris, H. L.; and Armstrong, S. T. US 9,891 (July 26, 1853)

17. Chen, S. F.; and NG, C. S. The Natural Higher Fatty Acid Soaps in Natural Rubber Latex and Their Effect on the Mechanical Stability of the Latex*, Rubb. Chem. Technol*. **57(2)**, 1984: 243.

18. Ho, C. C.; Kondo, T.; Muramatsu, N.; and Ohshima, H. Surface Structure of Natural Rubber Latex Particles from Electrophoretic Mobility Data*, J. Colloid Interf. Sci.* **178(2)**, 1996: 442-445.

19. Hasma, H. Lipids Associated with Rubber Particles and Their Possible Role in Mechanical Stability of Latex Concentrates, *J. nat. Rubb. Res.* **6(2)**, 1991: 105-114.

20. Kovuttikulrangsie, S.; and Tanaka, Y. NR Latex Particle Size and its Molecular Weight from Young and Mature Hevea trees, *J. Rubb. Res*. **2(3)**, 1999: 150-159.

21. Moir, G. F. J. Ultracentrifugation and staining of Hevea Latex, *Nature* **184**, 1959: 1626.

22. Eng, A. H.; Tangpakdee, J.; Kawahara, S.; and Tanaka, Y. Distribution and Origin of Abnormal Group in Natural Rubber, *J. nat. Rubb. Res*. **12(1)**, 1997: 11-20.

23. Gregg, Jr. E. C.; and Macey, J. H. The Relationship of Properties of Synthetic Poly (isoprene) and Natural Rubber in the Factory. The Effect of Non-rubber Constituents of Natural Rubber, *Rubb. Chem. Technol*. **46**, 1973: 47.

24. Burfield, D. R. Epoxy Groups Responsible for Crosslinking in Natural Rubber, *Nature* **249**, 1974: 29-30.

25. Burfield, D. R.; Chew, L. C.; and Gan, S. N. Distribution of Abnormal Group in Natural Rubber, *Polymer* **17**, 1976: 713-716.

26. Eng, A. h.; Kawahara, S.; and Tanaka, Y. Trans-isoprene Units in Natural Rubber, *Rubb. Chem. Technol*. **67**, 1993: 159-168.

27. Tangpakdee, J. Structure Characterization of Natural Rubber: Analysis of Biosynthesis Mechanism, Braching Formation and Role of Rubber in Hevea tree, PhD Thesis on Engineering submitted to the Tokyo University of Agriculture and Technology, Japan. 1998.

28. Komuro, K.; Ueda. A.; Wada, K.; and Waku, T. Isoprene Rubber, Tokyo: Taiseisha, 1975.

29. Subramaniam, A. Molecular Weight and Other Properties of Natural Rubber: a Study of Clonal Variations, *Proc. Int. Rubb. Conf. Kuala Lumpur* **4**, 1975: 3.

30. Subramaniam, A. Molecular Weight and Molecular Weight Distribution of Natural Rubber, *Rubb. Res. Inst. Malaysia Technol*. **Bull. No.4**, April 1980.

31. Allen, P. W.; and Bristow, G. M. The Gel Phase in Natural Rubber, *J. Appl. Polym. Sci*. **7**, 1963: 603-615.

32. Tangpakdee, J.; and Tanaka, Y. Purification of Natural Rubber, *J. nat. Rubb. Res.* 12(2), 1997: 112-119.

33. Food and Drug Administration, *FDA Medical Alert*, MDA 91-1, 1991.

34. Tomazic, V. J.; Withrow, T. J.; Fisher, B. J.; and Dillard, S. F. Latex Associated Allergies and Anaphylactic Reaction, *Clin. Immunol. Immunopath*. **64(2)**, 1992: 89.

35. Wyss, M.; Elsner, P.; Wuthrich, B.; and Burg. G. Allergic Contact Dermatitis from Natural Latex without Contact Urticaria, *Contact Dermatitis* **28**, 1993: 154-156.

36. Rietschel, R. L.; and Fowler, J. F. Fisher's Contact Dermatitis, 4th ed., Baltimore: Williams & Wilkins, 1995, pp. 642, 676-8.

37. Hasma, H. Proteins of Natural Rubber Latex Concentrate, *J. nat. Rubb. Res*. **7(2)**, 1992: 102-112.

38. Yip, E.; Turjanma, K.; Ng, K. P.; and Mok, K. L. Allergic Responses and Levels of Extractable proteins in NR Latex Gloves and Dry Rubber Products, *J. nat. Rubb. Res.* **9(2)**, 1994: 79.

39. Bristow, G. M. Composition and Cure Behaviour of Skim Block Natural Rubber, *J. nat. Rubb. Res.* **5(2)**, 1990: 114-134.

40. Nithi-Uthai, B.; Nithi-Uthai, P.; Vithisuwankul, R.; Promna, J.; Promnok, J.; and Boonrasri, S. Uncoagulation Problem of Skim Latex, The 7th international seminar on elastomer (December 16-17, 1998)

41. Dunlop Rubber Co, Ltd. Recovery of Rubber from Latex Skim, British Patent Specification No.695,813 (August 19, 1953)

42. The Firestone Tire & Rubber Co. Improvements in or relating to high grade rubber and method of making same, British Patent Specification No.739,750 (November 2, 1955)

43. Rungvichaniwat, A.; Nithi-Uthai, B.; and Nithi-Uthai, P. The Colour and Nitrogen Content of Skim Rubber, The 7th international seminar on elastomer (December 16-17, 1998)

44. Jayachandran, K.; and Chandrasekaran, M. Biological Coagulation of Skim Latex Using Acinetobacter sp. Isolated from Natural Rubber Latex Centrifugation Effluent, *Biotechnology letters* **20(2)**, 1998: 161-164.

45. Tanaka, Y.; Tangpakdee, J.; Miyamoto, Y.; Hayashi, M.; Kanamaru, E.; and Mihara, T. Method of Producing Particulate Natural Rubber, US 6,025,451, (February 15, 2000)

46. Klinptuksa, P.; and Wongboontarig, P. Preparation and Some Properties of deproteinized natural rubber, *Songklanakarin J. Sci. Technol.* **19(2)**, 1997: 224-229.

47. ASTM D1076 Standard Specification for Rubber-Concentrated, Ammonia Preserved, Creamed, and Centrifuged Natural Latex, 1997: 196-198.

48. Tong, N. Y. RRIM Test Methods for Standard Malaysian Rubbers: SMR bulletin No. 7 Rubber Research Institute of Malaysia, 1992.

49. Eng, A. H.; Tanaka, Y.; and Seng-Neon, G. FTIR studies on Amino Groups in Purified Hevea Rubber, *J. nat. Rubb. Res.* **7(2)**, 1992: 152-155.

50. Arnold, A. R.; and Evans, P. Role of Fatty Acids in Autoxidation of Deproteinised Natural rubber, J. *nat. Rubb. Res*. **6(2)**, 1991: 76.

51. Kawahara, S.; Kakubo, T.; Sakdapipanich, J. T.; Isono, Y.; and Tanaka, Y. Characterization of Fatty Acids Linked to Natural Rubber-Role of Linked Fatty Acids on Crystallization of the Rubber, *Polymer* **41(20)**, 2000: 7483-7488.

52. Walker, G. M. Yeast Physiology and Biotechnology. Chichester: John Wiley & Sons, 1998, pp. 148-149.

53. Hasma, H.; and Othman, A. B. Role of Some Non-Rubber Constituents on Thermal Oxidative Ageing of Natural Rubber. *J. nat. Rubb. Res*. **5(1)**, 1990: 1-8.

54. Silvabalasundevam, J.; and Nadarajah, M. Factor Affecting the Plasticity Retention Index, Proc. *Int. Rubb. Conf. Sri Lanka* 1965: 13-28.

APPENDICES

APPENDIX A

Determination of Total Solids Content (TSC) and Dry Rubber Content (DRC)

Total Solids Content (TSC) and Dry Rubber Content (DRC) are the general specification requirements for latex. The determination of TSC and DRC of skim latex was analyzed following ASTM D-1076.

A-1 Total Solids Content (TSC)

Procedure:

Skim latex was weighed accurately with microbalance near 2.5 ± 0.5 g to nearest 1 mg was in the covered weighing dish. The latex was distributed over the bottom of the dish and dried in a vented air oven for 16 hours at 70 ± 2°C. The dried rubber was cooled in a desiccator to room temperature, and weigh. Drying and weighing was repeated until the mass is constant to 1 mg or less. The triplicate of sample was done for the average result.

$$\text{Total solid, \%} = \left[\frac{(C - A)}{(B - A)}\right] \times 100 \qquad \text{(A - 1)}$$

Calculations:

The percentage of total solids content was as follows:

Where:

A = the weight of the weighing dish, g;

B = the weight of the dish plus the original sample, g; and

C = the weight of the dish plus the dried sample, g.

A-2 Dry Rubber Content (DRC)

Procedures:

The skim latex was weighed approximately 10 g to the nearest 1 mg into a porcelain evaporating dish. For completely coagulation of skim rubber, sufficient 2% (w/v) of acetic acid was added while stirring constantly over a 5 minutes period. The dish was stand at room temperature until a clear serum appeared. A coagulated latex particle was pick up with the main body of the coagulum. The coagulum was washed with running water, passed between rolls to a thickness of 2 mm and dried at 70 ± 2°C in a vented air oven atmosphere. The dried rubber was cooled in a desiccator to room temperature and weigh. Drying and weighing was repeated until the mass is constant to 1 mg or less. The triplicate of sample was done for the average result.

Calculations:

$$\text{Dry rubber content, \%} = \frac{\text{weight of dry coagulum}}{\text{weight of sample}} \times 100 \qquad \text{(A - 2)}$$

APPENDIX B

Determination of Nitrogen and Ash Contents

B-1 Nitrogen Content

Nitrogen in rubber can be used to provide an indication of the protein content. The general formula for protein content, protein = 6.25 × nitrogen content, is widely used.

Nitrogen content is analyzed using modified Kjeldahl method from RRIM Test Method part B7. The rubber is oxidized by digestion of rubber with concentrated sulfuric acid in the presence of a catalyst, thereby converting nitrogen compounds into ammonium hydrogen sulphate. After making the solution alkaline, the ammonia is removed by steam distillation, absorbed in boric acid solution and titrated with standard acid.

Procedures:

The rubber samples weighed accurately about 2.5 g, were gently digested with 10 cm^3 of concentrated sulfuric acid in the presence of 0.85 g catalyst (K_2SO_4 : $CuSO_4 \cdot 5H_2O$: Se = 15 : 4 :2 by weight) until the digest becomes clear green color or almost colorless with no yellow tint after cooling. The solution was allowed to cool before diluting with 40 cm^3 of deinonized water. The 40% (w/v) sodium hydroxide of 30 cm^3 was added before passing steam to the distillation vessel. The distillate was collected in 10 cm^3 of 2% (w/v) boric acid, containing two or three drops of methylred-methylene blue indicator. Distillation was done for 5 minutes so the boric acid turns green. The distillate in receiving flask was immediately titrated with 0.0101M sulfuric acid by automatic titrator. A blank determination was conducted using all the reagents but omitting the sample. Result was averaged from triplicate analysis.

Calculations:

Calculation of nitrogen content was performed with computer software following the equation bellowed.

$$\text{Nitrogen}, \% = \frac{(V_1 - V_B)N \times 0.0140}{W} \times 100 \qquad \text{(B - 1)}$$

Where:

V_1 = the volume of H_2SO_4 required for titration of the contents of the receiving flask, cm^3;

V_B = the volume of H_2SO_4 required for titration of the blank, cm^3;

N = the normality of the H_2SO_4, N; and

W = the weight of sample taken in grams.

B-2 Ash Content

The ash content in rubber was determined following RRIM Test Method part B6. The ash from natural rubber refers to the oxides, carbonates and phosphates of potassium, magnesium, calcium, sodium and other trace elements. The ash may also contain silica or silicates.

The determination of ash content in rubber involves incinerating the test sample in muffle furnace at about 550°C.

Procedures:

A portion of the rubber sample, weighed accurately with microbalance near 5-10 g to the nearest 0.1 mg, was wrapped in ashless filter paper and placed in a crucible, which has been previously ignited and weighed. The crucible and its content was charred over a small frame before introduced into a muffle furnace, controlled at a temperature of 550 ± 20°C until free from carbon. When ashing is complete, the crucible was cooled in desiccator and weighed to the nearest 0.1 mg. The ash content

was calculated from percentage of weight ratio of ash and original rubber. Result was averaged from triplicate analysis.

Calculations:

$$\text{Ash content, \%} = \left[\frac{(B - A)}{W}\right] \times 100 \qquad \text{(B - 2)}$$

Where:

A = the weight of empty crucible, g;

B = the weight of crucible plus ash, g; and

W = the weight of original rubber, g.

APPENDIX C

Data for Calibration Curve of Ester Content

Methyl stearate (MW 298.51) of 0.0132 g in chloroform of 50 cm^3 was prepared as stock solution. The stock solution was pipetted in the amount of 0.00, 0.20, 0.25, 0.50, 1.00 and 2.00 cm^3 for Std-1, Std-2, Std-3, Std-4, and Std-5, respectively.

Table C-1 Intensity ratio for standard calibration curve of ester content

Sample name	Methyl Stearate (mmol)	Polyisoprene weight (g)	Methyl Stearate (mmol/kg rubber)	A_{1738}	A_{1664}	Intensity ratio
Std-1	0	0.0230	0.00	0.031	1.584	0.0196
Std-2	0.000177	0.0231	7.65	0.016	0.373	0.0429
Std-3	0.000221	0.0225	9.82	0.182	1.879	0.0969
Std-4	0.000442	0.0252	17.54	0.105	0.799	0.1314
Std-5	0.000884	0.0225	39.29	0.279	1.060	0.2632
Std-6	0.001768	0.0209	84.59	0.463	0.693	0.6681

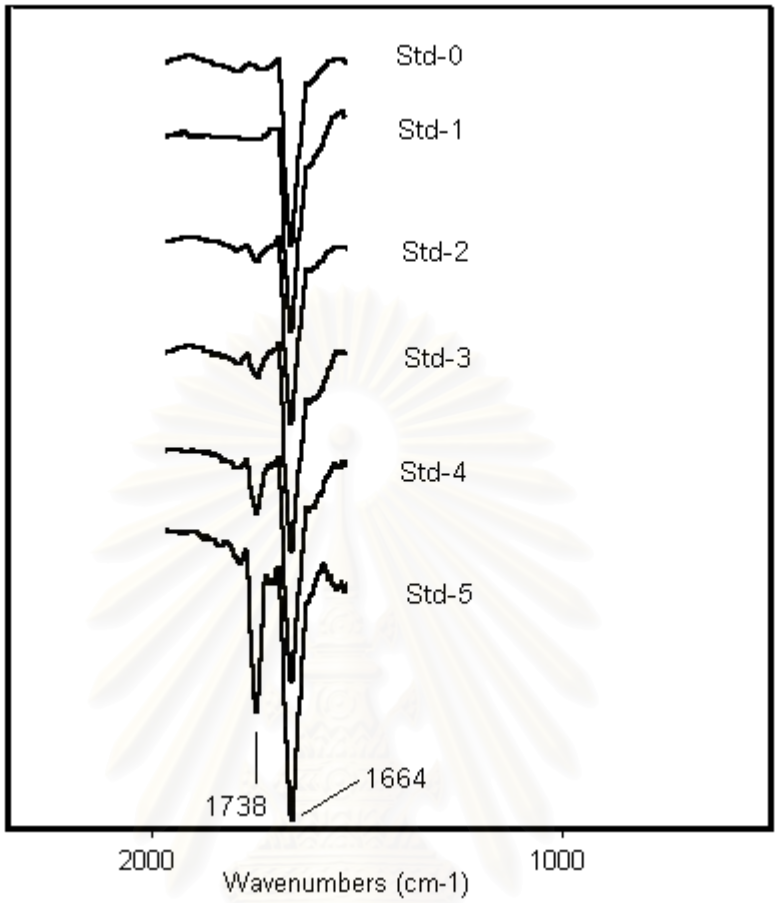


Figure C-1 FTIR spectra of the mixture of methyl stearate and *cis*-1,4 polyisoprene at various methyl stearate concentrations

APPENDIX D

Data of the Average Particle Size of the Purified Skim Latices and Yeast-Treated Latices

Table D-1 Average particle size of rubber particles in skim latices deproteinized with 0.04% enzyme in the presence of various NaCl concentrations at 30°C for 48 h.

Sample name	1st	2nd	3rd	Average particle size (μm)	Std. Dev.
Control	0.11	0.11	0.11	0.11	0.00
DP-0	0.25	0.24	0.24	0.24	0.01
DP-1	0.90	0.89	0.85	0.88	0.03
DP-3	3.21	3.32	3.39	3.31	0.09
DP-5	4.03	4.55	4.87	4.48	0.42
DP-7	4.07	4.22	4.33	4.21	0.13
DP-9	3.25	3.42	3.50	3.39	0.13

Table D-2 Average particle size of rubber particles in skim latices saponified with various NaOH concentrations at 50°C for 5 h and incubated at 30°C for 24 h.

Sample name	1st	2nd	3rd	Average particle size (μm)	Std. Dev.
Control	0.11	0.11	0.11	0.11	0.00
SAP-1	0.11	0.11	0.11	0.11	0.00
SAP-2	0.11	0.11	0.12	0.11	0.01
SAP-3	0.12	0.12	0.12	0.12	0.00
SAP-4	0.12	0.12	0.13	0.12	0.01
SAP-5	0.13	0.12	0.13	0.13	0.01

Table D-3 Average particle size of rubber particles in skim latices saponified with various NaOH concentrations in the presence of 1% (w/v) NaCl at 50°C for 5 h and incubated at 30°C for 24 h.

Sample name	1st	2nd	3rd	Average particle size (μm)	Std. Dev.
Control	0.11	0.11	0.11	0.11	0.00
SAP-C1	0.67	0.59	0.65	0.64	0.04
SAP-C2	0.66	0.61	0.65	0.64	0.03
SAP-C3	0.56	0.56	0.52	0.55	0.02
SAP-C4	0.64	0.55	0.60	0.60	0.05
SAP-C5	0.56	0.62	0.63	0.60	0.04

Table D-4 Average particle size of rubber particles in yeast-treated latices (incubated at 30°C for 48 h)

Sample name	Average particle size (μm)		
	All	Small	Large
Control pH7	1.82	0.35	3.23
	1.81	0.34	3.33
	1.80	0.31	3.35
Average =	1.81	0.33	3.30
Std. Dev. =	0.01	0.02	0.06
Control pH8	0.33	0.24	2.05
	0.37	0.28	2.06
	0.33	0.25	2.07
Average =	0.34	0.26	2.06
Std. Dev. =	0.02	0.02	0.01

Table D-4 (Continued)

Sample name	Average particle size (μm)		
	All	Small	Large
Control pH9	0.36	0.27	2.11
	0.37	0.28	2.08
	0.35	0.25	2.07
Average =	0.36	0.27	2.09
Std. Dev. =	0.01	0.02	0.02
Yeast-treated pH7	17.52	-	17.52
	17.62	-	17.62
	17.48	-	17.48
Average =	17.54	-	17.54
Std. Dev. =	0.07	-	0.07
Yeast-treated pH8	9.64	0.61	20.31
	9.69	0.56	20.35
	9.65	0.63	20.28
Average =	9.66	0.60	20.31
Std. Dev. =	0.03	0.03	0.04
Yeast-treated pH9	3.28	0.23	12.76
	3.33	0.24	12.75
	3.29	0.29	12.78
Average =	3.30	0.25	12.76
Std. Dev. =	0.03	0.03	0.02

Table D-5 Average particle size of rubber particles in yeast-treated latices in the presence of SDS (incubated at 30°C for 48 h)

Sample name		Average particle size (μm)		
		All	Small	Large
Control pH10		0.11	0.11	-
		0.11	0.11	-
		0.11	0.11	-
	Average =	0.11	0.11	-
	Std. Dev. =	0.00	0.00	-
Control pH7 (SDS)		0.92	0.14	5.51
		0.94	0.14	5.56
		0.88	0.16	5.56
	Average =	0.91	0.15	5.54
	Std. Dev. =	0.03	0.01	0.03
F01		0.14	0.19	3.23
		0.18	0.20	3.17
		0.16	0.14	3.28
	Average =	0.16	0.18	3.23
	Std. Dev. =	0.02	0.03	0.06
F02		0.84	0.15	4.64
		0.83	0.17	4.62
		0.80	0.17	4.59
	Average =	0.82	0.16	4.62
	Std. Dev. =	0.02	0.01	0.03
F03		3.26	0.97	3.40
		3.21	0.99	3.31
		3.24	0.99	3.39
	Average =	3.24	0.98	3.37
	Std. Dev. =	0.03	0.01	0.05
F04		5.58	-	5.58
		5.57	-	5.57
		5.61	-	5.61
	Average =	5.59	-	5.59
	Std. Dev. =	0.02	-	0.02

APPENDIX E

Data of Nitrogen and Ash Contents of the purified skim rubber

Table E-1 Nitrogen content of the purified skim rubber by various methods: deproteinization, saponification, and saponification in toluene solution, and skim rubber from yeast-treated latices.

sample	1st	2nd	3rd	Average	Std. Dev.
Control-DP	2.974	2.962	2.970	2.969	0.006
DP-0	0.488	0.503	0.507	0.499	0.010
DP-1	0.446	0.428	0.432	0.435	0.009
DP-3	0.553	0.544	0.540	0.546	0.007
DP-5	0.668	0.663	0.683	0.670	0.010
DP-7	0.712	0.715	0.689	0.705	0.014
DP-9	0.672	0.668	NA	0.670	-
DP-C0	0.147	0.157	0.151	0.152	0.005
DP-C1	0.133	0.124	0.130	0.129	0.005
DP-C3	0.046	0.036	0.040	0.041	0.005
DP-C5	0.051	0.037	0.039	0.042	0.008
DP-C7	0.051	0.037	0.044	0.044	0.007
DP-C9	0.056	0.040	0.025	0.040	0.016
Control-SAP	2.712	2.701	2.698	2.704	0.007
SAP-1	2.486	2.040	2.264	2.263	0.223
SAP-2	0.928	1.231	1.068	1.076	0.152
SAP-3	0.589	0.555	0.563	0.569	0.018
SAP-4	0.369	0.377	0.354	0.367	0.011
SAP-5	0.333	0.335	0.334	0.334	0.001

Table E-1 (Continued)

sample	1st	2nd	3rd	Average	Std. Dev.
SAP-C1	0.092	0.091	0.090	0.091	0.001
SAP-C2	0.087	0.074	0.080	0.080	0.007
SAP-C3	0.037	0.032	0.022	0.030	0.007
SAP-C4	0.030	0.035	0.026	0.030	0.005
SAP-C5	0.021	0.019	0.020	0.020	0.001
Control pH10	2.712	2.701	2.698	2.704	0.007
Control pH7	2.712	2.701	2.698	2.704	0.007
F01	2.621	2.623	2.615	2.620	0.004
F02	2.672	2.694	2.645	2.670	0.025
F03	2.725	2.659	2.598	2.661	0.063
STR-5L	0.312	0.314	0.304	0.310	0.005
SAP-STR-5L	0.020	0.018	0.017	0.018	0.002
control-SS	2.569	2.574	2.568	2.570	0.003
SS-06	0.025	0.024	0.024	0.024	0.001
SS-10	0.034	0.038	0.038	0.037	0.002
SS-12	0.034	0.038	0.038	0.037	0.002
SS-15	0.191	0.095	0.07	0.119	0.064
SS-00	1.732	2.238	1.862	1.944	0.263
SS-05	1.171	1.043	1.104	1.106	0.064
SS-20	0.032	0.032	0.032	0.032	0.000
SS-40	0.033	0.038	0.026	0.032	0.006
SS-60	0.022	0.042	0.031	0.032	0.010
SS-80	0.036	0.041	0.032	0.036	0.004
SS-100	0.034	0.038	0.038	0.037	0.002

Table E-2 Ash content of the purified skim rubber by various methods: deproteinization, saponification, and saponification in toluene solution

Sample	1st	2nd	3rd	Average	Std. Dev.
Control-DP	0.84	0.93	0.68	0.82	0.13
DP-0	0.58	0.65	0.30	0.51	0.19
DP-1	0.46	0.52	0.74	0.63	0.16
DP-3	1.22	0.96	0.90	1.03	0.17
DP-5	1.10	0.94	1.18	1.07	0.12
DP-7	1.42	1.67	1.59	1.56	0.13
DP-9	1.32	1.83	1.15	1.43	0.35
DP-C0	0.23	0.18	0.18	0.18	0.03
DP-C1	0.3	0.29	0.28	0.29	0.01
DP-C3	0.11	0.36	0.47	0.31	0.18
DP-C5	0.60	0.50	0.41	0.50	0.06
DP-C7	0.27	0.71	0.73	0.57	0.26
DP-C9	0.62	0.58	0.67	0.62	0.04
Control-SAP	0.54	0.55	0.55	0.55	0.01
SAP-1	0.38	0.35	0.38	0.37	0.02
SAP-2	0.41	0.41	0.39	0.40	0.01
SAP-3	2.06	2.10	2.59	2.25	0.30
SAP-4	2.09	2.71	2.56	2.45	0.32
SAP-5	2.42	2.90	2.60	2.64	0.24
SAP-C1	0.55	0.55	0.54	0.55	0.01
SAP-C2	0.41	0.38	0.41	0.40	0.02
SAP-C3	0.26	0.23	0.26	0.25	0.02
SAP-C4	0.65	0.71	0.67	0.68	0.03
SAP-C5	0.35	0.41	0.37	0.38	0.03

Table E-2 (Continued)

Sample	1	2	3	Average	Std. Dev.
STR-5L	0.16	0.17	0.15	0.16	0.01
SAP-STR-5L	0.16	0.10	0.14	0.13	0.03
control-SS	0.9	0.94	0.97	0.94	0.04
SS-06	0.22	0.29	0.27	0.26	0.04
SS-10	0.35	0.39	0.35	0.36	0.02
SS-12	0.45	0.43	0.43	0.44	0.01
SS-15	0.71	0.60	0.50	0.60	0.11
SS-00	0.61	0.73	0.83	0.72	0.11
SS-05	0.56	0.52	0.58	0.55	0.03
SS-20	0.15	0.14	0.15	0.15	0.01
SS-40	0.32	0.30	0.31	0.30	0.01
SS-60	0.20	0.27	0.27	0.25	0.04
SS-80	0.32	0.38	0.30	0.33	0.04
SS-100	0.35	0.39	0.35	0.36	0.02

APPENDIX F

^{13}C-NMR Spectra of Purified Skim Rubber

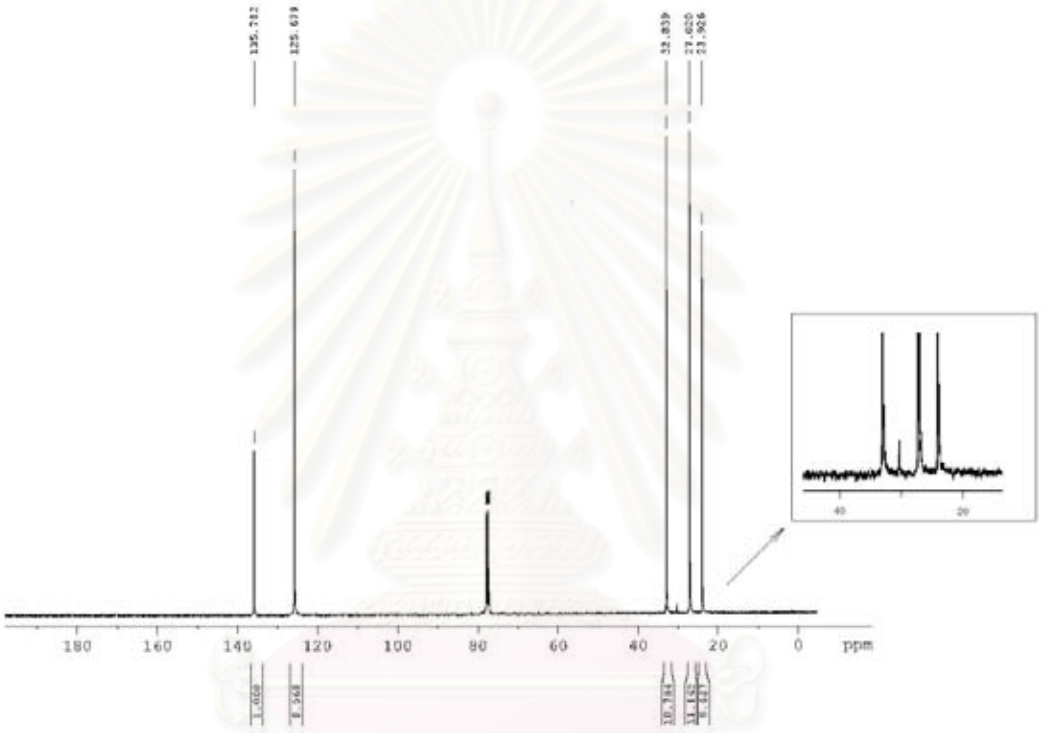


Figure F-1 ^{13}C-NMR spectra of the purified skim rubber obtained by enzymatic deproteinization (0.04% enzyme/ 7% NaCl)

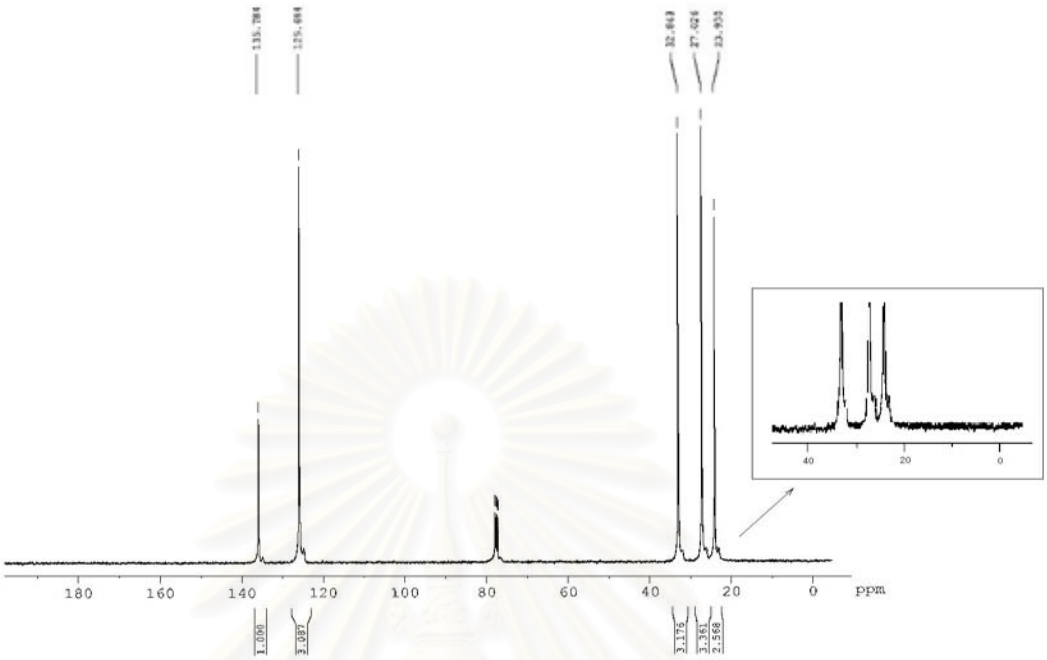


Figure F-2 ^{13}C-NMR spectra of the purified skim rubber obtained by saponification of skim latex (4% NaOH)

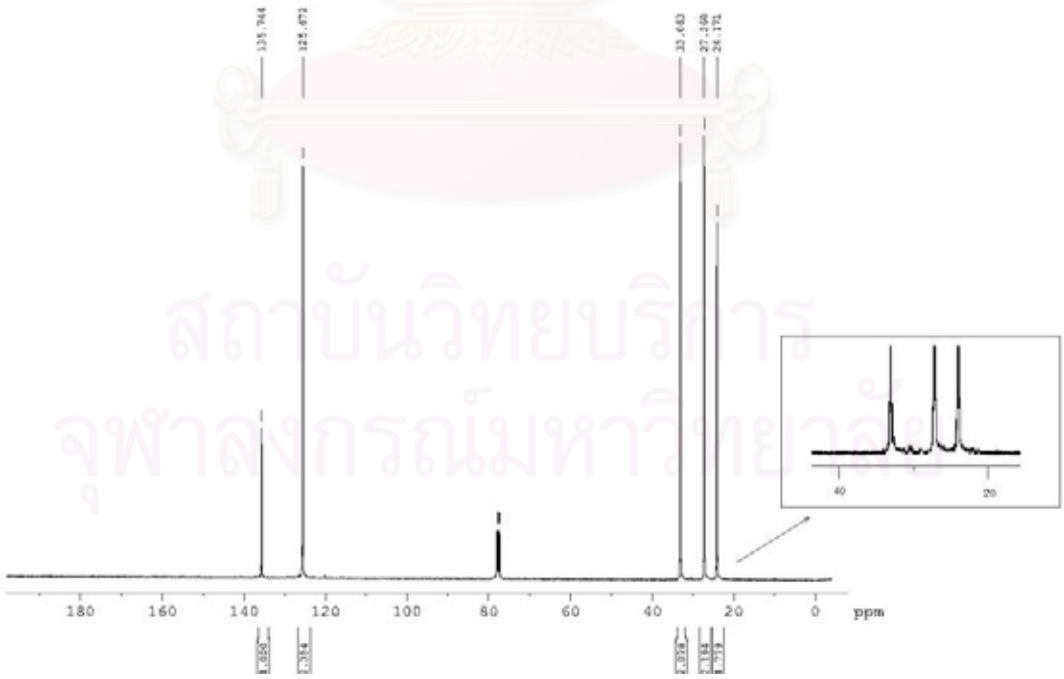


Figure F-3 ^{13}C-NMR spectra of the purified skim rubber obtained by saponification of skim rubber in toluene solution (10% rubber, 0.34% NaOH)

APPENDIX G

The Mooney Viscosity of Raw Rubbers

Table G-1 Classification of Mooney viscosity of raw rubbers from different clones

Raw rubber viscosity (CV)	Mooney viscosity range [ML (1+4) min,100 °C]	Number of clones		
		Class I	Class II	Class IIIA
Low	< 45	Nil	1	Nil
Medium-low	45-55	Nil	1	4
Medium	55-65	3	8	4
Medium hard	65-75	2	6	4
Hard	> 75	1	7	2

These clones are classified base on the growth and yield characteristics of the trees:

Class I clones : High performance materials recommended for large-scale planting e.g. GT1, RRIM 600.

Class II clones : Promising materials, suitable for moderated scale planting e.g. RRIM 623, RRIM 725.

Class IIIA clones : Experimental materials planted up to 10 ha per clone e.g. RRIM 709, RRIM 710.

VITA

Miss Kanokwan Jumtee was born on August 2, 1976 in Bangkok, Thailand. She received her Bachelor's degree of Science in Chemistry, from the Faculty of Science, Kasetsart University in 1996. Since that time she has been a scientist at the Analytical Chemistry Training Division, Department of Science Service, Ministry of Science Technology and Environment. She has pursued Master Degree of Science in Petrochemistry and Polymer Science, Program of Petrochemistry and Polymer Science, Graduate School, Chulalongkorn University since 1998 and finished her study in 2000.