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QUANTITATIVE ANALYSIS OF ORGANIC ACIDS IN AQUEOUS EXTRACTS OF *TAMARINDUS INDICA* PULP AND PREPARATION OF TAMARIND POWDERS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biomedicinal Chemistry
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Thesis Title	QUANTITATIVE ANALYSIS OF ORGANIC ACIDS IN AQUEOUS EXTRACTS OF *TAMARINDUS INDICA* PULP AND PREPARATION OF TAMARIND POWDERS
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Field of Study	Biomedicinal Chemistry
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มะขาม (*Tamarindus indica* L.) ถูกนำมาใช้เป็นยาสมุนไพรเป็นเวลานาน เพื่อวัตถุประสงค์ต่างๆ เช่น ใช้ลดไข้ ต้านเชื้อแบคทีเรีย และโดยเฉพาะอย่างยิ่งการใช้เป็นยาระบายแก้อาการท้องผูก กรดอินทรีย์ในเนื้อมะขามเป็นสารที่มีฤทธิ์เป็นยาระบาย ในการศึกษาครั้งนี้มีจุดประสงค์เพื่อวิเคราะห์หาปริมาณกรดอินทรีย์ในน้ำสกัดเนื้อมะขาม และเตรียมผลิตภัณฑ์ผงมะขามจากน้ำสกัดมะขามกับพอลิแซ็กคาไรด์ของเนื้อในเมล็ดมะขาม การวิเคราะห์ปริมาณกรดอินทรีย์ในมะขามเปรี้ยวและมะขามหวานด้วยเทคนิค HPLC ผลการทดลองพบว่ากรดทาร์ทาริกเป็นกรดที่พบมากในมะขามเปรี้ยว ขณะที่ กรดทาร์ทาริกและมาลิก เป็นกรดที่พบมากในมะขามหวาน ส่วนกรดอินทรีย์อื่นๆที่พบเป็นส่วนน้อยได้แก่ กรดออกซาลิก ซิตริก ซักซินิก และฟูมาริก เป็นต้น พบกรดทาร์ทาริกมีปริมาณสูงในมะขามเปรี้ยวยักษ์จากจังหวัดเพชรบูรณ์ และมะขามเปรี้ยวจากจังหวัดนครราชสีมา (โคราช) ขณะที่มะขามหวานพันธุ์ปลูกสีทองหนักจากจังหวัดนครราชสีมา มีปริมาณกรดทาร์ทาริกต่ำสุด ซึ่งปริมาณกรดทาร์ทาริกที่พบในมะขามแต่ละพันธุ์ปลูกมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) มะขามหวานพันธุ์ปลูกสีทองหนักมีปริมาณกรดออกซาลิก กรดซิตริก กรดฟูมาริกและกรดซักซินิกสูงที่สุด และมีปริมาณแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) เทียบกับมะขามชนิดเปรี้ยว ทำการสกัดพอลิแซ็กคาไรด์จากเนื้อมะขามและเนื้อในเมล็ดมะขามด้วยน้ำร้อน แล้วตกตะกอนด้วยแอลกอฮอล์ การตรวจสอบชนิดของน้ำตาลที่เป็นองค์ประกอบในพอลิแซคคาไรด์โดยวิธีการย่อยด้วยกรดและวิเคราะห์ด้วยเทคนิค HPLC พบว่าพอลิแซ็กคาไรด์จากเนื้อมะขามมีน้ำตาลกรดกาแลคทูโรนิก แรมโนส ไซโลส อราบิโนส ฟรุกโตส และกลูโคส/กาแลกโทส เป็นองค์ประกอบ ส่วนพอลิแซคคาไรด์จากเนื้อในเมล็ดมะขามมีน้ำตาลไซโลสและกลูโคสเป็นองค์ประกอบ การเปรียบเทียบรูปแบบ FT-IR ของพอลิแซ็กคาไรด์จากเนื้อมะขามกับเพคติน พบว่า มีรูปแบบ FT-IR คล้ายกัน พอลิแซ็กคาไรด์จากเนื้อในเมล็ดมะขามที่ความเข้มข้น 2 % มีพฤติกรรมการไหลเป็นแบบซูโดพลาสติก เมื่อเพิ่ม shear rate ความหนืดจะลดลง การเตรียมผงมะขามด้วยวิธีพ่นแห้ง จากน้ำสกัดเนื้อมะขามและพอลิแซ็กคาไรด์ของเนื้อในเมล็ดมะขาม ผงมะขามพ่นแห้งในตำรับที่ประกอบด้วยน้ำสกัดเนื้อมะขามเปรี้ยวยักษ์ และ เนื้อมะขามหวานขันตีอย่างละ 15 กรัม/ลิตร, น้ำตาลฟรุกโตส 1.35 กรัม/ลิตร, โซเดียมคลอไรด์ 0.4 กรัม/ลิตร, มอลโตเดกซ์ตริน 25 กรัม/ลิตร,พอลิแซ็กคาไรด์จากเนื้อในเมล็ดมะขาม 5 กรัม/ลิตร และซิลิคอนไดออกไซด์ 0.3 กรัม/ลิตรใน 1 ลิตรของน้ำดีไอโอไนซ์ พบว่าได้ผงมะขามมีลักษณะเป็นอนุภาคละเอียด ผงแห้งสีมีเหลืองนวล มีปริมาณความชื้น 8.05±0.02 % เมื่อผสมผงมะขาม 10 กรัมในน้ำร้อน 100 มิลลิลิตร ผงมะขามกระจายในน้ำได้หมดภายใน 10 นาที

ภาควิชา : .....ชีวเคมี.....ลายมือชื่อนิสิต : .....วิโรจน์ ชัยพรโภคิน.....

สาขาวิชา : .....ชีวเวชเคมี.....ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก : .....

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WIROD CHAIPORNPOKIN : QUANTITATIVE ANALYSIS OF ORGANIC ACIDS IN AQUEOUS EXTRACTS OF *TAMARINDUS INDICA* PULP AND PREPARATION OF TAMARIND POWDERS. THESIS PRINCIPAL ADVISOR: ASSOC. PROF. SUNANTA PONGSAMART Ph.D., 112 pp.

Tamarind (*Tamarindus indica* L.) has long been used in traditional medicine for several purposes such as antipyretic, antimicrobial and especially as a laxative for constipation. Organic acids in tamarind pulp are the laxative active component. This study aimed to quantitatively determine organic acids in tamarind pulps and prepare tamarind powder from tamarind pulp extracts with tamarind seed polysaccharide (TSP) as carrier. Organic acid contents in sour and sweet tamarind pulps were determined by HPLC. The results showed that tartaric acid was a major acid in sour tamarinds while tartaric acid and L-malic acid were the major acids in sweet tamarinds. The other minor organic acids were oxalic, citric, succinic and fumaric acids. High content of tartaric acid was found in sour tamarinds, "Priao-Yak" from Phetchabun (P) province and "Priao" from Nakhon Ratchasima (Khorat/K), whereas sweet tamarind "Sithong-nak" from Nakhon Ratchasima (Khorat/K) province contained the lowest content of tartaric acid. Tartaric acid contents in tamarind pulp between different tamarind cultivars were significantly different ($P<0.05$). The sweet tamarind "Sithong-nak" contained the highest content of oxalic acid, L-malic acid, fumaric acid and succinic acid; these values were significantly higher ($P<0.05$) than those of sour tamarinds. Tamarind pulp polysaccharide was extracted from tamarind pulp with hot water extraction, followed by acid-alcohol precipitation and Tamarind seed polysaccharide (TSP) was extracted from tamarind seed kernel with hot water, followed by ethanol precipitation. Sugar composition analysis of polysaccharide was determined by acid hydrolysis and HPLC. Tamarind pulp polysaccharide showed the presence of galacturonic acid rhamnose, xylose, arabinose, fructose and glucose/galactose, while tamarind seed polysaccharide showed the presence of xylose and glucose. FT-IR pattern of tamarind pulps polysaccharide was similar to that of commercially available pectin. TSP of sour and sweet tamarind at a concentration of 2% w/v in water exhibited a pseudoplastic flow behavior, increasing shear rate resulted in decreasing viscosity. Tamarind pulp extracts and tamarind seed polysaccharides were formulated to prepare tamarind powder by spray-drying technique. Tamarind powder formulation contained extracts of "Priao-Yak" and "Khantee" 15 g/L each, 1.35 g/L fructose, 0.45 g/L NaCl, 25 g/L maltodextrin, 5 g/L TSP and 0.3 g/L silicon dioxide in 1 L of DI water was prepared by spray drying. The product obtained was light yellow powder with 8.05±0.02 %. moisture content. Tamarind powder product (10 g) was completely dispersed in 100 mL hot water in 10 minutes.

Department : .....Biochemistry.....Student's Signature : WIROD CHAIPORNPOKIN

Field of Study : ..Biomedicinal Chemistry..Thesis Principal Advisor's Signature : Sunanta Pongsamart

Academic Year : .....2008.....

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LIST OF ABBREVIATIONS

C	Concentration
°C	Degree celcius
CA	Citric acid
cm^{-1}	$Centrimeter^{-1}$
cv	Cultivar
d	Day
DE	Degree of Esterification
DI	Deionized
ELSD	Evaporative laser scattering detector
et. al.	Et alii, and Others
F	Shear stress
FA	Fumaric acid
ft	Feet
FT-IR	Fourier Transform Infrared
g	Gram
G	Shear rate
GA	Gallic acid
Gal	Galactose
Gal A	Galacturonic acid
Glc	Glucose
Glc A	Glucuronic acid
HGs	Homogalacturonan I
HPLC	High performance liquid chromatography
hr	Hour
KBr	Potassium bromide
L	Liter
L-MA	L-Malic acid
LOD	Limit of detection

M	Molarity
m^3	Cubic meter
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
NaCl	Sodium chloride
NaH_2PO_4	Sodium dihygrogen phosphate
$(NH_4)H_2PO_4$	Ammonium dihygrogen phosphate
nm	Nanometer
No.	Number
OA	Oxalic acid
P	Pressure
Pa	Pascal
pKa	Acid dissociation constant
r^2	Correlation coefficients
RG I	Rhamnogalacturonan I
RG II	Rhamnogalacturonan II
RH	Relative humidity
RID	Refractive index detector
RSD	Relative standard deviation
S	Physico-chemical nature of substance
SA	Succinic acid
SiO_2	Silicon dioxide
T	Temperature
t	Time
TA	Tartaric acid
TI-K/P	*Tamarindus indica* - Khantee/ Phetchabun
TI-P/K	*Tamarindus indica* – Priao/ Nakhon Ratchasima (Khorat,K)

TI-PY/P	*Tamarindus indica* – Priao- Yak/ Phetchabun
TI-SP/K	*Tamarindus indica* – Srichomphu/ Nakhon Ratchasima (Khorat,K)
TI-STH/K	*Tamarindus indica* – Sithong-nak/ Nakhon Ratchasima (Khorat,K)
TKP	Tamarind kernel powder
TSP	Tamarind seed polysaccharide
USP	The Untied States Pharmacopeia
UV	Ultraviolet
V_{max}	Maximum velocity
w	weight
w/v	weight/volume
Xyl	Xylose
η	Viscosity
%	Percent

CHAPTER I

GENERAL BACKGROUND

Introduction

Now a day, daily lives of constant rush and fat food habits have turned constipation into a common problem of epidemic proportions. The cause of constipation on a physical level is human behavior of eating food which is difficult to digest. Our lifestyle is also an important factor such as sleeping rate, rushing off to work in the morning without giving proper time for evacuation (Haubrich W.S., 1995). The people are rarely able to undertake these important and effective basic measures, as most of them suffer from increasing fatigue, immobility and loss of appetite. There is a clear medical indication for the application of laxative (Klaschik, 2003). There are medicines which induce and facilitate defecation such as lactulose, macrogol, castor oil but the side effect of medicines are flatulence, abdominal cramps and bloating (Klaschik, 2003). Therefore, herb medicines such as tamarind may be good alternative treatment for constipation.

Tamarind (*Tamarindus indica* Linn.) is a member of the dicotyledonous family Fabaceae (Leguminosae), subfamily Caesalpinioideae, genus *Tamarindus*, species indica. Tamarind is a diploid species with a chromosome number of 2n=24 (Purseglove, 1968). Tamarind is native to tropical eastern Africa and is extensively cultivated in tropical areas of the world. Tamarind is widely spread throughout South Asia such as India and Southeast Asia such as Thailand and Malaysia (Gamble, 1922, Chaturvedi, 1985). More recently, Thailand has become a major producer of tamarind, with sweet and sour varieties. Thailand is particularly prominent due to the availability of the sweet tamarind types. In the central, north and northeast of the country small sweet tamarind orchards have been established by smallholders to produce fresh fruits. The main areas of propagation of tamarind in Thailand are in Phetchabun, Loei, Lampang, Chiang Mai, Nakhon Ratchasima and Ubon Ratchathani provinces. There are many kinds of cultivated tamarinds such as Preaw-Yak, Pragaythong, Khantee, Sithong, Srichomphu and Muenjong.

Tamarind is a nutritious fruit with a variety of uses. The tamarind fruit pulp has been an important culinary ingredient in Thailand. Tamarind pulps constitute 30-50% of the ripe fruit (Purseglove, 1987, Shankarachrya, 1998), 11-30% for the shell and fiber and the seed about 25-40% (Chapman, 1984, Shankarachrya, 1998). Tamarind pulps contain low water content and high protein content, carbohydrates, vitamins, minerals and organic acids. The organic acids in tamarind pulps are oxalic acid, tartaric acid, succinic acid and citric acid (Lewis and Neelakantan, 1964, Singh, 1973, Anon, 1976). The sour and sweet tamarind pulps contain tartaric acid 10%, potassium bitartarate 8% and invert sugars 25-40% (British Pharmaceutical Codex, 1911). Besides, the tamarind pulp was used as a mild laxative, organic acid contents in tamarind pulp extract seemed to be an active ingredient for laxative activity of the tamarind extract. Quantitative analysis of organic acid components in tamarind pulp will be necessary in order to control dosage of tamarind extracts for laxative use.

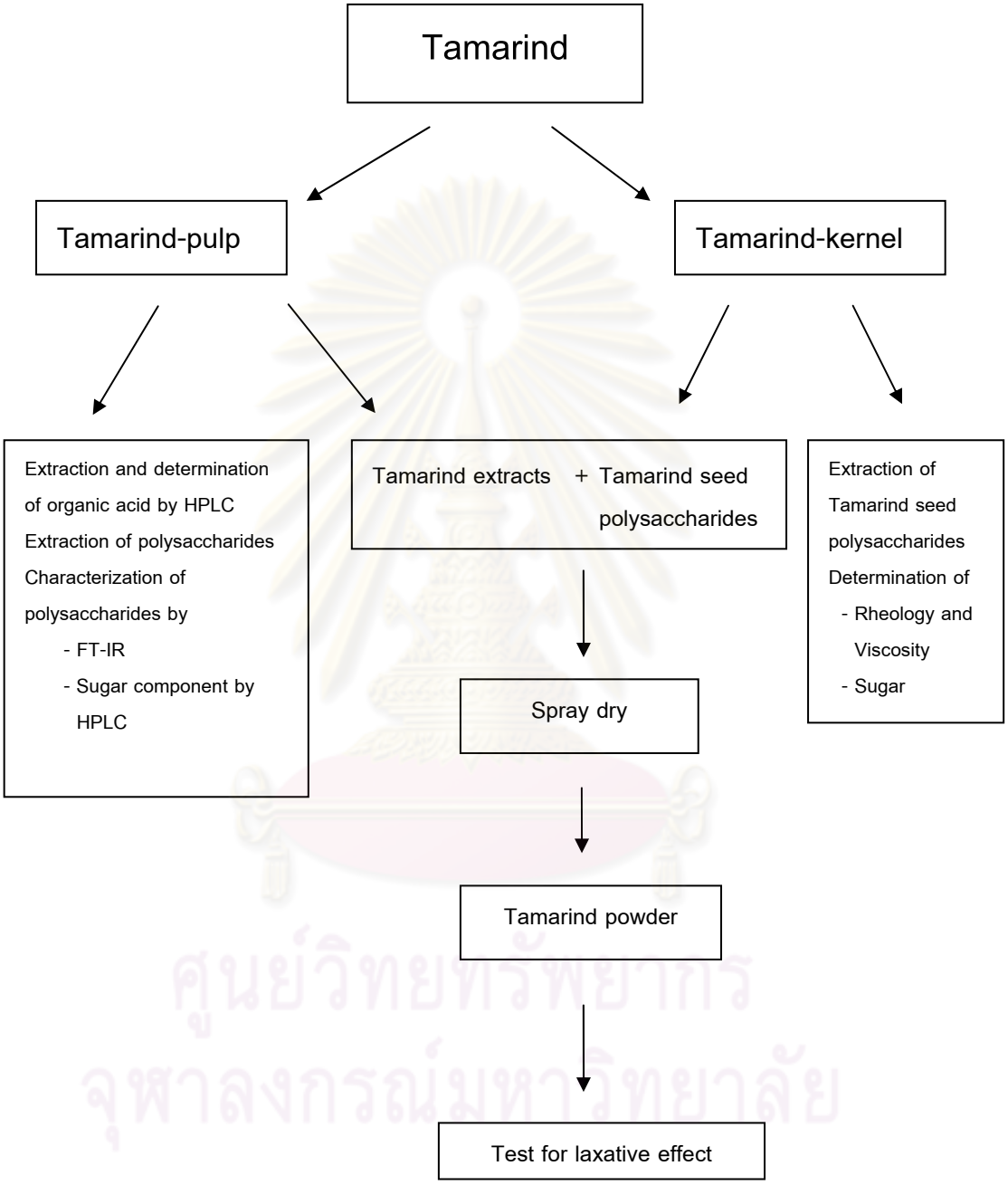
Tamarind seed is a by product of the commercial utilization of the fruit, however, it also has several uses. The seed is also used in the vegetable and food processing industries. Tamarind xyloglucan is the major component of tamarind kernel powder (TKP), TKP forms a stiff gel that has been used for thickening, stabilizing and gelling agents in food. It is commercially available as a food additive for improving the viscosity and texture of processed foods (Sone and Sato, 1994)

The objective of study was to separate and determine of organic acids in tamarind pulp extracts by using HPLC with UV detector and prepare tamarind powder from tamarind pulp extracts with tamarind seed polysaccharide by spray drying technique.

Objective

1. To separate and determine of organic acids in tamarind pulp extracts by using HPLC with UV detector.
2. To study the extraction of tamarind pulp polysaccharide from tamarind pulp.
3. To study the processes for the preparation of tamarind seed polysaccharide (TSP) from tamarind kernel powder.
4. To prepare tamarind powder from tamarind pulp extracts and tamarind seed polysaccharide by spray drying technique.

Conceptual Framework



Anticipated Outcomes

1. Method for quantitative determination of organic acids from tamarind pulps extracts.
2. Formulation of tamarind powder from tamarind pulps extracts with tamarind seed polysaccharide by spray drying technique.
3. Value added product of tamarind.

Chapter II

Literature Review

1. Constipation

The term constipation indicates a subjective impression that describe by Edwards (1993) as follows:

- The frequency of bowel movements is dissatisfying.
- There is a sensation of incomplete evacuation.
- The consistency of the stool is too hard.
- The stool is passed with discomfort.

Indications for constipation are given when there are less than three bowel movements per week, less than 35 g of stool per day, stool water weight is less than 70% and gastrointestinal transit time is longer than five days (Klaschik, 2003).

1.1 Cause of constipation

The possible causes of constipation may be classified into two main categories.

1.1.1 Somatopathic causes

Somatopathic constipation may be a result of diseases such as diverticulitis, tumours, inflammatory processes in the anal area, neurological diseases, endocrinal diseases, recto-anal diseases or metabolic changes.

1.1.2 Functional causes

Functional constipation may be due to prolonged colon passage, defecation disorder, poor intake of fluids or dietary fibers, medication or situational factors such as way of life, lack of exercise.

A large number of substances are known to cause medication-induced constipation such as antibiotics, anticholinergics, antihypertensives, anticonvulsants, diuretics, excessive use of laxative, muscle relaxants, opioids.

1.2 Diagnostics

Diagnostic measures include patient history, medical examination including inspection of the anal area, digital examination of the sphincter muscles and the rectum, stool examination and laboratory tests.

1.3 Therapy

Basic therapeutic measures include :

- Information for the patient about the nature, cause and option for the treatment of constipation
- Information about physiotherapeutic measures
- Promotion of exercise
- Intake of an adequate amount of dietary fiber (to approximately 20-35 g/d) in order to enhance the water-binding capacity of the faeces
- Intake of the required amount of fluid (to about 1.5-2 l/d)
- Avoidance of constipation-promoting food such as flour products, chocolate and tea.

However, patients are rarely able to undertake these important and effective basic measures, as most of them suffer from increasing fatigue, immobility and loss of appetite. So, there is a clear medical indication for using laxatives.

1.4 Laxatives

Laxatives are substances that accelerate defecation. According to their mode of action, they are divided into 4 categories (Klaschik, 2003) :

1.4.1 Bulk-forming laxatives

They consist of natural or synthetic polysaccharides. By absorbing water in the intestine, bulk-forming laxatives increase the volume and softness of faeces, which subsequently increases the propulsive motor function.

1.4.2 Osmotic laxatives

The common denominator of osmotic laxatives is that they are not marginally reabsorbed during the bowel transit. Water intakes as a component of food remains bound and is transferred to the extracellular space in the bowel. Osmotic laxatives are divided into magnesium salts, saccharine, alcohols and macrogols.

1.4.3 Stimulants

Antireabsorptive and secretagogue acting agents inhibit the reabsorption of liquid and sodium from the bowel lumen. They induce an inflow of sodium, chloride, calcium and liquid into bowel lumen. Stimulants include anthracenes, diphenols, castor oil.

1.4.4 Lubricants

Lubricating agents are stool softeners and ease defecation due to their surfactant effect. This substance group includes non-reabsorbable oils, oils are very difficult to reabsorb such as paraffin.

2. Botanical Laxatives

Laxatives of botanical origin include anthraquinones (senna, cascara, frangula, aloe, rhubarb), bulk-forming agents (bran, psyllium, agar), sugar containing herbs (tamarind, cassia and plum) (Capasso, 2002).

2.1 Anthraquinones

The anthraquinones usually occur in nature as glycosides, which behave like pro-drug, liberating the aglycone that acts as the laxative. The metabolism takes place in the colon, where bacterial glycosidases remove sugar. The product obtained are poorly absorbed and act by evoking secretory and motility changes in the colon.

- Senna (*Cassia acutifolia*)

Senna is taken in the form of tea prepared from 0.5-2 g leaves or fruits, fluid extract or syrup.

2.2 Bulk-forming agents

The celluloses, hemicelluloses and lignins contained in bulk-forming laxatives are resistant to human digestive enzymes so they pass unchanged through the small intestine in the colon. In colon retain water and hence stimulate sensory receptor of peristalsis in the intestinal wall. This stimulation produces increased motility.

- Psyllium (*Plantago psyllium*)

Seed of psyllium is used in the amounts of 5-10 g, one to three times daily, usually suspend in a considerable amount of water.

2.3 Herb

2.3.1 Cassia (*Cassia fistula*)

The pulp contains citric acid, tannic substances, pectin, anthraquinone derivatives and fructose. It is used in the form of jam or syrup as a mild laxative for children.

2.3.2 Plums (*Prunus domestica*)

Plums have an excellent laxative action at doses of 50-100 g. Their laxative effect is due to organic acid, invert sugar and oxyphenisatin (Capasso, 2002).

2.3.3 Tamarind (*Tamarindus indica*)

The pulp contains organic acid in the free form and potassium salts, polysaccharide and sugar. Tamarind laxative is taken in the form of juice, jam or syrup.

Table 1. Main botanical laxatives supported by German Commission E to treat constipation

Common name	Latin name	Part(s) of plant used	Key constituents	Daily dose
Aloe	Aloe *spp.*	Juice of the leaves	Anthraquinones, flavonoids	0.05-0.2 g
Buckthorn	*Rhamnus catharticus*	Fruit	Anthraquinones, flavonoids, tannins	2-5 g
Cascara	*Rhamnus purshiana*	Bark	Anthraquinones	1 g
Flax	*Linum ustiatissimum*	Seeds	Mucilages, fatty oil, protein, lignans	1-2 g
Frangula	*Rhamnus frangula*	Bark	Anthraquinones	1 g
Manna	*Fraxinus ornus*	Juice of the bark	Mannitol, oligosaccharide	20-30 g
Psyllium	*Plantago psyllium*	Seeds	Fatty oil, iridoids(aucubin), mucilages, proteins	12-40 g
Rhubarb	*Pheum palmatum*	Bark	Anthraquinones, flavonoids, tannins	1-2 g
Senna	*Cassia spp.*	Leaves, fruits	Anthraquinones(sennosides)	*

* 20-60 mg sennosides

3. Tamarind (*Tamarindus indica* Linn.)

The tamarind is native to tropical Africa and grows wild throughout Sudan. It was introduced into India so long ago, it has often been reported as indigenous in India. It is extensively cultivated in tropical areas worldwide.

3.1 Taxonomy of tamarind

Name	:	*Tamarindus indica* Linn.
Class	:	Dicotyledonae
Order	:	Leguminales
Family	:	Leguminosae
Subfamily	:	Caesalpinioideae
Genus	:	*Tamarindus*
Species	:	indica

Tamarinds are slow-growing, long-lived, evergreen trees that under optimum conditions can grow 80 feet high with a spread of 20 to 35 ft in native eastern Africa and Asia (Gunasena and Hughes, 2000).

3.2 Uses of tamarind.

Tamarinds have useful plant

3.2.1 Twigs and barks

The bark contains tannin and is often employed in tanning hides and in dyeing, and is burned to make an ink. Bark from young trees has low-quality fiber used for twine and string. Bark is used as an astringent for the treatment of diarrhoea, as a cure for asthma and acts as a digestive aid (Gunasena and Hughes, 2000).

3.2.2 Wood

The wood is valued for fuel, for brick kilns. It also yields a charcoal for the manufacture. The wood ashes are employed in tanning and in de-hairing goatskins. Young stems and slender roots of the tamarind tree are fashioned into walking-sticks (Gunasena and Hughes, 2000).

3.2.3 Leaves

Tamarind leaves are useful as mordants in dyeing. Tamarind leaves in boiling water are employed to bleach the leaves of the buri palm (*Corypha elata* Roxb.) to prepare them for hat-making. Young leaves are used as seasoning vegetable (Gunasena and Hughes, 2000).

3.2.4 Flowers

The flowers are used internally as a remedy to cure jaundice and externally to cure eye diseases and skin ulcers.

3.2.5 Pulp

The most valuable and commonly used part of the tamarind tree is the fruit. Tamarind pulp is a mild laxative. Active ingredients of pulp are acids such as citric acid, tartaric acid and malic acid. Tamarind pulp alone or in combination with milk is used as a remedy for constipation (British Pharmaceutical codex, 1911). It is also aid in the cure of malarial fever (Timyan, 1996). Tamarind pulp is also rich in minerals : high in potassium, phosphorus and calcium. It excels in riboflavin and is a good source of thiamin and niacin (Leung and Flores, 1961).

3.2.6 Seed

The seed comprises the seed coat and the kernel. Tamarind seed is the raw material used in the manufacture of tamarind seed kernel powder (TKP), polysaccharide (jellose). Seed powder has also been externally applied on eye diseases and ulcers. Boiled, pounded seeds are reported to treat ulcers and bladder stones and powdered seed husks are used to treat diabetes (Rao, 1975).

3.3 Organic acids in tamarind

3.3.1 Tartaric acid

Tartaric acid ($C_4H_6O_6$) is dihydroxydicarboxylic acid which pKa_1 and pKa_2 at 25°C = 2.98 and 4.34 respectively. It is a white crystalline organic acid. It occurs naturally in many plants, particularly grapes, bananas and tamarinds. Tartaric acid is the highest acid in sour tamarind. Tamarind cultivated in Thailand, the tartaric acid content varied from 2.5 to 11.3%. The tartaric acid content of sweet tamarind was low as 2.0 to 3.2% (Feungchan et al., 1996). Tartaric acid stimulates sensory receptor of peristalsis in the intestinal wall (Martindale, 1989). The table below shows tartaric acid content in “sweet” tamarind cultivars in Thailand (Feungchan et al., 1996).

Table 2. Tartaric acid content in “sweet” tamarind cultivars in Thailand

Tamarind cultivar	Tartaric acid (%)
Sithong	3.18
Piyai	2.01
Praroj	2.70
Srichomphu	2.39
Kru-in	2.70

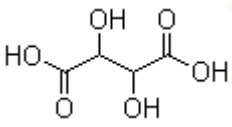
3.3.2 Malic acid

Malic acid ($C_4H_6O_5$) is dihydroxydicarboxylic acid which pKa_1 and pKa_2 at 25°C = 3.40 and 5.11 respectively. Malic acid has two isomeric L-form and D-form. In biological sources, malic acid exists L-form. Malic acid stimulates sensory receptor of peristalsis in the intestinal wall (Martindale, 1989).

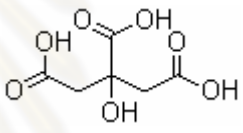
3.3.3 Citric acid

Citric acid ($C_6H_8O_7$) is hydroxytricarboxylic acid which pKa_1, pKa_2 and pKa_3 at 25°C = 3.14, 4.77 and 6.93 respectively. It is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things. It exists in a variety of fruit and vegetables, most notably citrus fruits and tamarind. Citric acid stimulates sensory receptor of peristalsis in the intestinal wall (Martindale, 1989).

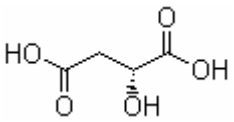
Molecular structures of organic acids in tamarind are shown below



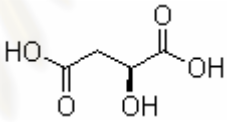
tartaric acid



citric acid



D-malic acid



L-malic acid

Figure 1. Molecular structure of organic acids in tamarind

4. Analysis of organic acid in plants by high performance liquid chromatography (HPLC)

Chromatography is the most useful technique for the separation of organic acids. In chromatographic analysis sample would distribute between a stationary phase and mobile phase. The separation occurrs under an optimum condition such that each component in the mixture would differently partition between the two phases relying upon its affinity. High performance liquid chromatography (HPLC) has simplified the analysis for various food constituents including organic acid. It allows the fast, sensitive and specific analysis. A number of ion exchange, ion exclusion, ion pair and reversed-phase chromatographic methods have so far been developed for the separation and determination of organic acids in various types of sample (Nollet, 1992). Six important organic acids such as oxalic acid, tartaric acid, malic acid,

vitamin C, citric acid and succinic acid in *Fructus mume* can be efficiently separated using reversed-phase high performance liquid chromatography with a mobile phase containing a dilute aqueous solution of $(NH_4)H_2PO_4$ and quantifying with a UV detector (Zhanguo, 2002).

5. Polysaccharide in tamarind

5.1 Pectin in tamarind pulps

5.1.1 Pectin

Pectin substances are a large family of structural elements of primary cell walls and intracellular regions of higher plants. Pectins are among the cell-wall components whose collective ability to contain the turgor pressure of the cell wall determines its growth (Jarvis 1984). The term pectin substance is used to include the methoxyl ester pectin, the deesterified pectic acid and its salts, pectates. Other neutral polysaccharides likes arabinans, arabinogalactans, and galactans lacking the galacturonan backbone are often found in association with pectic substances in the cell wall (Mccan and Robert 1991; Neill et al. 1990). The main component of pectin is backbone chain structure of α-(1→4) – linked D-galacturonic acid units interrupted by the insertion of (1→2)- linked L-rhamnopyranosyl residues in adjacent positions (Aspinall 1980). Another important of galacturonans is the esterification of carboxylic groups in galacturonic acid residues with methanol in certain pectin. The degree of esterification (DE) is defined as the number of moles of methanol per 100 moles of galacturonic acid. Pectins are called high-methoxyl pectins when the value for DE is 50 or higher. While DE is below 50, the pectin is called low-methoxyl pectin. Other constituent sugars are attached in side chains, the most common being D-galactose, L-arabinose and D-xylose.

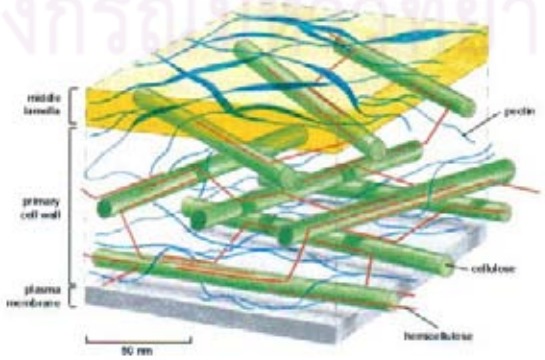


Figure 2. Cell wall and component of cell wall plant. (McCann and Roberts 1991)

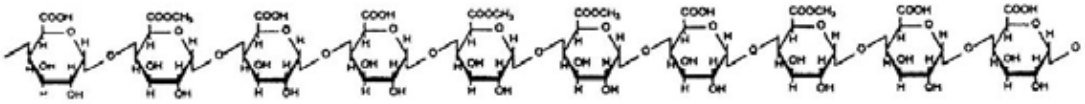


Figure 3. Structure of α-(1→4) – linked D-galacturonic acid of pectin in plant

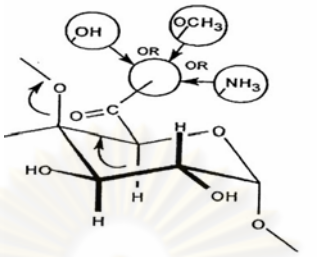


Figure 4. Position of substitute by methoxy or ammonia group

5.1.2 Pectin extraction

Maini (1996) extracted pectin from Galgal (*Citrus pseudolimon* Tan.) peel using aqueous solution of hydrochloric acid as an extractant in dried peel powder:extractant ratio of 1:10 with two extractions of 60 min each followed by alcohol precipitation.

Pagan (1999) extracted pectin from fresh peach pomace. The highest yield of extract were obtained at the highest temperatures and the lowest pH. The yield of extracted pectin were correlated with pH of extraction by a polynomial third degree equation.

Ralet (2007) extracted pectin from citrus peels with water, oxalate, hot dilute hydrochloric acid and dilute sodium hydroxide. Homogalacturonans (HGs) were isolated from the four pectins by mild acid hydrolysis after deesterification. Rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) were isolated from the oxalate- and the acid-extracted pectin using endo-1,4-α-polygalacturonase followed by anion-exchange and size-exclusion chromatographies.

5.2 Polysaccharide in tamarind seed

5.2.1 Tamarind kernel powder (TKP)

TKP is a white powder without testae. It contains crude protein, fat and carbohydrate. TKP is composed of three types of fraction P1, P2 and P3 which differ in their solubilities and power of gelatinization (Rao, 1959; Tamura 1964; Martindale

1982). Fraction P1 had no gelling property, while Fraction P2, P3 had excellent jellying and sizing properties.

Fraction P1 was soluble in water within 2-3 minutes at 5°C, yielded 2-4% of TKP.

Fraction P2 was soluble in water at room temperature within 45 minutes, when the seed meal was vigorously stirred with ten times its weight of water, yielded 20-30% of TKP.

Fraction P3 was insoluble in cold water but completely soluble in boiling water within 20 minutes, yielded 30-50% of TKP.

5.2.2 Characterization of polysaccharide in tamarind seed

Polysaccharide in tamarind seed is called tamarind seed polysaccharide (TSP) or xyloglucan. TSP is a white, fine powder, gives a transparent and high viscous dispersion in water. TSP is not pectin because it does not contain methyl ester groups and galacturonic acid but it can form jellies similar to fruit pectin. TSP is a neutral polysaccharide but contains xylose and forms jellies with sugar concentrates in a wide pH range. It is suggested that jellies forming property at neutral and acidic pH is characteristic of TSP and it was classified as a new class named jellose (Rao, 1956). TSP is most valuable for sizing, printing cotton, artificial silk and textile industries in India. It is excellent for use as fruit pectin in jam, jelly and marmalades (Lawrence, 1976; Lewis, 1970). It can also be used as a good suspending and emulsifying agent in pharmaceutical preparations.

5.2.3 Tamarind seed polysaccharide (TSP) extraction

Degichi and Shiba (1966) extracted polysaccharide from tamarind seed using hot water followed by with sodium sulfate, magnesium sulfate and aluminium sulfate precipitation. The precipitates were washed with lower aliphatic alcohol such as ethyl alcohol solution 20-40% by weight. The coagulation of the polysaccharide took place while sulfate concentration in the extraction exceeded the critical concentration.

Johanson and Pichitkul (1967) tried to extract and purify TKP by boiling fresh TKP with polymetaphosphate and kieselguhr was used as purifying agent. This method gave higher yield than the others and resulted in lighter colored TSP from alcoholic precipitation.

Sandford (1984) extracted TSP by mixing TKP for 2 hours with an alkali aqueous solution to form an aqueous slurry at pH 13. The slurry is diluted with

five volumes of water and stirred for another 2 hours. The diluted slurry is neutralized with hydrochloric acid and the polysaccharide is precipitated with three volumes of 99% isopropyl alcohol. The precipitate is dried for about three hours at about 60°C and milled. The precipitated product is in the alkali metal salt form.

Nitayanont (1979) extracted TSP by mixing fresh TKP with hot water at 80°C for 55 minutes. The TKP dispersion was kept in a water bath at 55°C, enzyme diastase 0.3% by volume was added and left to digest for 2 hours, then the dispersion was filtered and the polysaccharide was precipitated with 95% ethyl alcohol (2 volumes of the filtrate). This method gave 84% yield.

Suttananta (1986) extracted polysaccharide from TKP by hot water at 92-98°C for 1-1.5 hours, then the dispersion was centrifuged and the supernatant was collected. Polysaccharide was precipitated from the supernatant liquid with 1.5 volumes of 95% ethyl alcohol and dried in hot air oven at 50-60°C and milled. This method gave 43% yield.

Shankaracharya (1998) prepared TSP by adding TKP to 30-40 times it weight of boiling water, containing citric or tartaric acid at concentration 0.2%. It is then stirred vigorously and boiled for 30-40 minutes. The resultant solution is kept overnight for setting and the supernatant liquid is siphoned off and concentrated under vacuum, pass through a filter press and then dried in a drum drier. The product is pulverized in a ball mill.

5.2.4 Chemical composition and structure of TSP

Many methods were used to investigate the composition of TSP.

Savur (1959), Srivastava and Singh (1967) investigated the composition of polysaccharide by acid hydrolysis. The hydrolysates contained glucose, xylose, galactose and arabinose in molar ratio 8:2:4:1.

Edward, Iain (1984) were examined xyloglucan polysaccharide from tamarind seed using X-ray diffraction. The result showed that TSP was composed of glucose (G), xylose(X) and galactose(Gal) occurred in the molar ratios of 4:3:1. Chemical structure of xyloglucan suggested by Edward and Iain is shown in Figure 5.

Michael (1991) suggested that the composition of TSP by small-angle-X ray scattering. The major polysaccharide was a galactoxyloglucan for which the ratio galactose:xylose:glucose are 1:2.25:2.8. A minor polysaccharide (2-3%)

contained branched α-(1→5)-L-arabinofuranan and unbranched β-(1→4)-D-galactopyranan features.

York (1993) studied of enzyme degradation of xyloglucan from tamarind seed. Xyloglucan consists of four β-D-glucose residues (Glc) bonded by β-(1→4)-glycosidic linkages, three α-D-xylose residues (Xyl) substituted to each glucose by 1-6 linkages, and one β-D-galactose residue (Gal) substituted to xylose by a β- (1→2) linkage. Chemical structure of xyloglucan suggested by York is shown in Figure 6.

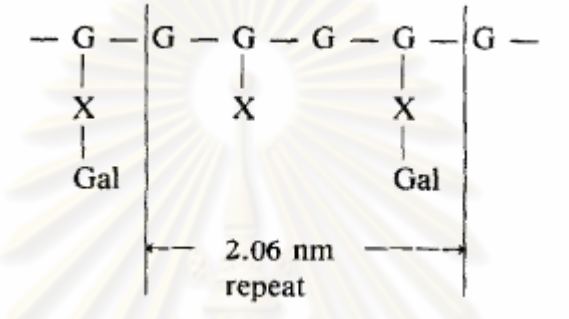


Figure 5. Chemical structure of xyloglucan suggest by Edward, Iain (1984)

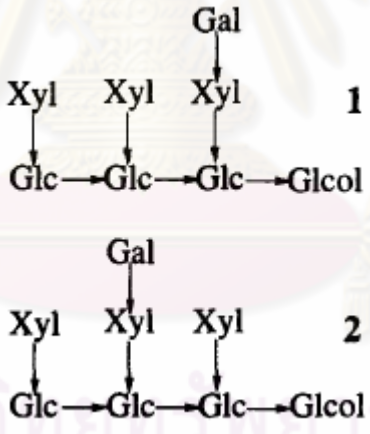


Figure 6. Chemical structure of xyloglucan suggested byYork (1993)

5.2.5 Factors affecting on viscosity of TSP dispersion

- Effect of concentration

TSP dispersion was a non-newtonian flow at the concentration higher than 1.75% (Suttananta, 1986). Nittayanout (1979) compared the relative viscosity of TSP with other gums, TSP dispersion was more viscous than sodium alginate, pectin and acasia at the same concentration but less viscous than carboxymethylcellulose.

- Effect of temperature

Viscosity of TSP dispersion decreased exponentially with temperature (Suttananta, 1986).

- Effect of pH

TSP dispersion gave maximum viscosity at a pH range 4-5 but TSP dispersion was not stable in alkali pH, especially at pH more than 8 (Suttananta, 1986).

- Effect of other substances

TSP was compatible with sorbitol, syrup but incompatible with alcohol, glycerol, polyethylene glycol 400. It was more tolerable to salts or electrolytes as compared with other gums (Suttananta, 1986).

6. Rheology

Shear stress (F)

Shear stress is a force (F') applied to an area (A) being the interface between the upper plate and the liquid layer. The viscosity of flow that can be maintained for a given force will be controlled by the internal resistance of the liquid (Schramm, 1981).

$F = (F'/A) \quad = Pa$ (Pascal)

Shear Rate (G)

The shear stress causes the liquid to flow in a special pattern. A maximum flow speed, V_{max} will be found at upper boundary. The speed drops across the gap size, whereas the $V_{max} = 0$ at the lower boundary contacting the stationary plate. Laminar flows mean that infinite laminar than liquid layers slide on the top of each other. One laminar layer is then displaced with respect to the adjacent ones by a fraction of the total displacement encountered in the liquid between both plates. Shear rate is defined by

$G = (dv/dr) \quad = s^{-1}$

η is the coefficient of viscosity, usually referred to simply as viscosity.

$\eta = (F/G)$

Flow and viscosity curves

The correlation between shear stress and shear rate defining the flow behavior of a liquid graphically can be display in a diagram of shear stress on the ordinate and shear rate on the abscissa. Flow curve is shown in Figure 7.

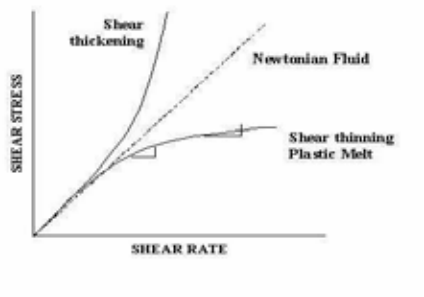


Figure 7. Flow curve

Viscosity parameters

Viscosity describing the physico-chemical properties of a liquid resisted to shear induced flow may depend upon 5 independent parameters

$$\eta = \text{function of } S, T, P, G, t$$

S : The physico-chemical nature of a substance being the primary influence on viscosity, whether the liquid is water, oil, honey.

T : The temperature of the substance, viscosity is heavily influenced by changes of temperature.

P : Pressure, the pressure compresses fluids and thus increases intermolecular resistance. Liquid are compressible under the influence of very high pressure similar to gases but much less. Increase of pressure tends to increase the viscosity.

G : Shear rate is decisive factor influenced the viscosity of very small liquids. Increasing shear rates may decrease or increase the viscosity.

t : Time, denotes the phenomenon that the viscosity of some substances, usually dispersions, depend on the previous shear history.

The measurement of viscosity required test condition providing :

- The applied shear must lead only to laminar flow.

- In Newton's law of viscosity, F = G, the applied shear stress was correlated to shear rate. The shear stress means the one that was just sufficient to sustain a constant flow rate.

- The applied shear stress must be transmitted from the moving plate across the liquid boundary layer into liquid, if the adherence between the moving plate and the liquid is insufficient to transmitted the shear stress, the moving plate would slip above the non-moving liquid sample.

- Samples must be homogeneous

- No physical or chemical change in the sample during testing

- No elasticity

7. Basic principle of spray drying process

The main purpose of drying process is to extend the shelf life of foods by a reduction in water activity that can inhibit the microbial growth and enzyme activity, but the processing temperature is usually insufficient to cause their inactiviation. Therefore any increase in moisture content during storage will result in rapid spoilage. In addition, the reduction in weight and bulk of food reduces transport and storage costs. For some types of food, dehydration provides a convenient product for the consumer or more easily handled ingredients for food processors. Drying causes deterioration of both the eating quality and the nutritional value of the food (Fellow, 2000).

The heat from drying air is absorbed by food and provides the latent heat needed to evaporate water from the surface. An increase in air temperature, or reduction in relative humidity (RH), causes water to evaporate more rapidly from a wet surface and therefore produces a greater fall in temperature. When hot air is blown over a wet food, water vapor in the food diffuses through a boundary film of air surrounding the food and is carried away by the moving air. A water vapor pressure gradient is established from the moist interior of the food to the drying air. The characteristic of air, are moderately high-bulb temperature, low relative humidity and high air velocity (Fellow, 2000).

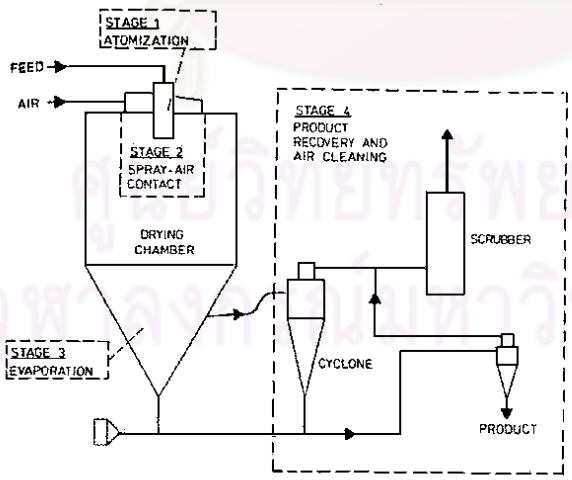


Figure 8. Spray drying process (Master, 1991)

Spray drying consists of four process stages. Schematic diagram of spray drying process is shown in Figure 8. The first stage is atomization of feed into a spray. The second is spray-air contact stage. This stage water in droplet would evaporate by hot air in chamber. The third is evaporation stage which mass and heat transfer occurs in this stage. Finally, product recovery stage, cyclone separator is used for powder recovery from air flow (Master, 1991).

The main advantages of spray drying process are rapid drying, large-scale continuous production, low labors costs and relatively simple operation and maintenance. The major limitations are high capital costs and the requirement for a relatively high-feed moisture content to ensure that the food can be pumped to the atomizer. This results in higher energy costs and higher volatile losses.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Chemicals

Chemical	Grade	Supplier/ Manufacturer
Acetonitrile	HPLC reagent grade	Labscan., Ireland
Ammonnium dihyrogen orthophosphate	Analytical reagent grade	Ajax Finchem., Australia
Barium hydroxide	Analytical reagent grade	Fisher Scientific., UK.
Citric acid	Analytical reagent grade	Fisher Scientific., UK.
D-arabinose	Analytical reagent grade	Sigma-Aldrich., U.S.A.
D-fructose	Analytical reagent grade	E. Merck., Germany.
D-glucose anhydrate	Analytical reagent grade	E. Merck., Germany.
D-galactose	Analytical reagent grade	E. Merck., Germany.
D-xylose	Analytical reagent grade	E. Merck., Germany.
Ethanol	Commercial grade	The Government Pharmaceutical Organization., Thailand.
Fumaric acid	Analytical reagent grade	Fluka., Switzerland.
Gallic acid	Analytical reagent grade	Sigma-Aldrich., U.S.A.
L-ascorbic acid	Analytical reagent grade	Fisher Scientific., UK.
L-malic acid	Analytical reagent grade	Fluka., Switzerland.
L-rhamnose	Analytical reagent grade	Fluka., Switzerland.
Maltodextrin	Food grade	CT Laboratory., Thailand.
Methanol	HPLC reagent grade	Fisher Scientific., UK.
Orthophophoric acid	Analytical reagent grade	Ajax Finchem., Australia
Oxalic acid	Analytical reagent grade	Fisher Scientific., UK.
Pectin	Food grade	Danisco., Mexico.
Potassium bromide	Analytical reagent grade	E. Merck., Germany.
Silicon dioxide	Commercial grade	Maxway Co., Ltd., Germany.
Sodium chloride	Analytical reagent grade	E. Merck., Germany.
Sodium dihydrogen phosphate	Analytical reagent grade	E. Merck., Germany.
Succinic acid	Analytical reagent grade	Ajax Finchem., Australia
Sulfuric acid	Analytical reagent grade	J.T. Baker., U.S.A.
Tartaric acid	Analytical reagent grade	Ajax Finchem., Australia

2. Equipments

Equipments	Model	Supplier/Manufacturer
Balance	XT 620M	Presica Instruments Ltd., Switzerland.
Fourier Transform Infrared Spectrometry (FT-IR)	Spectrum 2000	Perkin Elmer., U.S.A.
HPLC	Class VP software 6.1, System controller SCL-10Avp Pump LC-10ADvp Auto sampler SIL-10ADvp Column oven CTO-10Asvp UV-Vis detector SPD-10Avvp	Shimadzu., Japan.
HPLC	ELSD Detector	Alltech., U.S.A.
HPLC Amino-Column	size 250x4.6 mm.	Alltech., U.S.A.
HPLC Amino-Column –CHO	size 250x4.6 mm.	Alltech., U.S.A.
HPLC C18-column	Hypersil Gold size 250x4.6 mm	Thermo Scientific., U.K.
Magnetic Stirrer	SP 46920-26	Branstead/Hermolyne., U.S.A.
Mini Spray Dryer	B-290	Buchi., Switzerland.
Moisture balance	XM 60	Presica Instruments Ltd., Switzerland.
Oven	Memmert	Becthai Co.Ltd., Thailand.
pH meter	SevenMulti	Mettler Toledo Gmbh., Switzerland.
Refrigerated centrifuge	HIMAC, SCR20B	Hitachi Koki Co. Ltd., Japan.
Rheometer	Rheowin-RV1 software	HAAKE Rheowin., Germany
Rotary evaporator	R-200	Buchi., Switzerland.
Scanning Electron Microscope	JEOL-JSM5410LV	Japan
Suction apparatus, Buchner Funnel, Aspirator, Circulating aspirator	WJ-20	Sibata., Japan.
Ultra sonicator bath	TRASSONIC 890	Becthai Co.Ltd., Thailand
Water bath	Memmert	Becthai Co.Ltd., Thailand.

3. Plant Materials

Fruits of *Tamarindus indica* L. cultivars sour types, “Priao” (TI-P/K) and sweet types, “Srichomphu” (TI-SP/K), “Sithong-nak” (TI-STH/K) were collected during February 2005 from Pak-chong Nakhon Ratchasima (Khorat,K) while “Priao-Yak” (TI-PY/P) and “Khantee” (TI-K/P) were collected during January-April 2005 from Chanika farm, Phetchabun (P) provinces, Thailand.

Methodology

1. Preparation of Dried Pulps and Seeds of Tamarind

Fruit of tamarind were broken and pulps were separated. Tamarind seed was removed from tamarind pulp. The freshed tamarind pulps were dried with hot air oven at 50°C until the constant weight was obtained. Dried tamarind pulps were stored a freezer at -20°C until used. The tamarind seeds were parched and removed the testa (seed coat). The kernels of tamarind seeds were blended. The kernel powder of tamarind seed was kept in dry place at room temperature until used.

2. Development quantitative determination of organic acids in fresh pulps of tamarind

2.1 Sample preparation

Dried pulps of each sample was weighed at equivalent to 75 g of fresh pulp and blended in 200 mL of DI water, and incubated with constant stirring at 90°C for 1 hour, using water-bath. The mixture was centrifuged at 6800xg for 20 min and the supernatant was collected. The precipitate was repeatedly extracted with 100 mL of DI water, and centrifuged to separate the supernatant. The supernatants were collected and pooled together. The water extracts of tamarind pulps were concentrated at 70°C under reduced pressure by Rotavapor R-200. The extract was transferred to a 100 mL volume volumetric flask, diluted to volume with DI water and stored at 4°C, until used.

2.2 Quantitative determination of organic acids in tamarind extracted by High Performance Liquid Chromatography (HPLC)

2.2.1 Chromatograhic condition

The organic acids were determined according to the method developed by Zhanguo et al.(2002), with slight modification. Briefly, the conditions were modified by using C18 column (Hypersil gold, 5 µm, 250x4.6 mm. i.d.) with 0.5% $(NH_4)H_2PO_4$, pH 2.6 as the mobile phase at the flow rate of 1 mL/min, column temperature was 25°C and the UV detector was set at 210 nm. Gallic acid (GA) was used as an internal standard.

2.2.2 Organic acids and internal standard stock solutions

Standard organic acids were oxalic acid (OA), tartaric acid (TA), L-malic acid (L-MA), citric acid (CA), fumaric acid (FA), succinic acid (SA). Gallic acid (GA) was an internal standard. Each of standard organic acids was dissolved in ultrapure water to prepare stock standard solutions. Oxalic acid, tartaric acid, L-malic acid, citric acid, fumaric acid, succinic acid and gallic acid were separately dissolved in ultrapure water to make each of final concentration of the stock standard solutions at 6, 40, 40, 10, 2, 35 and 0.8 g/L, respectively. The standard stock solutions of organic acid were stored at 4°C.

2.2.3 Analytical method validation

The validation of an analytical method was proceeded for the specificity, linearity, sensitivity, accuracy and precision as follows.

(1) Specificity

The specificity of the method is indicated by the resolution value of the standard mixture organic acid ≥ 1.5 (USP 27, 2004). The separation is essentially completed.

The standard mixture solution was prepared by transferring the standard stock solution of 1 mL OA, 1mL TA, 1mL L-MA, 1 mL CA, 1 mL FA and 2 mL SA into 10 mL volumertric flask, make up to volume with mobile phase. One milliliter of standard mixture solution was mixed with 100 µL of 0.8 g/L gallic acid in a 2 mL volumetric tube and adjusted to volume with mobile phase. The final concentration of standard mixture solution was 0.3 g/L OA, 2 g/L TA, 2 g/L L-MA, 0.5 g/L CA, 0.005g/L FA, 3.5 g/L SA and 0.04 g/L GA, respectively. The standard mixture was filtered through a 0.45 µm, 13 mm. nylon syringe filter. The injection volume was 15µl. Resolution value (R) was calculated by using the following equation.

$$R = 2(t_2-t_1)/(W_2+W_1)$$

R = resolution value
t_2 = retention time of peak two
t_1 = retention time of peak one
W_2 = peakwidth of peak two
W_1 = peakwidth of peak one

(2) Linearity

The linearity was defined as the relationship between the peak area ratio of organic acid to internal standard and the concentrations of standard organic acids.

The linearity of the method was determined by analyzing a serial concentration of each 6 organic acid standards with the replicate of 5 concentrations. The mixture of standard solution was prepared at concentrations of 0.02-0.30 g/L for OA, 1.00-6.00 g/L for TA, 0.10-2.90 g/L for L-MA, 0.01-0.21 g/L for CA, 0.00005-0.03100 g/L for FA, 0.03-0.35 g/L for SA. The gallic acid as internal standard was

added in the concentration of 0.04 g/L. The concentration of standard mixture of organic acid was prepared in 5 concentrations below. The standard solution mixture was filtered through a 0.45 µm, 13 mm. nylon syringe filter. Each standard solution mixture 15 µL was injected to the column in tripicate. A graph between peak area ratio (y) and concentrations of standard acids (x) was plotted. The linearity was assessed by means of linear regression.

Standard Mixture	Concentration of organic acid (g/L)						
	OA	TA	L-MA	CA	FA	SA	GA
Concentration 1	0.02	1.00	0.10	0.01	0.0001	0.03	0.04
Concentration 2	0.09	2.25	0.80	0.06	0.0080	0.11	0.04
Concentration 3	0.16	3.50	1.50	0.11	0.0160	0.19	0.04
Concentration 4	0.23	4.75	2.20	0.16	0.0240	0.27	0.04
Concentration 5	0.30	6.00	2.90	0.21	0.0320	0.35	0.04

(3) Sensitivity

Sensitivity is the ability of the test method to quantitate small differences in solute concentration. It can be evaluated by determining the smallest change in concentration that will give a significantly different detector response.

The standard organic acid mixture was prepared at concentration 0.02-0.30 g/L for OA, 1.00-6.00 g/L for TA, 0.10-2.90 g/L for L-MA, 0.01-0.21 g/L for CA, 0.00005-0.03125 g/L for FA, 0.025-0.345 for SA and 0.04 g/L for GA. The same calibration runs were used to determine the limit of detection (LOD) which were calculated considering a signal-to-noise ratio (S/N) of 3.3

(4) Accuracy and Precision

Precision of the method composed of intra-day and inter-day precision. The relative standard deviation (RSD) was calculated.

4.1 Intra-day accuracy and precision

The accuracy of the method was evaluated in term of recovery. Percentage recoveries were calculated from peak spiked tamarind extracts of known amounts with the standard mixture solution.

% recovery = $(C_2 \times 100)/C_1$

C_2 = concentration of organic acid analyzed

C_1 = concentration of organic acid added

4.1.1 The prepared tamarind extract was diluted with the mobile phase. All sample contained an internal standard of 0.04 g/L gallic acid.

4.1.2 The standard mixture of organic acid was prepared in 3 concentrations below.

Standard Mixture	Concentration of organic acid (g/L)						
	OA	TA	L-MA	CA	FA	SA	GA
Concentration 1	0.03	1.00	0.39	0.02	0.001	0.04	0.04
Concentration 2	0.09	1.51	0.80	0.10	0.003	0.08	0.04
Concentration 3	0.15	2.00	1.20	0.12	0.005	0.12	0.04

4.1.3 Tamarind extract was spiked with known amounts of the standard organic acid mixture.

The solution was filtered through a 0.45 µm, 13 mm. nylon syringe filter. Each solution was injected 3 times in a volume of 15 µL. To determine :

- Concentration of organic acid in tamarind extracted (C_1)
- Concentration of organic acid in mixed standard organic acid (C_2)
- Concentration of organic acid in tamarind extract spiked with standard organic acid mixture (C_3)

Percentage recovery was calculated from peaks of organic acids in tamarind extracts of know amounts with its standard spiked.

% recovery = $(C_3)/(C_1+C_2) \times 100$

4.2 Inter-day precision

For inter-day precision evaluation only the precision of the method was performed. Five concentrations of the mixture of standard solution were prepared to make concentration 0.05-0.25 g/L for OA, 1.60-4.00 g/L for TA, 0.70-1.90 g/L for L-MA, 0.075-0.195 g/L for CA, 0.00015-0.01200 g/L for FA, 0.09-0.21 for SA and 0.04 g/L for GA. Each standard mixture was prepared in three samples (n=3) Five concentrations of the mixture of standard solution were also injected 3 times everyday for three days. The inter-day RSD of each organic acid concentration analyzed in different three days were calculated.

2.2.4 Determination of organic acids in tamarind pulp extracts

The water extract of tamarind samples were diluted with the mobile phase in a ratio of 1:29, 1:29, 1:10, 1:14, 1:5 for TI-P/K, TI-PY/P, TI-K/P, TI-SP/K and TI-STH/K, respectively. All samples contained an internal standard of 0.04 g/L of gallic acid. The sample solution was filtered through a 0.45µm, 13 mm nylon syringe filter prior to injection into the HPLC under chromatographic conditions described. Organic acid peaks were identified by comparing the retention time in the sample solution with that of the standard solution. The concentration of organic acids in the samples were calculated by comparing peak area ratios with that of standard in calibration curves.

2.2.5 Statistical analysis

The result of organic acids concentrations in sample tamarind extracts was analyzed statistically using ONE-WAY analysis of variance (ANOVA) with, TURKEY HSD statistic using SPSS 14. The values were considered to be significantly different when the P value was less than 0.05.

3. Isolation of tamarind pulp polysaccharides from tamarind pulps

The isolation of polysaccharide from tamarind pulps was slightly modified from the method of Pagan et al. (1999). Briefly, Dried pulps of each sample were weighed equivalent to 300 g of fresh pulp and blended in 1200 mL of DI-water. The mixture was stirring at 90°C in water bath for 1 hour, and centrifuged at 6800xg for 20 min. The supernatant was collected. The precipitated was repeat extracted with 300 mL of DI water, by using the same process, centrifuged and separated the supernatant. The supernatant was pooled and concentrated at 70°C under reduced pressure with Rotavapor R-200 in order to reduce approximately 4 times of the total volume. A viscous liquid was mixed in three volumes of cold acid-ethanol (4% HCl in 75% ethanol) with continuous stirring for 10 minutes until the precipitation was completed. The precipitate of crude polysaccharide was collected by filtering through a fine nylon sieve. The precipitate was washed 3 times with cold 75% ethanol, filtered and pressed to remove excess solvent. The precipitate was finally washed with 95% ethanol, filtered and pressed to remove excess solvent. Crude extract of polysaccharide of tamarind pulp was dried in hot air oven at 50°C for 5 hours. The sugar components of polysaccharide in tamarind pulp were determined by performing acid hydrolysis and analyzed using HPLC.

The result of percent yield extracted of polysaccharide was analyzed statistically using ONE-WAY analysis of variance (ANOVA) with, TURKEY HSD statistic using SPSS 11.5. The values were considered to be significantly different when the P value was less than 0.05.

3.1 Determination of tamarind pulp polysaccharides from tamarind pulps

3.1.1 FT-IR spectra of tamarind pulp polysaccharides

The infrared spectra of polysaccharides were evaluated utilizing Fourier Transform Infrared Spectrometry (FT-IR). The KBr disc containing polysaccharides powder was prepared at the ratio of KBr : Polysaccharide to be 90 : 10. The mixture was ground with an agate mortar and pestle to obtain uniform mixture by speeding it in the die of 7 mm diameter and compressed with Qwik Handi-Press. The spectra of were obtained by scanning in the range of 4000-450 cm^{-1} for 16 scans. The mean spectra were obtained with the resolution at 4 cm^{-1}.

3.1.2 Determination of sugars composition of polysaccharide from tamarind pulp

(1) Sample preparation

The acid hydrolyzate solutions of polysaccharide from tamarind pulp were prepared by acid hydrolysis of 0.3 g tamarind pulp polysaccharide of 10 mL DI water with 0.75 M sulfuric acid in autoclave at 121°C for 20 minutes. After hydrolysis, Barium hydroxide powder was slowly added to acid hydrolyzate solution and stirred until neutral. Solutions of acid hydrolyzate were collected by centrifugation at 6800xg 20 min. The solution of acid hydrolyzate was concentrated by Rotavapor R-200 and adjusted to volume of 10 mL. The acid hydrolyzate was filtered through a 0.45µm, 13mm. nylon syringe filter and analyzed for sugar composition by HPLC-ELSD and RID techniques. Sugar peaks were identified by comparing the retention time in the sample solution with that of the standard sugars solution such as glucose, fructose, rhamnose, arabinose and xylose.

(2) Determination of uronic acid, glucuronic and galacturonic acid composition by HPLC-RID

The glucuronic acid and galacturonic acid were determined according to the method by Leitao (1995). The chromatographic conditions were slightly modified by amino column (carbohydrate NH_2 column, 5 µm, 250x4.6 mm., i.d.) with NaH_2PO_4 buffer at pH 4.6 as the mobile phase at flow rate 1.50 mL/min, column temperature at 35°C and Refractive Index Detector (RID) was used.

(3) Determination of neutral sugars composition by HPLC-ELSD

The chromatographic conditions were slightly modified from Gerddit (2002) by using amino column (NH_2 column, 5 µm, 250x4.6 mm. i.d.) with 90% acetonitrile in water as the mobile phase at flow rate 1.90 mL/min, column temperature at 80°C and Evaporative Laser Scattering Detector (ELSD) was used. A volume of 5 µL for standard and samples was injected and monitor in HPLC (Alltech, U.S.A.).

4. Isolation of tamarind seed polysaccharide (TSP) from tamarind seed kernel

The isolation of polysaccharide was slightly modified from the method of Suttananta (1986). Dried kernels of each sample were equivalent to 50 g and blended. Tamarind kernel powder was extracted with 2 L of hot DI water and stirred in water bath at 90°C for 2 hour. The mixture was centrifuged at 6800xg for 20 min. The supernatant was collected. The sediment was repeatedly extracted with 1 L of hot DI water and stirred in water bath at 90°C for 1 hour. The mixture was centrifuged at 6800xg for 20 min and separated the supernatant. The supernatant was pooled and concentrated at 70°C under reduced pressure with Rotavapor R-200 in order to reduce 40 times of the total volume. A viscous liquid was precipitated in 1.5 volumes of cold 95% ethanol. TSP was collected by filtration though a fine nylon sieve and dried in hot air oven at 50°C, and then pulverized to fine powder of TSP. Sugar components in TSP were determined by acid hydrolysis and followed by HPLC.

The percent yield extracted of tamarind seed polysaccharide was analyzed statistically using ONE-WAY analysis of variance (ANOVA) with, TURKEY HSD statistic using SPSS 11.5. The values were considered to be significantly different when the P value was less than 0.05.

4.1 Determination of tamarind seed polysaccharides from tamarind seeds

4.1.1 Determination of sugars composition of tamarind seed polysaccharide from tamarind kernel

(1) Sample preparation

The acid hydrolyzate solutions of polysaccharide from tamarind pulp were prepared by acid hydrolysis of 0.1 g tamarind seed polysaccharide of 10 mL DI water, using the same method at described.

(2) Determination of neutral sugars composition by HPLC-ELSD

The chromatographic conditions were the same method as described, in method 3.1.2, (3).

4.2 Rheology and viscosity of TSP

Solution at 2% w/v TSP in distilled water was scanned the viscosity at shear rate from 0 to 6000 1/s by Rheometer (Rheowin-RV1 software, HAAKE Rheowin) using 35/1 Ti a sensor.

5. Preparation of tamarind powder by spray drying technique

5.1 Sample preparation

Dried pulps of Thai tamarind cultivars Priao-Yak (TI-PY/P) and Khantee (TI-K/P) were weighed (each of equivalent to 120 g of fresh pulp) and blended in 500 mL of DI-water, and then stirred in water bath at 90°C for 1 hour, centrifuged at 6800xg for 20 min and the supernatant was collected. The sediment was repeatedly extracted with 500 mL of DI water by the same process, centrifuged and separated the supernatant. The pooled supernatant was adjusted to 1000 mL in volumetric flask. The extract was kept at 4°C, and used within 7 days.

5.2 Formulation of tamarind powder preparation

Ingredients	Function	Content (g)
Tamarind extract	Active ingredient	30
Tamarind seed polysaccharide (TSP) or Pectin	Carrier	5-10
Maltodextrin	Carrier	15-25
Silicon dioxide	Flow aid	0.3
Fructose	Flavoring agent	1.35
Sodium chloride	Flavoring agent	0.45

All ingredients were mixed in a 1 liter of DI-water. TSP or pectin suspension was autoclaved at 121°C 30 minutes before mixing into tamarind extract mixture. The tamarind powder was prepared by spray drying technique in a spray dryer. Tamarind extract mixture for spray drying was peristaltically pumped into a sprinkler at rate 2.30 ml/min. Air inlet and outlet temperatures were 140°C and 85°C respectively. The aspirator rate was 90 m^3/hr.The tamarind powder was subjected to morphology study under scanning electron microscope.

The products were measure moisture content and solubility in hot water.

5.3 Quantitative Determination of organic acids in tamarind powder

Three samples of tamarind powder (1 g) were weighed and dissolved in 20 mL of DI water, each solution mixture was treated to precipitated TSP and pectin in 1.5 volumes of cold 95% ethanol and centrifuged at 6800xg for 20 min. The supernatant was collected. The supernatant was concentrated at 40°C under reduced pressure by Rotavapor R-200, in order to eliminate ethanol. The volume of test solution was adjusted to 10 ml in volumetric flask and stored at 4°C. The test solution was determined for organic acids quantitatively by HPLC.

The test solutions of three samples of tamarind powders were diluted with the mobile phase in a ratio of 1:3. All samples contained an internal standard of 0.04 g/L of gallic acid. Each sample solution was filtered through a 0.45µm, 13mm. nylon syringe filter before injection into HPLC system under the specified chromatographic conditions.

CHAPTER IV

RESULTS AND DISCUSSION

1. Development and optimization of organic acids analysis by HPLC method

1.1 Chromatographic column

This experiment was to achieve a separation of the organic acids such as oxalic acid, tartaric acid, L- malic acid, citric acid, fumaric acid and succinic acid using HPLC method by C18 column (Hypersil gold, 5µm, 250x4.6 mm.) with an aqueous phosphate buffer as mobile phase. C18 column (Hypersil gold, Thermo Scientific, UK) composed of highly pure silica. It provides the ultimate in symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistry. In addition, it allows the separation of relatively organic acids by using aqueous phosphate buffer at a suitable pH to prevent the ionization of the species to be resolved. Therefore, C18 column (Hypersil gold) was chosen in this experiment.

1.2 Mobile phase composition

Zhanguo (2002) found that when a mobile phase aqueous 0.5% (w/v) of containing KH_2PO_4 was used, the six organic acids such as oxalic acid, tartaric acid, L- malic acid, ascorbic acid, citric acid and succinic acid were not completely resolved. When the solution of 0.5% KH_2PO_4 was replaced by an aqueous solution of 0.5% $(NH_4)H_2PO_4$, the six organic acids can be completely resolved. Therefore, buffer solution of 0.5% $(NH_4)H_2PO_4$ (w/v) was selected as the buffer in the mobile phase in this experiment and appropriate sensitivities was obtained.

From previous study, Zhanguo (2002) found that the pH value of the mobile phase was more than 3, the separation degree between oxalic acid and tartaric acid, tartaric acid and L- malic acid, L- malic acid and ascorbic acid, ascorbic acid and citric acid, citric acid and succinic acid were reversely smaller. Variation of pH of 0.5% $(NH_4)H_2PO_4$ (w/v) buffer was performed from 2.4, 2.5, 2.6, 2.7, 2.8 and 2.9, respectively, as shown in Figure 9, the experiment indicated that the suitable pH was 2.6. Therefore, Ammonium phosphate buffer, pH 2.6 was chosen as a mobile phase.

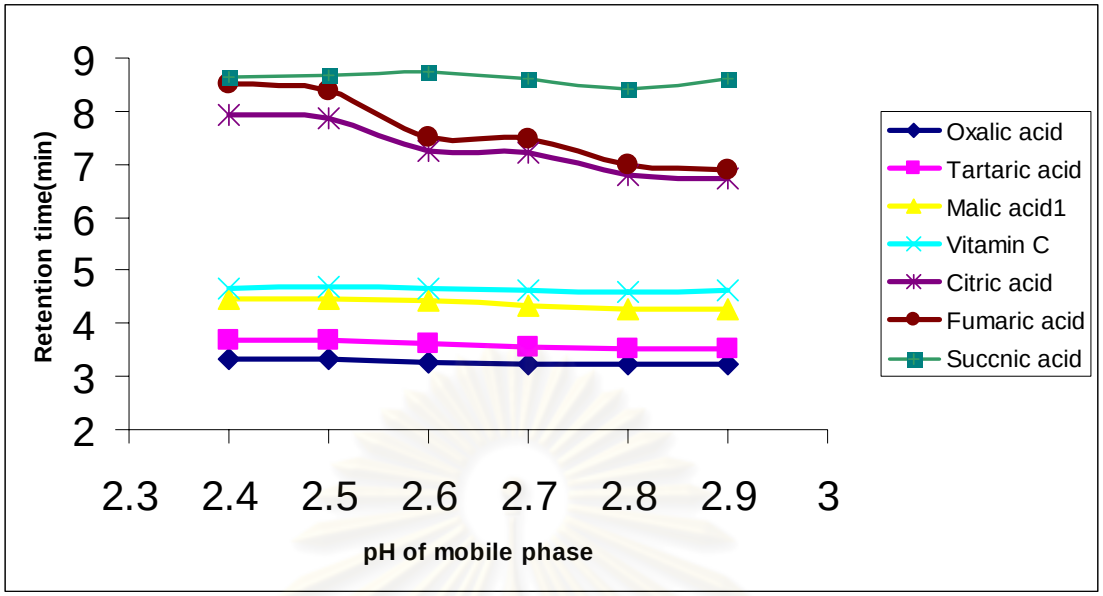


Figure 9. Effect of mobile phase pH on the separation of organic acids by Hypersil gold, C18 column (5µm, 250x4.6 mm., i.d.) at 210 nm.

1.3 Ultraviolet detector

Ping (2006) found that seven organic acids, oxalic acid, tartaric acid, L- malic acid, citric acid, succinic acid, acetic acid and malonic acid have strong UV absorption at 200-240 nm. Moreover, Khanthapok (2007) found that oxalic acid, tartaric acid, L-malic acid, ascorbic acid, citric acid and succinic acid have strong UV absorption at 210 nm. Therefore, the UV detector at 210 nm was chosen in this experiment.

Summary of chromatographic condition for quantitative determination of organic acids in tamarind extract is shown in Table 3.

2. Analytical method validation

2.1 Specificity

Specificity is the ability of the test method to measure the analytical component without interference from other sample-matrix components (Szepesi, 1990). Determination method is specificity when resolution value $\geq$ 1.5 (USP 27, 2004). The separation is essentially complete. Under the condition described oxalic acid, tartaric acid, L- malic acid, citric acid, fumaric acid and succinic acid could be separated on C18 column (Figure 10). The peaks of all organic acids were symmetrical and well separated. The resolution values of peak organic acids were in the ranges 1.64-4.54 (Table 4). Therefore, the method is specific for the determination of oxalic acid, tartaric acid, L- malic acid, citric acid, fumaric acid and succinic acid.

2.2 Linearity

Linearity is usually expressed in term of variance around the slope of the regression line calculated (Szepesi, 1990). Linearity was evaluate by plotting peak area ratio of organic acid and internal standard vs concentrations of standard organic acids. The coefficients of the regression curves and the square of the coefficients of determination (R^2) were calculated using the following equation.

$$y = ax + b$$

when y = Peak area ratio

x = Concentration of standard organic acids (g/L)

a = Slope

b = Intercept of the y axis

Table 3. Summary of chromatographic condition for quantitative determination of organic acids in tamarind extract

HPLC parameters	Optimized condition
Column	C18 column (5 µm, 250x4.6 mm)
Mobile phase	0.5% $(NH_4)H_2PO_4$ (w/v), pH 2.6
Flow rate	1 mL/min
Time	25 min
Detector	UV-detector at 210 nm
Temperature	25°C
Internal standard	Gallic acid

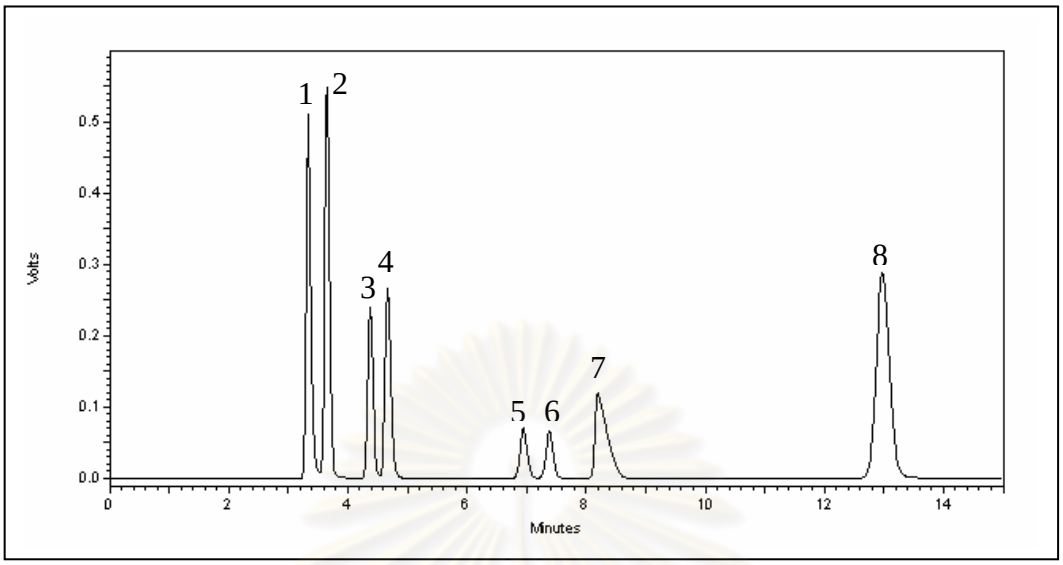


Figure 10. Chromatogram of standard mixture organic acids.
Peaks: 1 oxalic acid (OA), 2 tartaric acid (TA), 3 L-malic acid (L-MA), 4 ascosbic acid (AA), 5 citric acid (CA), 6 fumaric acid (FA), 7 succinic acid (SA) and 8 gallic acid (GA)

Table 4. Resolution (R) of organic acid standard and internal standard

Organic acid	Retention time (min)	Resolution
Oxalic acid	3.400	1.64
Tartaric acid	3.616	2.06
L-malic acid	4.343	4.54
Ascorbic acid	4.650	1.75
Citric acid	6.896	3.40
Fumaric acid	7.250	1.94
Succinic acid	8.450	2.57
Gallic acid	12.718	3.92

The linearity of the method was validated at five concentrations of each acid. The concentrations of the standard solutions of organic acids were chosen in such a way that the whole expected concentration range of each acid in the samples was covered. Linear regression parameters for calibration curves of all organic acid investigated are shown in Table 5.

2.3 Sensitivity

The limit of detection is the lowest concentration of organic acid in the tamarind pulp extracted which can be detected, but the necessarily quantified under the stated experimental conditions (Szepesi, 1990). Using the optimized conditions, the detection limit (signal-to-noise equal to 3.3) was 0.09 mg/L for oxalic acid, 0.88 mg/L for tartaric acid, 1.28 mg/L for L- malic acid, 1.44 mg/L for citric acid, 0.02 mg/ L for fumaric acid and 1.56 mg/L for succinic acid.

2.4 Accuracy and Precision

2.4.1 Intra-day accuracy and precision

Percentage recovery was examined to evaluate the accuracy of the method. Percentage recoveries were calculated from peak spiked tamarind extracts of known amounts with the standard mixture solution. Tamarind extracts were spiked with three different concentration of standard mixture. By comparing the found concentrations to the added concentrations, the relative standard deviation (RSD) was calculated for the determination of each acid.

The percent recoveries of organic acid and % RSD intra-day precision are shown in Table 6. Percentage recoveries were within the limit range 75-120 % (AOAC, 2002). Chromatograms of organic acids in tamarind pulp extract of sweet type *T.indica* “Srichomphu” (TI-SP/K) from Nakhon Ratchasima (Khorat,K) and the extract spiked with the mixture of organic acids are shown in Figure 11.

2.4.2 Inter-day precision

Five concentrations of the mixture of standard solution were injected continuously 3 times for 3 days. The inter-day %RSD values for organic acids are shown in Table 7.

Table 5. Linear range of organic acids standard

Acids	Linear range (g/L)	Regression equation[a]	R^2
Oxalic acid	0.0200-0.3000	y=2.3373x+0.0127	0.9999
Tartaric acid	1.0000-6.0000	y=0.3472x+0.0213	0.9995
L-malic acid	0.1000-2.9000	y=0.1797x+0.0013	0.9999
Citric acid	0.0100-0.2100	y=0.2370x+0.0003	0.9999
Fumaric acid	0.0001-0.0310	y=29.3640x-0.0016	0.9998
Succinic acid	0.0300-0.3500	y=0.1297x	1.0000

[a] y: peak area ratio, x: contentration of organic acid, g/L.

Table 6. Summary accuracy and intra day precision of recoveries for organic acids added to the tamarind pulp (TI-SP/K) extracts (n=3)

Organic acids	Background Concentration (g/L)	Concentration Added (g/L)	Concentration Analyzed (g/L)	Recovery (%)	Intra-day precision RSD (%)
OA	0.06	0.03 0.09 0.15	0.09±0.003 0.15±0.003 0.22±0.002	106.67 103.26 107.33	2.80 3.44 0.68
TA	1.36	1.00 1.51 2.00	2.37±0.005 2.86±0.014 3.27±0.015	101.50 99.47 95.50	0.22 0.50 0.47
L-MA	0.57	0.39 0.80 1.20	0.97±0.014 1.39±0.013 1.82±0.018	102.56 102.50 104.17	1.44 0.94 0.99
CA	0.04	0.02 0.07 0.12	0.06±0.003 0.10±0.002 0.15±0.003	104.35 92.86 94.17	5.00 1.50 2.00
FA	0.0006	0.0010 0.0030 0.0050	0.0015±0.0001 0.0035±0.0001 0.0055±0.0001	92.78 97.39 97.80	1.73 0.29 0.73
SA	0.10	0.04 0.08 0.12	0.14±0.002 0.17±0.004 0.22±0.002	97.5 92.5 103.33	1.43 2.35 0.18

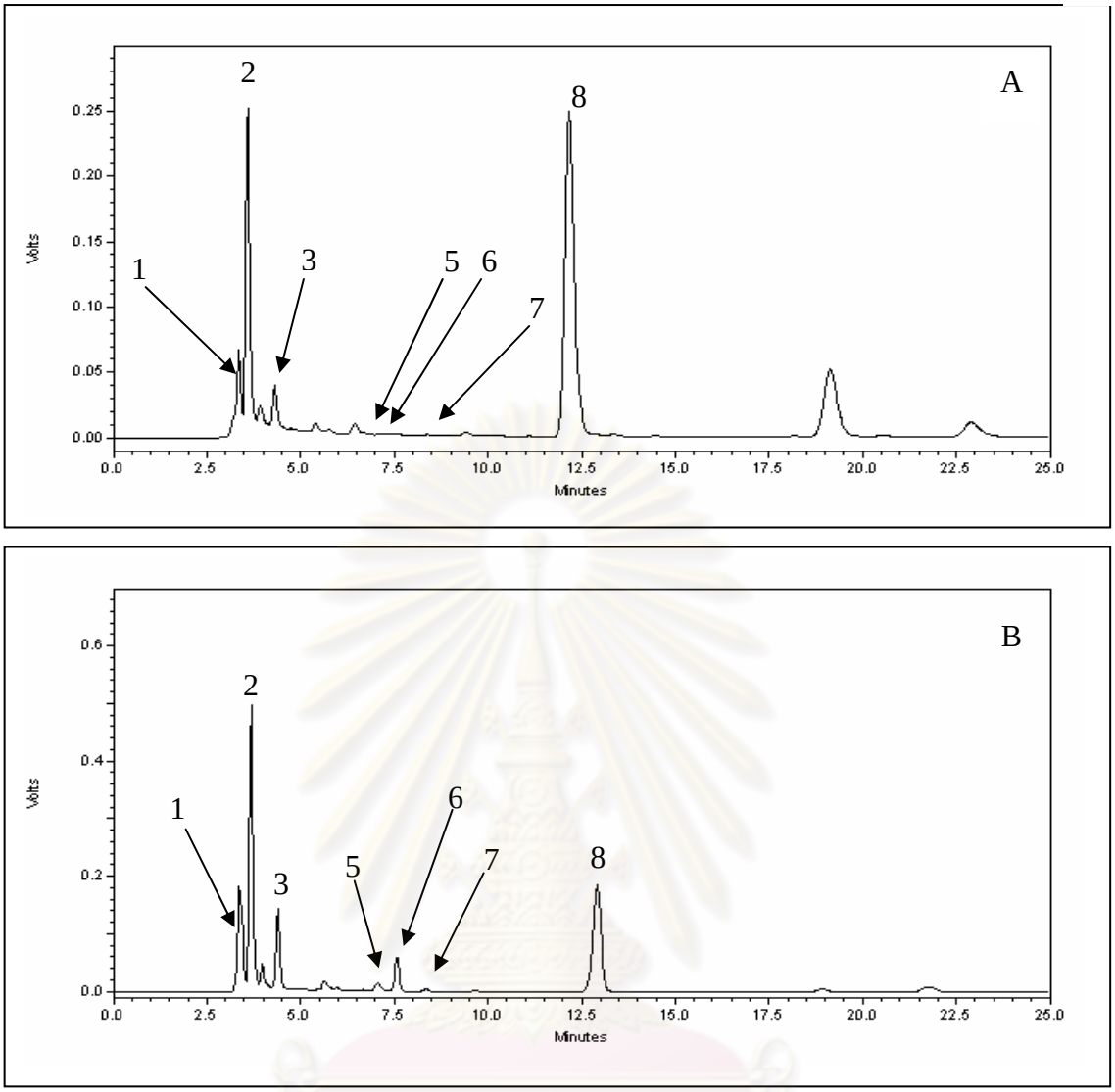


Figure 11. Chromatograms of organic acids in tamarind pulp extract of sweet type *T.indica* "Srichomphu" (TI-SP/K) from Nakhon Ratchasima (Khorat,K)
(A) chromatogram of tamarind pulp extract
(B) chromatogram of tamarind pulp extract spiked with the mixture of organic acids standard
Peaks: 1 oxalic acid (OA), 2 tartaric acid (TA), 3 L-malic acid (L-MA), 5 citric acid (CA), 6 fumaric acid (FA), 7 succinic acid (SA) and 8 gallic acid (GA)

Table 7. The inter-day precision (%RSD) n=3 of organic acids standard

Organic acids	Concentration Added (g/L)	Concentration Analyzed (g/L)	Inter day precision RSD (%)
OA	0.05	0.049±0.00031	0.66
	0.10	0.098±0.00091	0.94
	0.15	0.149±0.00160	1.11
	0.20	0.193±0.00190	1.03
	0.25	0.240±0.00350	1.48
TA	1.10	1.065±0.014	1.36
	2.20	2.155±0.037	1.72
	3.30	3.295±0.037	1.13
	4.40	4.382±0.051	1.16
	5.50	5.484±0.053	0.97
L-MA	0.70	0.678±0.003	0.49
	1.00	0.965±0.006	0.64
	1.30	1.260±0.014	1.13
	1.60	1.545±0.015	0.98
	1.90	1.889±0.025	1.33
CA	0.08	0.0772±0.0006	0.71
	0.11	0.1071±0.0006	0.55
	0.14	0.1382±0.0016	1.13
	0.17	0.1663±0.0014	0.82
	0.20	0.1954±0.0026	1.34
FA	0.003	0.0028±0.0001	0.15
	0.006	0.0057±0.0003	0.58
	0.009	0.0086±0.0002	1.26
	0.012	0.0115±0.0009	0.81
	0.015	0.0162±0.0008	0.51
SA	0.09	0.085±0.0006	0.68
	0.12	0.113±0.0020	1.70
	0.15	0.146±0.0020	1.27
	0.18	0.177±0.0023	1.30
	0.21	0.207±0.0024	1.18

Therefore, this analytical method revealed acceptable accuracy and precision. They are then indicated that the method are accurate and precise for oxalic acid, tartaric acid, L- malic acid, citric acid, fumaric acid and succinic acid determination in sample under the chromatographic conditions described.

3. Quantitative determination of organic acids in tamarind pulp extracts

Pulp extract of tamarind has long been used for the treatment of constipation. Organic acid contents in fruits are the active component (Suborough and Vridhachalam 1920, Verharar 1948, Reynolds 1989). The organic acid contents in tamarind cultivars type “sour” and “sweet” were determined. The organic acid contents in tamarind pulp extracts from the different growing areas, Phetchabun (P) and Nakhon Ratchasima (Khorat/K) province, are shown in Table 8, the chromatograms of tamarind cultivars sour type, “Priao-Yak” (TI-PY/P), “Priao” (TI-P/K), the sweet type, “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) are shown in Figures 12-16.

Tartaric acid was a major acid in sour tamarind “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) while tartaric acid and L-malic acid were the major acids in sweet tamarind “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K). The other minor organic acids were oxalic, citric, succinic and fumaric acids. The sour tamarind contained the highest tartaric acid content, but L-malic acid content in sweet tamarind was higher than that in sour tamarind.

As shown in Table 8, tartaric acid and citric acid contents of sour tamarind “Priao-Yak” (TI-PY/P) were significantly higher than that of “Priao” (TI-P/K), and also significantly higher than that of all sweet tamarind cultivars ($P< 0.05$). Moreover, tartaric acid content in each tamarind cultivar was significantly different ($P< 0.05$) in comparison between cultivars. The sweet tamarind “Sithong-nak” (TI-STH/K) contained the highest content of oxalic acid, L-malic acid, fumaric acid and succinic acid. Oxalic acid, L-malic acid, fumaric acid and succinic acid contents of sweet tamarind “Sithong-nak” (TI-STH/K) was significantly higher ($P<0.05$) than that of sour tamarind, “Priao” (TI-P/K) and “Priao-Yak” (TI-PY/P), and also other sweet tamarinds, “Khantee” (TI-K/P) and “Srichomphu” (TI-SP/K).

Table 8. Organic acids content in fresh pulp aqueous extracts of Thai Tamarind cultivars from Phetchabun (P) and Nakon ratchasima (Khorat,K) provinces

T.indica Cultivars	Organic acid, mean (SD)					
	mg/100g					
	Oxalic acid	Tartaric acid	Succinic acid	Fumaric acid	L-malic acid	Citric acid
Type "sour"						
Priao-yak (TI-PY/P)	95.78^{cd} (2.85)	17301.31^{a} (281.46)	-	1.34^{c} (0.02)	615.94^{d} (18.67)	231.00^{a} (23.70)
Priao (TI-P/K)	92.66^{d} (0.44)	8993.52^{b} (57.78)	160.85^{c} (4.34)	0.42^{e} (0.00)	575.99^{d} (1.80)	52.21^{c} (0.70)
Type "sweet"						
Khantee (TI-K/P)	100.85^{c} (0.82)	2785.64^{c} (0.52)	217.19^{ab} (2.09)	2.21^{b} (0.10)	971.12^{c} (7.92)	85.31^{b} (0.96)
Srichomphu (TI-SP/K)	119.00^{b} (1.04)	2402.32^{d} (1.99)	205.43^{b} (7.61)	1.13^{d} (0.02)	1141.02^{b} (1.62)	80.99^{b} (1.08)
Sithong-nak (TI-STH/K)	163.23^{a} (3.43)	1607.17^{e} (34.29)	235.37^{a} (9.13)	3.22^{a} (0.07)	1696.24^{a} (22.28)	79.36^{b} (3.79)

a,b,c,d,e show significant difference with in the same type acid of between cultivar at $P<0.05$

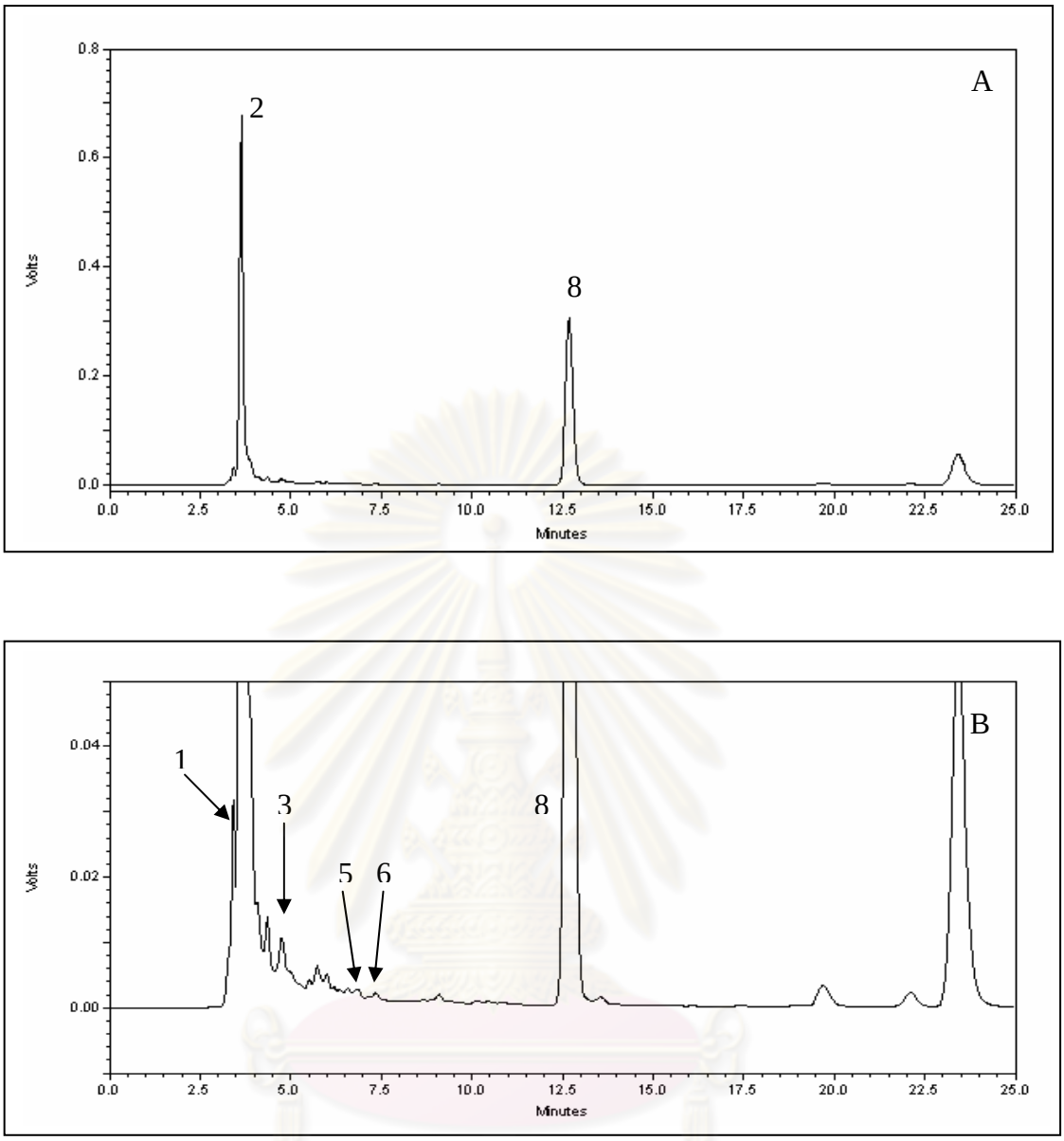


Figure 12. Chromatograms of organic acids in tamarind pulp extract of sour type *T.indica* "Preaw Yak" (TI-PY/P) from Phetchabun (P).
(A) chromatogram of tamarind pulp extract
(B) expanded chromatogram of (A)
Peaks: 1 oxalic acid (OA), 2 tartaric acid (TA), 3 L-malic acid (L-MA), 5 citric acid (CA), 6 fumaric acid (FA) and 8 gallic acid (GA)

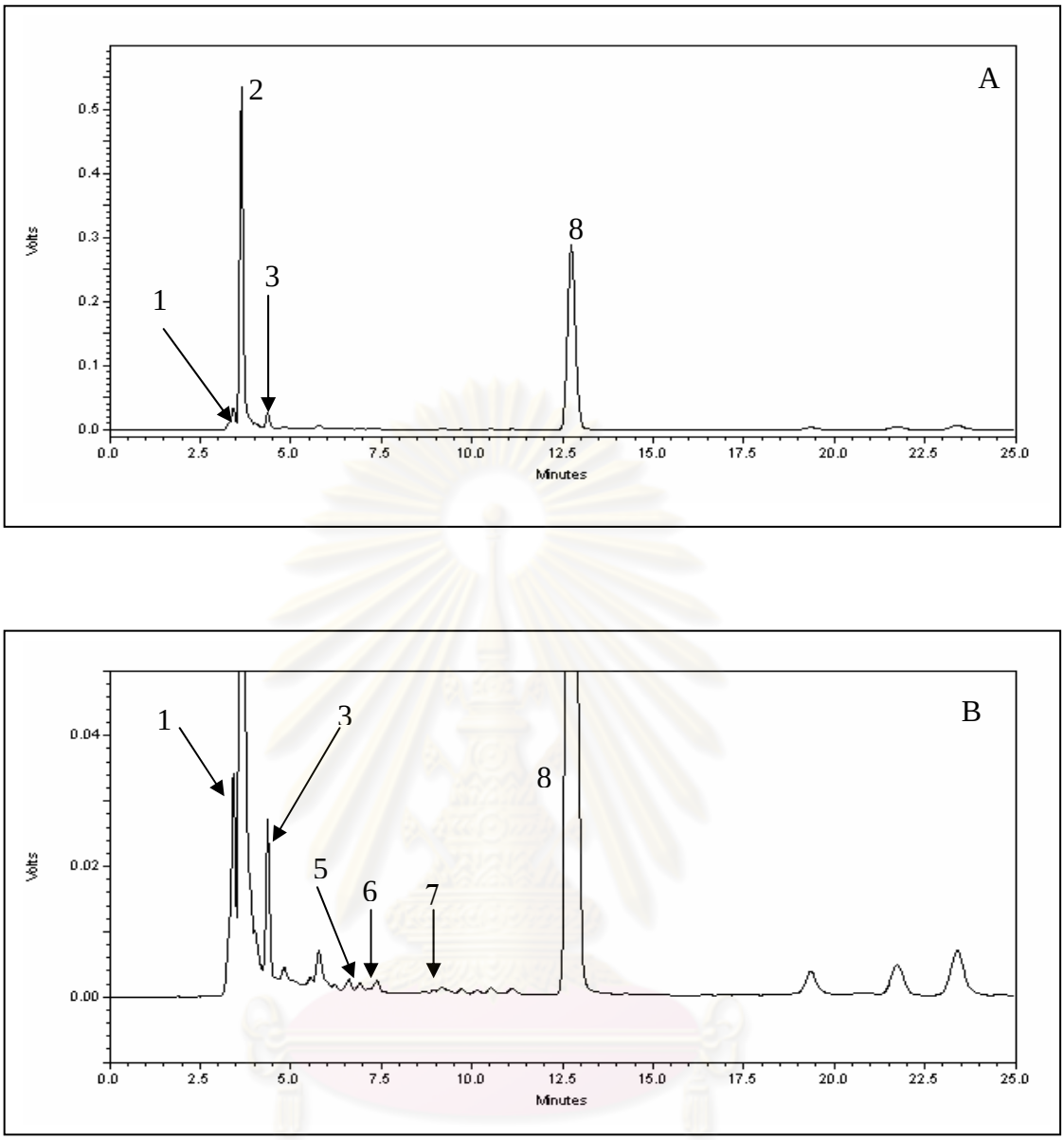


Figure 13. Chromatogram of organic acids in tamarind pulp extract of sour type *T.indica* "Preaw" (TI-P/K) from Nakhon Ratchasima (Khorat,K).
(A) chromatogram of tamarind pulp extract
(B) expanded chromatogram of (A)
Peaks: 1 oxalic acid (OA), 2 tartaric acid (TA), 3 L-malic acid (L-MA), 5 citric acid (CA), 6 fumaric acid (FA), 7 succinic acid (SA) and 8 gallic acid (GA)

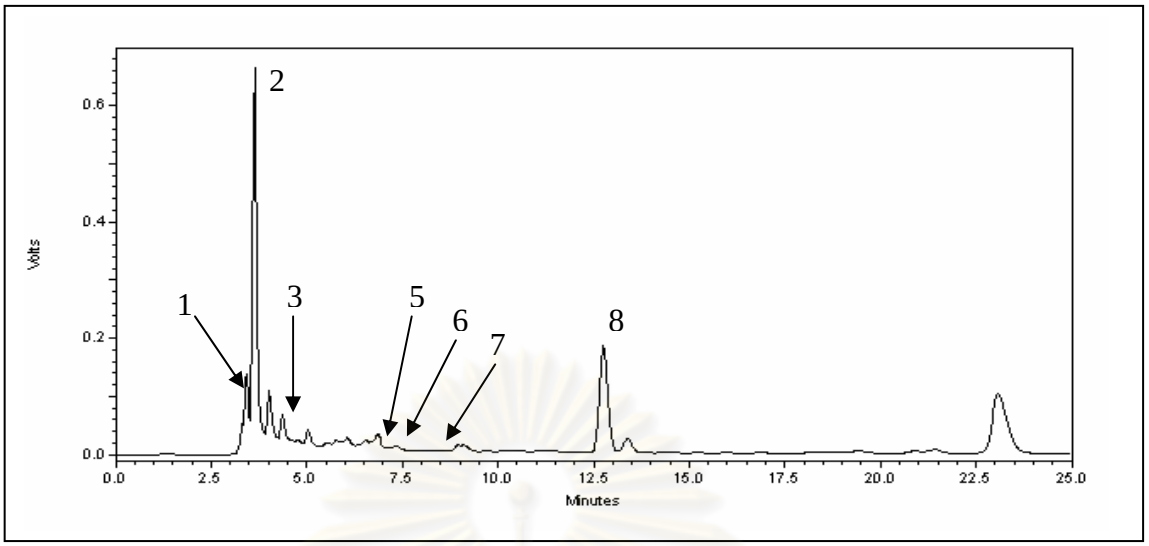


Figure 14. Chromatogram of organic acids in tamarind pulp extract of sweet type *T.indica* "Khantee" (TI-K/P) from Phetchabun (P).
Peaks: 1 oxalic acid (OA), 2 tartaric acid (TA), 3 L-malic acid (L-MA), 5 citric acid (CA), 6 fumaric acid (FA), 7 succinic acid (SA) and 8 gallic acid (GA)

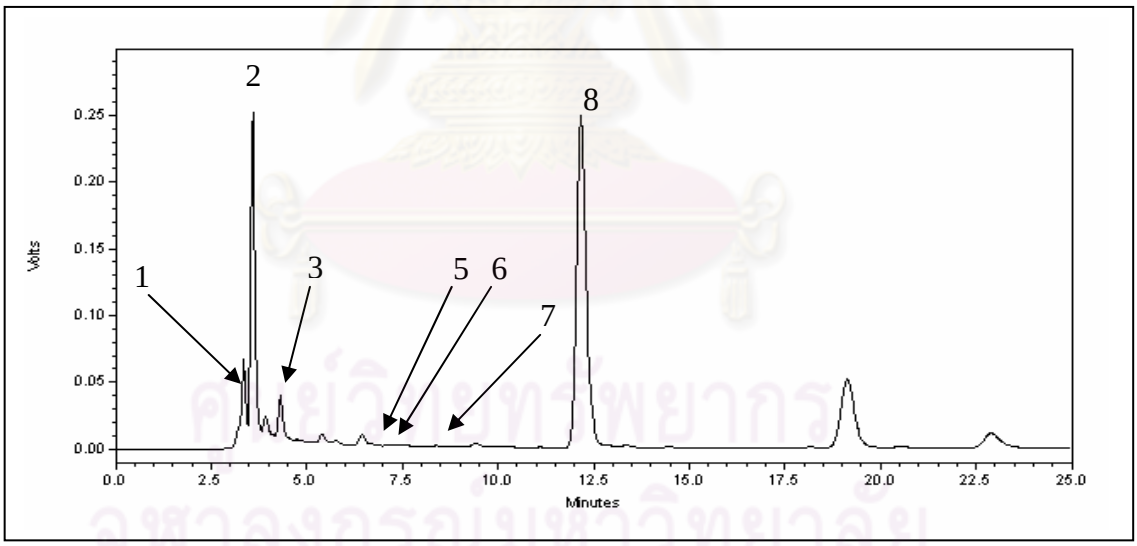


Figure 15. Chromatogram of organic acids in tamarind pulp extract of sweet type *T.indica* "Srichomphu" (TI- SP/K) from Nakhon Ratchasima (Khorat,K).
Peaks: 1 oxalic acid (OA), 2 tartaric acid (TA), 3 L-malic acid (L-MA), 5 citric acid (CA), 6 fumaric acid (FA), 7 succinic acid (SA) and 8 gallic acid (GA)

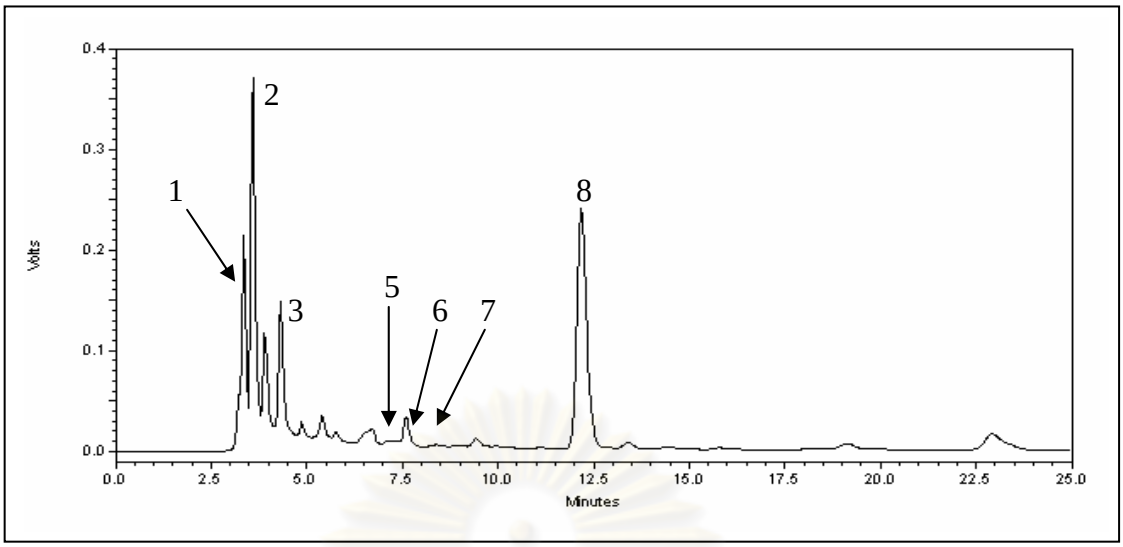


Figure 16. Chromatogram of organic acids in tamarind pulp extract of *T.indica* cv. Sithong- nak (TI- STH/K) from Nakhon Ratchasima (Khorat,K). Peaks: 1 oxalic acid (OA), 2 tartaric acid (TA), 3 L-malic acid (L-MA), 5 citric acid (CA), 6 fumaric acid (FA), 7 succinic acid (SA) and 8 gallic acid (GA)

In this experiment, ascorbic acid was not detected in tamarind pulp extracted because of the instability of the compound. From other reporter, ascorbic acid content in tamarind is very low (Ishola et al. 1990, Lefevre 1971). Therefore, ascorbic acid was not detected in the sample of tamarinds which were kept at -20°C for 6 months in the present study.

The high acid content is the most outstanding characteristic of tamarind, which mostly due to tartaric acid ranging from 12.2-23.8%, the very high organic acids is uncommon in other plant tissue (Ulrich, 1970). The other organic acids in tamarind pulp are oxalic acid, citric acid, succinic acid and quinic acid (Lewis and Neelakantan 1964, Singh 1973, Anon 1976) however, the ascorbic acid content in tamarind is low and wide ranges 2-20 mg/100g (Ishola et al. 1990, Lefevre 1971). For samples investigated, the tamarind extract contained oxalic acid, citric acid, fumaric acid and succinic acid but ascorbic acid was not detected. Different researchers have reported wide variations of tartaric acid content in tamarind pulp. In Pakistan, Hasan and Ijaz (1972) found that the tartaric acid content varied from 8.4-12.4% in sour tamarind. The tartaric acid content varied from 2.5-11.3% and tartaric acid content of sweet tamarind was low as 2.0-3.2% in tamarinds cultivated In Thailand (Feungchan et al. 1996). Tartaric acid content of sour tamarind varied from 12.0-17.0% (Satjapong et al. 1999). In this experiment, the tartaric acid content of sour tamarind “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) were similar to the values report by Satjapong (1999). In addition, tartaric acid content of sweet tamarind “Khantee” (TI-K/P) and “Srichomphu” (TI-SP/K) were similar to the values report by Feungchan et al. (1996). “Sithong-nak” (TI-STH/K) contained the lowest tartaric acid content.

Organic acids analysis of tamarind pulp extracts by the HPLC method demonstrated that the conditions used in this study for determination of organic acids contents including oxalic acid, tartaric acid, L- malic acid, citric acid, fumaric acid and succinic acid can be applied for the routine qualitative and quantitative analysis of organic acids in pulp extracts of tamarind cultivars.

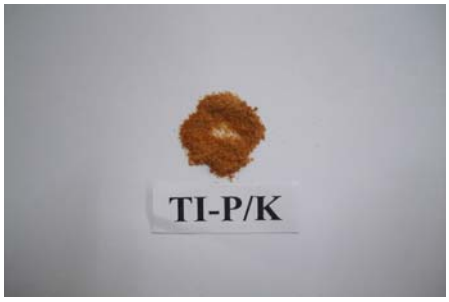
4. Isolation of tamarind pulps polysaccharide

4.1 Percentage of yield of tamarind pulps polysaccharide

In this experiment, the appearance of tamarind pulp polysaccharides of different cultivars including sour type, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) and sweet type, “Srichomphu” (TI-SP/K) and “Sithong-nak”(TI-STH/K) were shown in Figure 17. Table 9 illustrated the percentage of yield and statistic analysis of polysaccharides yield. Polysaccharides yield of sweet tamarind “Srichomphu” (TI-SP/K) from Nakhon Ratchasima (Khorat/K) gave significantly higher percent yield than that of “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima and “Priao-Yak” (TI-PY/P) from Phetchabun ($P<0.05$). On the other hand, polysaccharides yield of sweet tamarind “Srichomphu” (TI-SP/K) from Nakhon Ratchasima province was not significantly different from that of sour tamarind “Priao” (TI-P/K) in the same province ($P>0.05$). Polysaccharides yield of sour tamarind “Priao” (TI-P/K) from Nakhon Ratchasima province was higher than that of “Priao-Yak” (TI-PY/P) from Phetchabun ($P<0.05$). Whereas polysaccharides yield of sour tamarind “Priao-Yak” (TI-PY/P) from Phetchabun was not significantly different from that of sweet tamarind “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima.

An aqueous dispersion of polysaccharides of sour type, “Priao” (TI-P/K) and sweet type, “Srichomphu” (TI-SP/K), “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima were opaque light brown whereas and aqueous dispersion of polysaccharides “Priao-Yak” (TI-PY/P) from Phetchabun was grayish white.

(a)



(b)

(c)

(d)

(e)

Figure 17. Appearance of tamarind pulp polysaccharides from dried tamarind pulps
(a) “Priao-Yak” (TI-PY/P) from Phetchabun (P)
(b) “Priao”(TI-P/K) from Nakhon Ratchasima (Khorat,K)
(c) “Srichomphu” (TI-SP/K) from Nakhon Ratchasima (Khorat,K)
(d) “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima (Khorat,K)
(e) Comparison of tamarind pulp polysaccharides of tamarind cultivars

Table 9. Appearance and yield of the tamarind pulp polysaccharide isolated from dried tamarind pulps from Phetchabun (P) and Nakon ratchasima (Khorat,K) provinces

T.indica Cultivars	Appearance of polysaccharide isolated	Aqueous dispersion of polysaccharide	% yield of polysaccharide (mean (SD))
Type "sour"			
Priao-yak (TI-PY/P)	grayish white powder	grayish white opaque	1.74^{c} (0.03)
Priao (TI-P/K)	light brown powder	brown opaque	2.44^{ab} (0.16)
Type "sweet"			
Srichomphu (TI-SP/K)	brown powder	brown opaque	2.75^{a} (0.20)
Sithong-nak (TI-STH/K)	brown powder	brown opaque	1.98^{bc} (0.30)

a,b,c show significant difference between cultivar at $P<0.05$

4.2 Determine composition of tamarind pulp polysaccharides

4.2.1 FT-IR spectra of tamarind pulp polysaccharide

Infrared spectra of tamarind pulp polysaccharides from dried tamarind pulps were determined by using Fourier Transform Infrared Spectrometry (FT-IR). Tamarind pulp polysaccharide was directly examined using KBr disc. IR spectra of polysaccharide including sour type, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) and sweet type, “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) were compared with the FT-IR spectrum of commercially available pectin from citrus fruits. The FT-IR spectra of pectin standard and tamarind pulp polysaccharide sample were illustrated in Figure 18. It was found that FT-IR spectra of polysaccharide from tamarind cultivars sour type, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) and sweet type, “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K), and that of commercially available pectin standard from the citrus fruits were similar, the results suggested that polysaccharides from each tamarind cultivars contained pectic polysaccharide. This result was resemble with the study of Kulkarni et al.(1997) who have found that tamarind pulp composes of pectin, tartaric acid and potassium bitartrate.

As can be seen in Figures 19-23, Among the adsorption bands common to both spectra is the one at about 3400 cm^{-1} due to O-H stretching vibration, the 2930 cm^{-1} band corresponding to the C-H stretching of CH_2 groups and two bands at about 1630 and 1440 cm^{-1} which correspond to the vibrations of the aliphatic carboxylic acids (O=C-O) structure. In addition, the carbohydrates show high absorbances between 1200 and 950 cm^{-1} wave numbers values which constitutes the “finger print” region, specific for each polysaccharide. The band appears at about 1740 cm^{-1} can been assigned to C=O stretching vibration of methyl esterified carboxylic group. The specific band at 1740 and 1630 cm^{-1} indicated the ester carbonyl (COOR) and carboxylate ion (COO^-) groups, respectively. The band at about 1630 cm^{-1} is assigned to the symmetrical stretching vibration at COO^- groups, while the band at about 1740 cm^{-1} is assigned to carbonyl group from both COOH and $COOCH_3$ groups (Manrique and Lajolo, 2002).

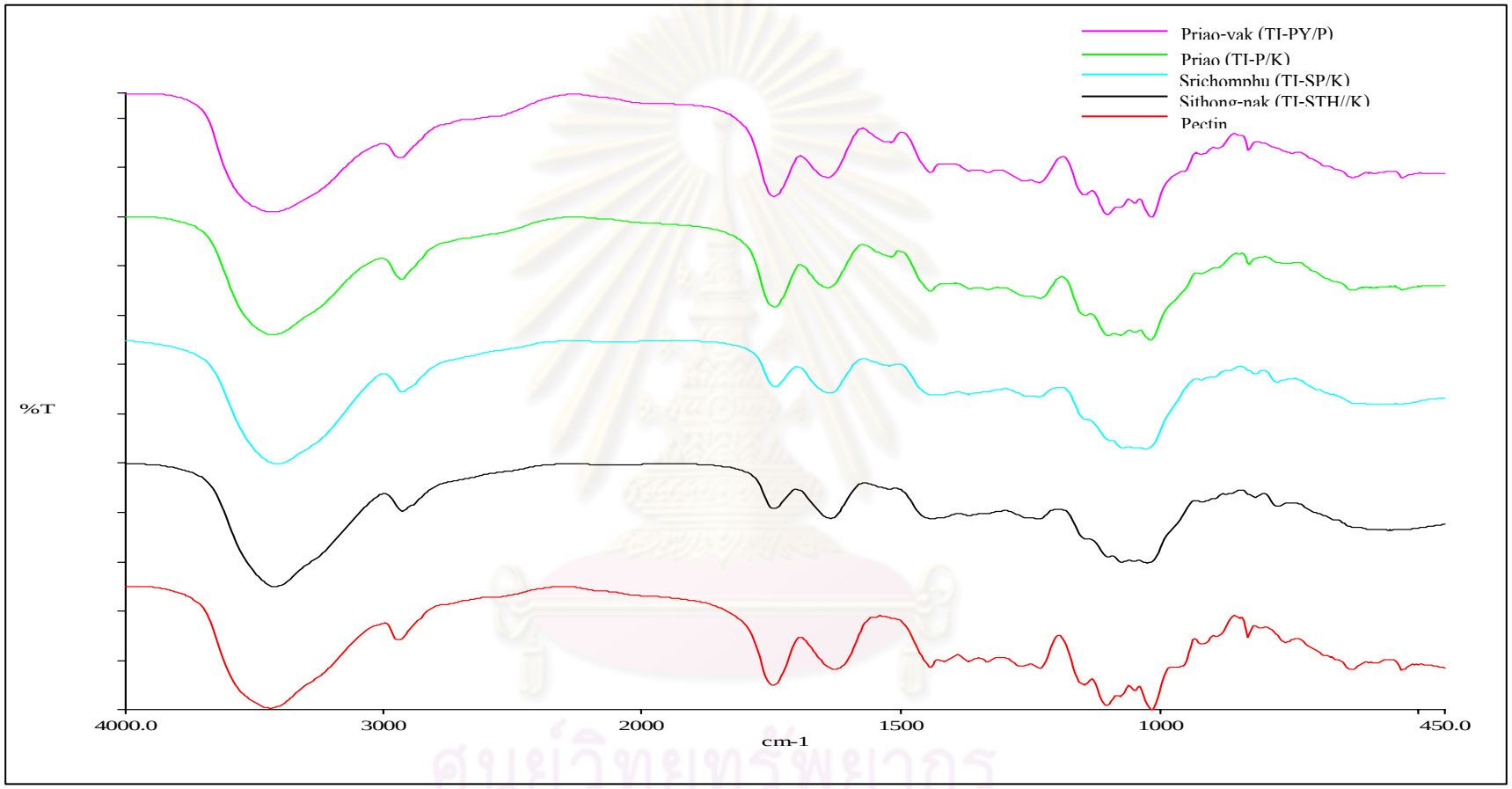


Figure 18. Fourier transform infrared spectra of tamarind pulp polysaccharide from pulps of tamarind cultivars, from the top shows the spectra of “Priao-Yak” (TI-PY/P) from Phetchabun, “Priao” (TI-P/K), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima (Khorat/K) compared to pectin standard from citrus fruits (at the bottom).

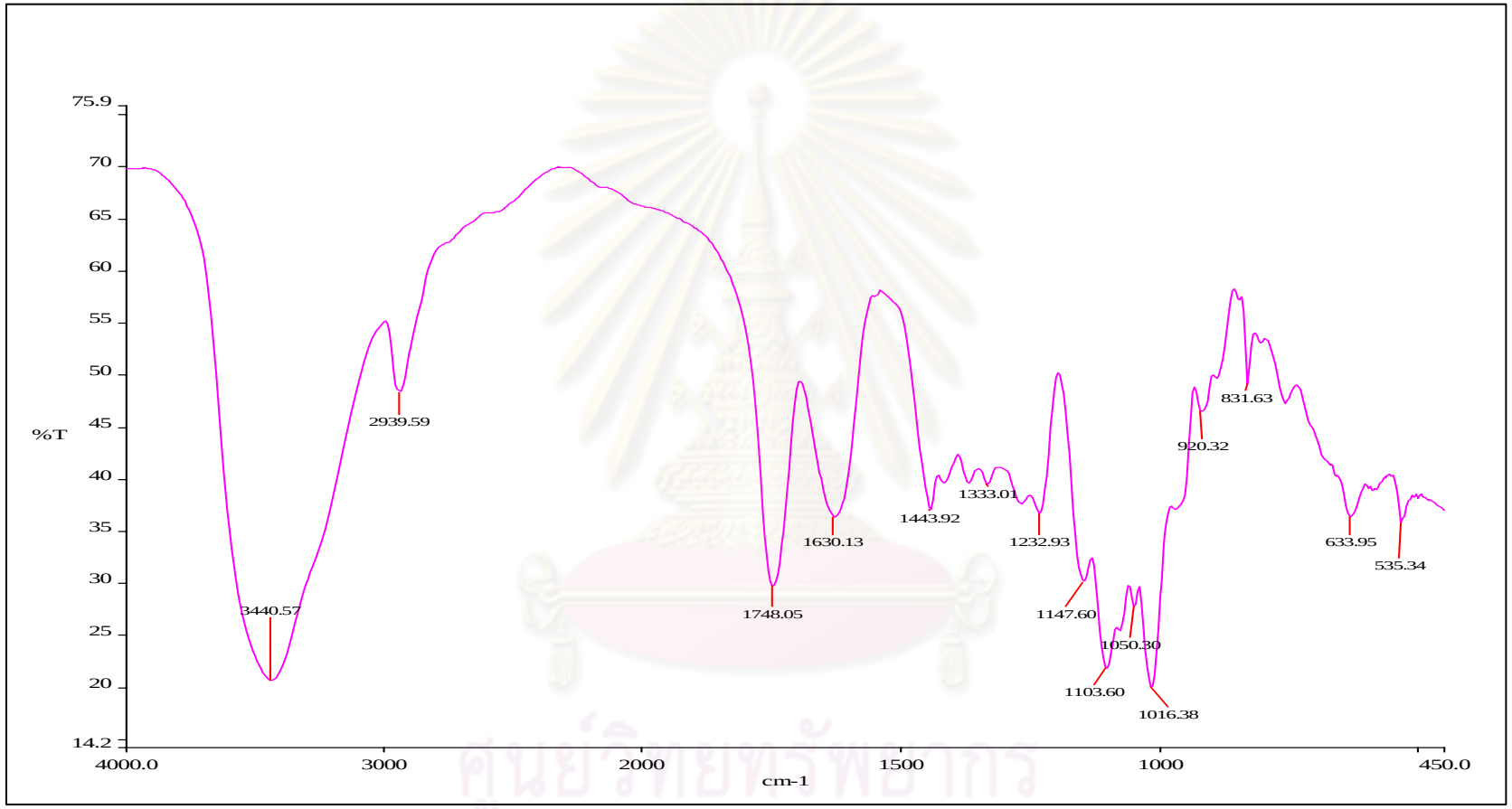


Figure 19. Fourier transform infrared spectra of pectin standard from citrus fruits

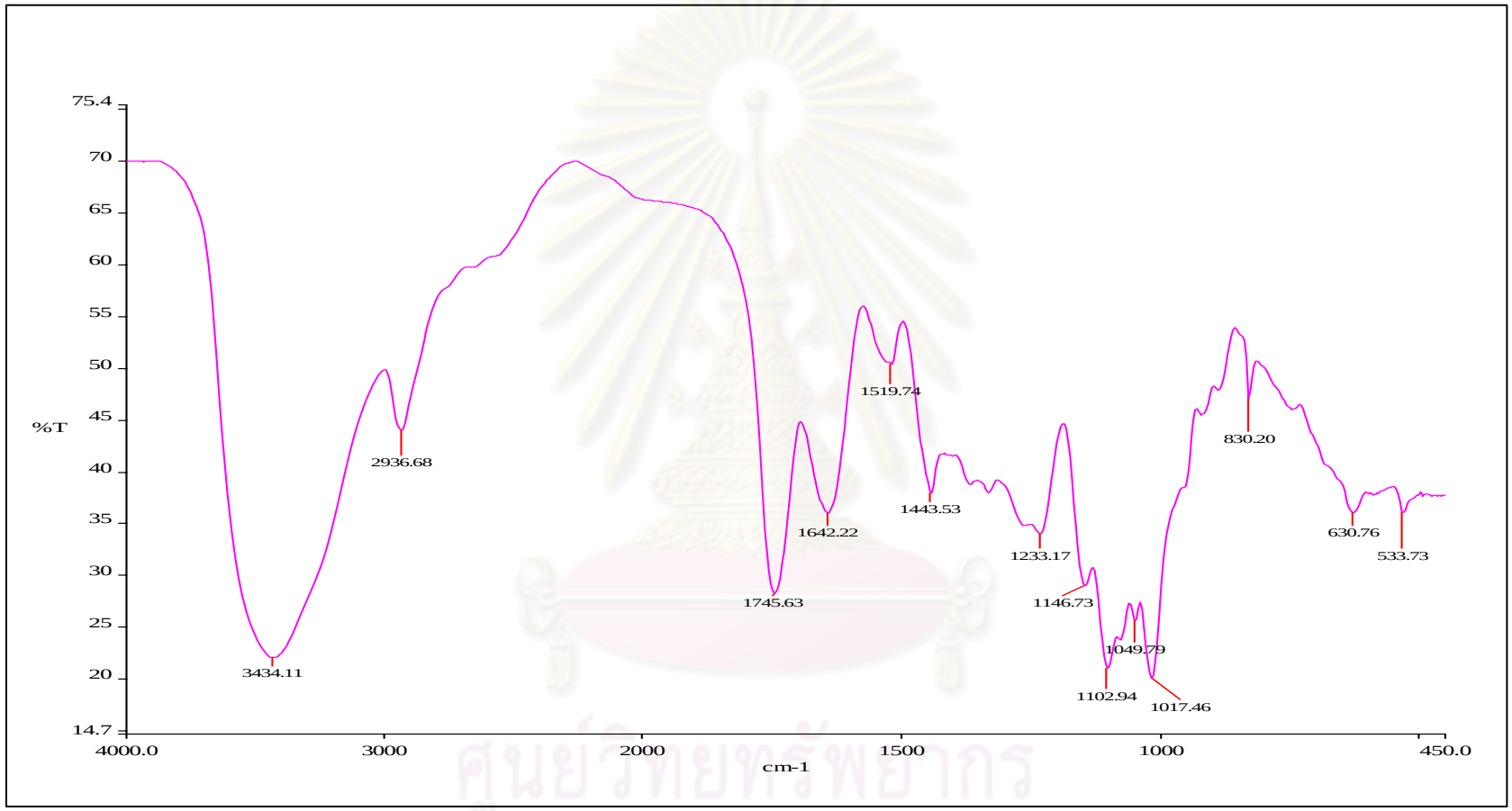


Figure 20. Fourier transform infrared spectra of tamarind pulp polysaccharide of tamarind cultivar, “Priao-Yak” (TI-PY/P) from Phetchabun province.

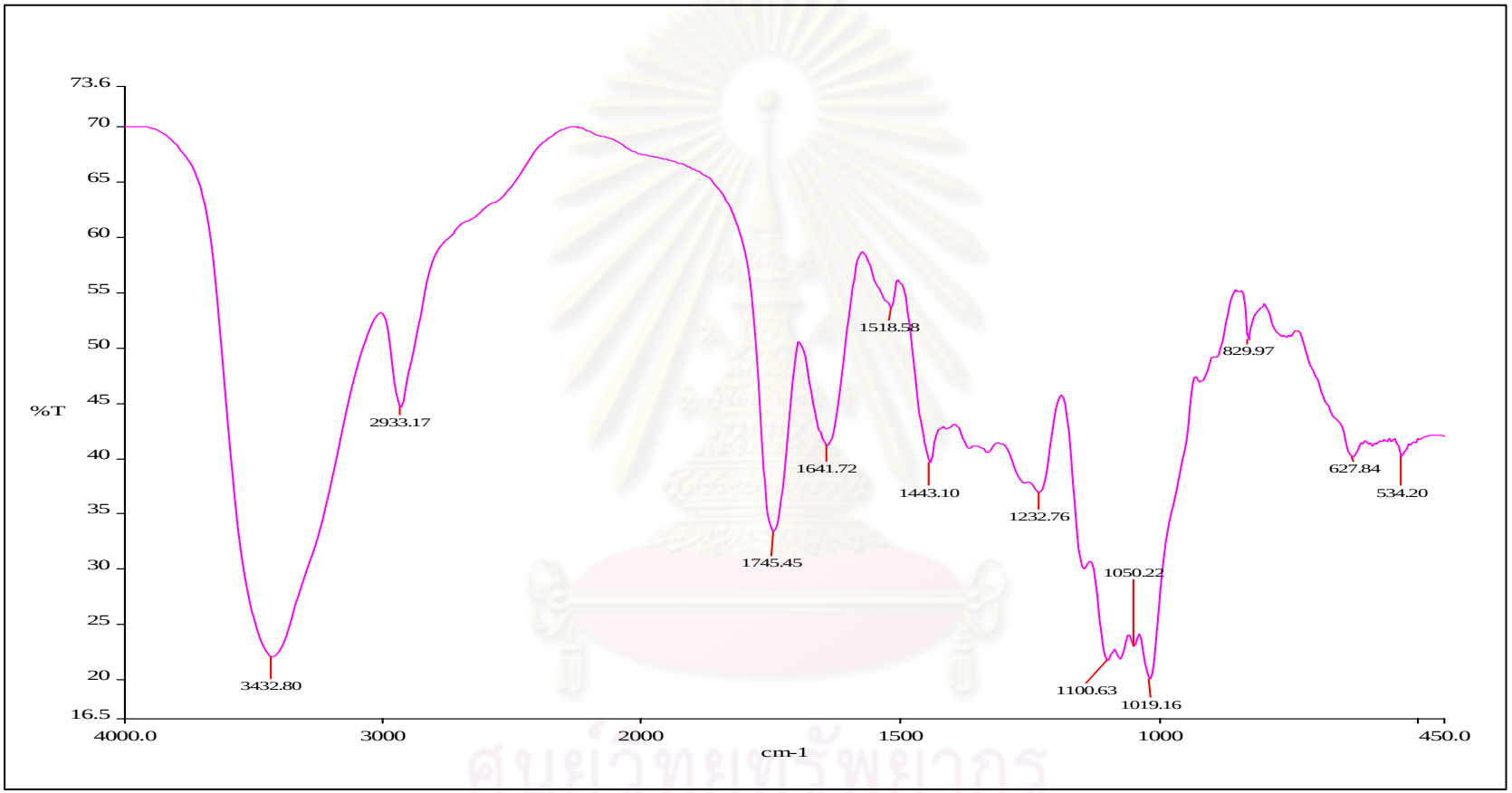


Figure 21. Fourier transform infrared spectra of tamarind pulp polysaccharide of tamarind cultivars, “Priao” (TI-P/K) from Nakhon Ratchasima (Khorat,K).

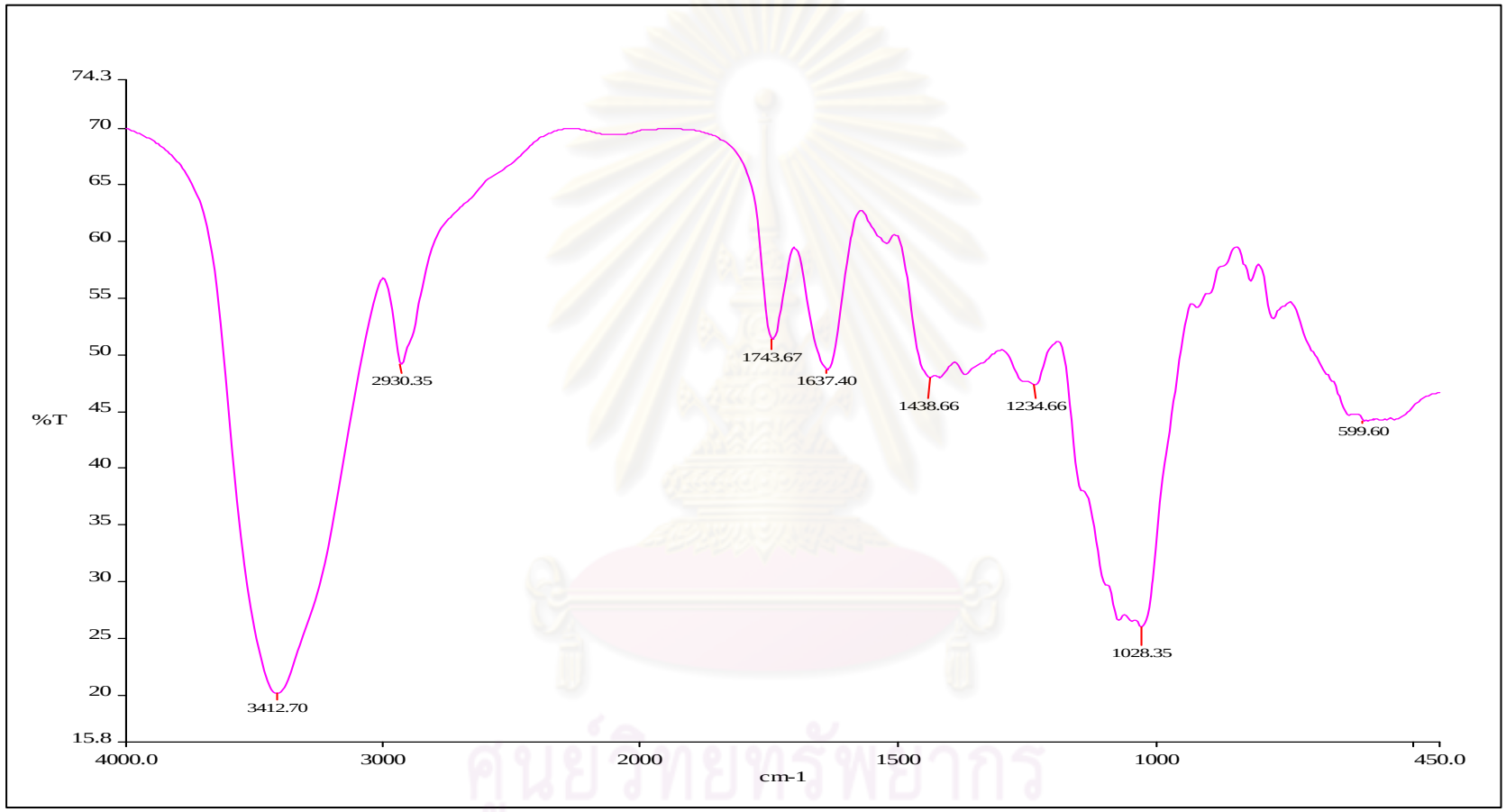


Figure 22. Fourier transform infrared spectra of tamarind pulp polysaccharide of tamarind cultivar, “Srichomphu” (TI-SP/K) from Nakhon Ratchasima (Khorat,K).

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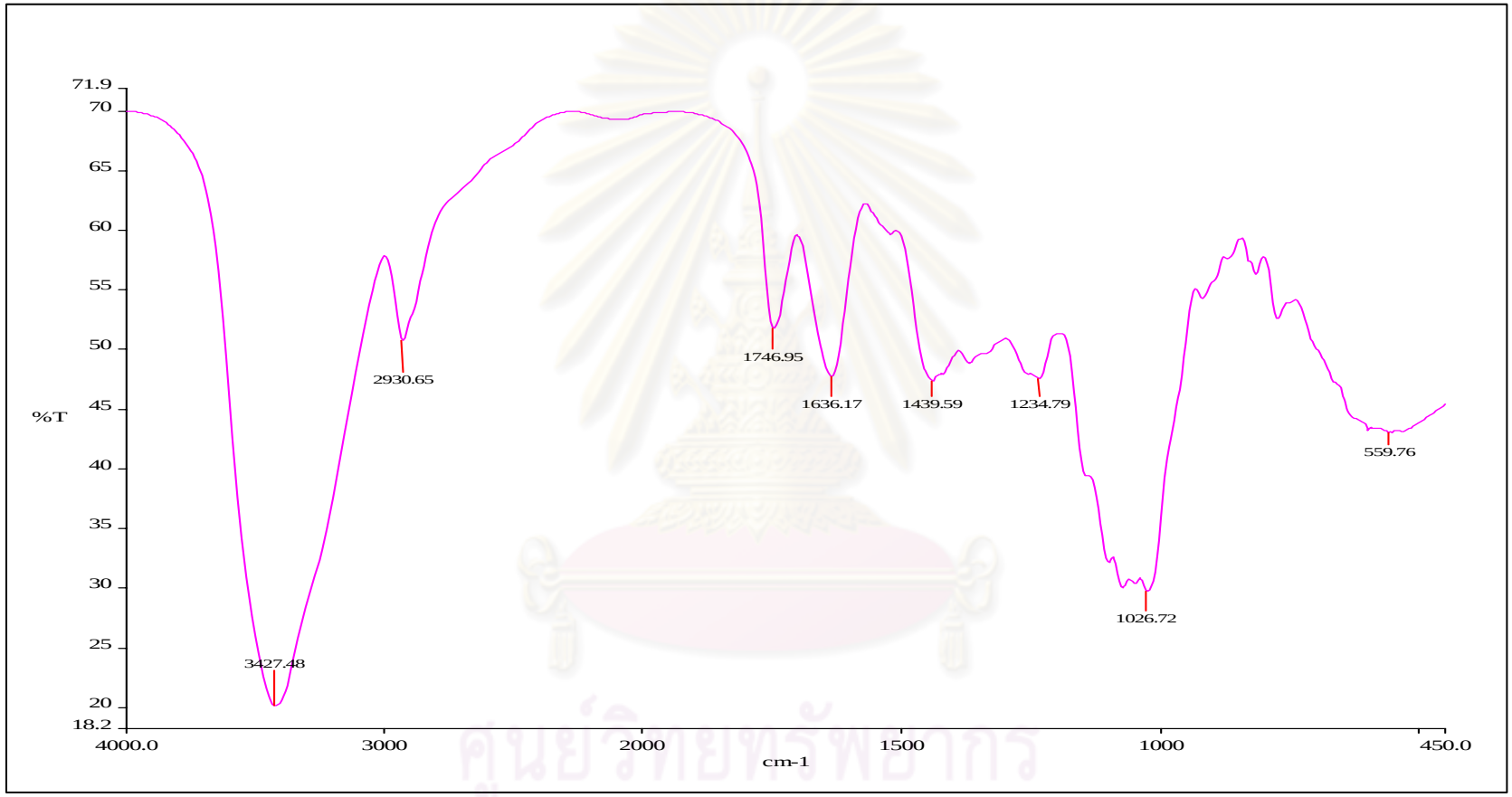


Figure 23. Fourier transform infrared spectra of tamarind pulp polysaccharide of tamarind cultivar, “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima (Khorat,K).

The results of FT-IR spectra of tamarind pulp polysaccharide from sour type “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K), and sweet type “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) were similar to standard FT-IR spectra of pectic polysaccharide from citrus fruits. The results showed that polysaccharides extract from different tamarind cultivars and different cultivated area contained pectic polysaccharide according to their profiles of FT-IR spectra, were similar to standard pectin from citrus fruits.

4.2.2 Determination of uronic acid composition by HPLC-RID

Table 10 indicates optimal conditions of uronic acids, glucuronic and galacturonic acid, analysis by HPLC method. Uronic acid composition in acid hydrolyzate of tamarind pulp polysaccharides was analyzed by HPLC method. Chromatograms of the standard mixture and chromatogram of acid hydrolyzate of sour tamarind, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K); and sweet tamarind, “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K), are shown in Figures 24, 25. Acid hydrolyzate of pulp polysaccharide of sour tamarind “Priao-Yak” (TI-PY/P) from Phetchabun, “Priao” (TI-P/K) from Nakhon Ratchasima showed one peak similar to standard galacturonic acid while sweet tamarind “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima composed of two peaks. First peak was not synchronized with the spiked glucuronic acid standard, where second peak was synchronized with the spiked galacturonic acid standard.

4.2.3 Determination of sugar composition of tamarind pulp polysaccharides from dried tamarind pulps

1. Conditions of sugar analysis by HPLC method

The sugar composition can be determined by HPLC method using amino column (NH2 column, 5 µm, 250x4.6 mm., i.d.) with 90% acetonitrile in water as the mobile phase at a flow rate of 1.90 mL/min, column temperature at 80°C and Evaporative Laser Scattering Detector (ELSD). Table 14 illustrated HPLC condition for sugar determination in polysaccharides of tamarind pulp.

Table 10. HPLC condition for glucuronic acid and galacturonic acid determination in polysaccharides of tamarind pulp

HPLC parameters	Optimized condition
Column	Amino column (Carbohydrate-NH_2, 250x4.6 mm.)
Mobile phase	NaH_2PO_4, pH=4.6
Flow rate	1.50 mL/min
Time	25 min
Detector	Refractive Index Detector (RID)
Temperature	35°C

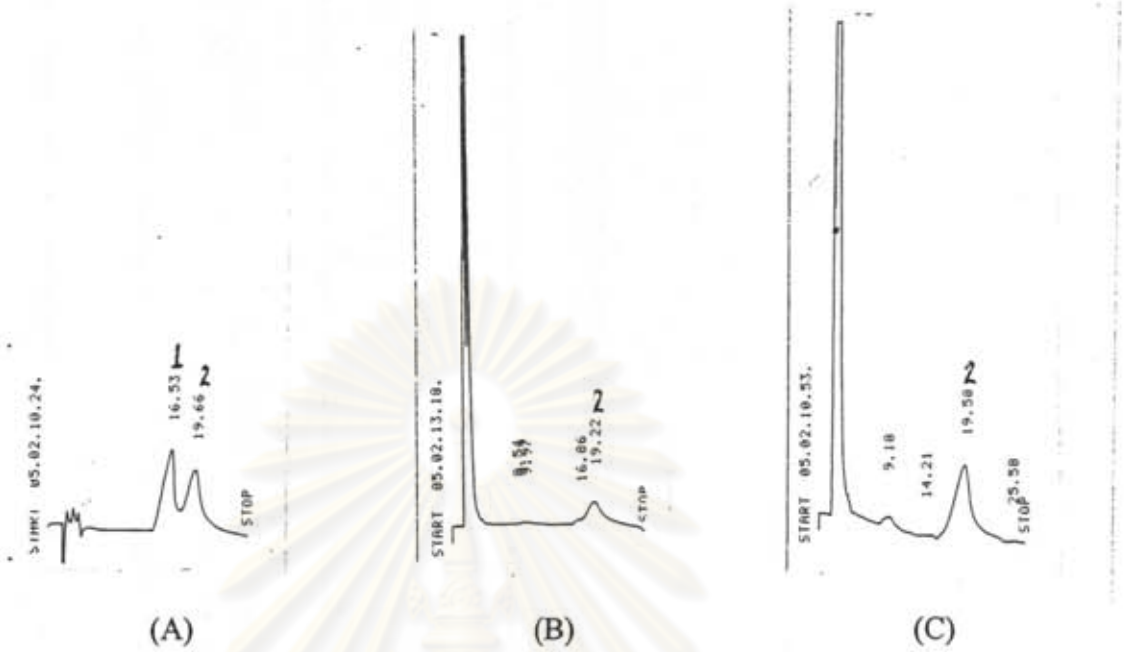


Figure 24. Chromatograms of uronic acids standard and acid hydrolyzate of pulp polysaccharides of sour tamarind cultivars:

(A) glucuronic acid and galacturonic acid standard

(B) "Priao-Yak" (TI-PY/P)

(C) "Priao" (TI-P/K)

Peaks: 1 = glucuronic acid (Glc A) 2 = galacturonic acid (Gal A)

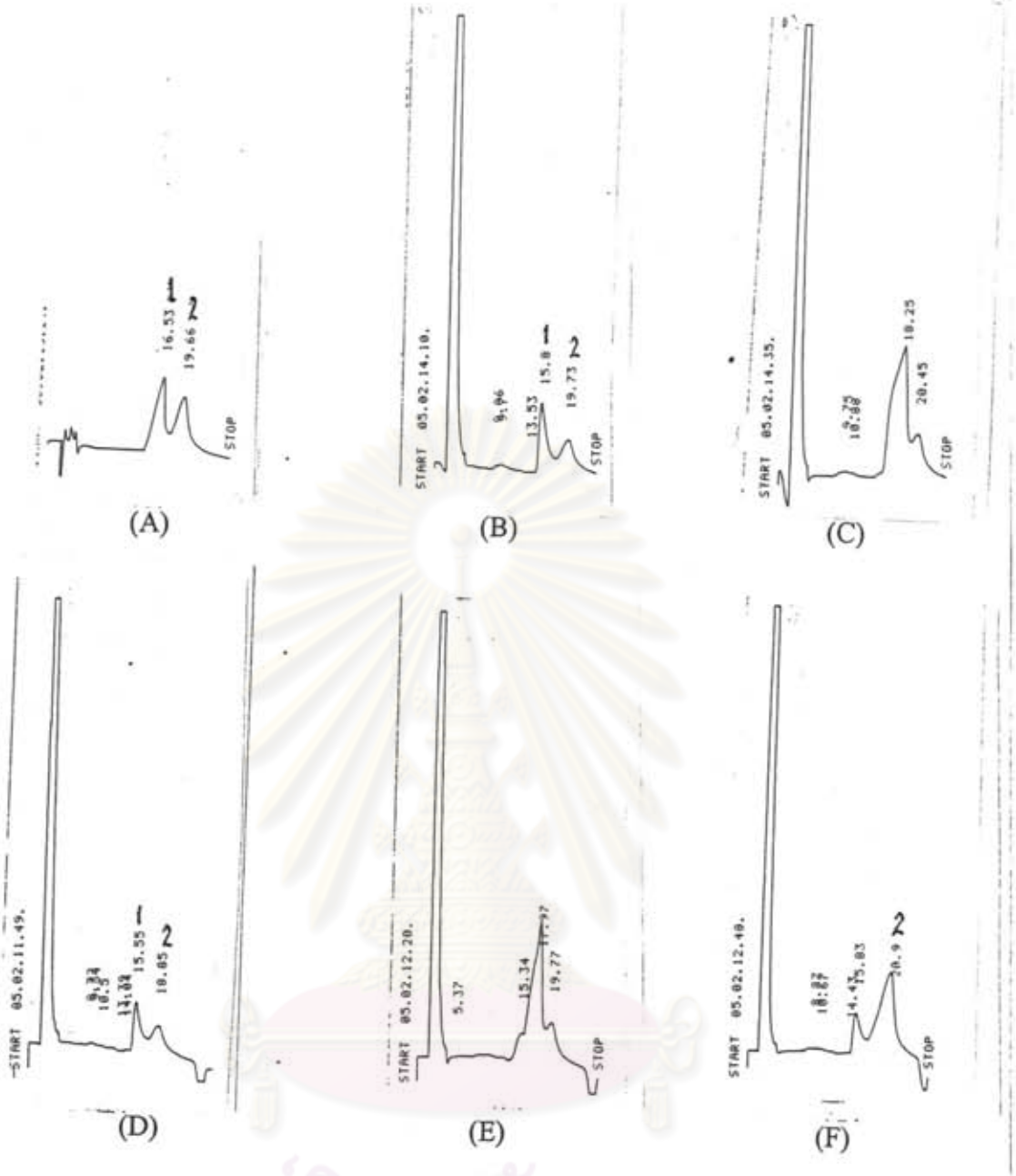


Figure 24. Chromatograms of uronic acids standard and acid hydrolyzate of polysaccharides of sweet tamarind cultivars:

(A) glucuronic acid and galacturonic acid standard

(B) "Srichomphu" (TI-SP/K)

(C) "Srichomphu" (TI-SP/K) spiked with Glc A

(D) "Sithong-nak" (TI-STH/K)

(E) "Sithong-nak" (TI-STH/K) spiked with Glc A

(F) "Sithong-nak" (TI-STH/K) spiked with Gal A

Peaks: 1 = glucuronic acid (Glc A) 2 = galacturonic acid (Gal A)

Table 13. HPLC condition for sugar determination in polysaccharides of tamarind pulp and tamarind seed polysaccharides (TSP) from tamarind kernel

HPLC parameters	Optimized condition
Column	Amino column (250x4.6 mm.)
Mobile phase	90% acetonitrile in water
Flow rate	1.90 ml/min
Time	12 min
Detector	Evaporative Laser Scattering detector (ELSD)
Temperature	80°C

Figure 26 shows HPLC chromatograms of the mixture standard sugars, Figure 26 (A); 5 g/L of rhamnose, 5 g/L xylose, 5 g/L arabinose, 5 g/L fructose and 5 g/L glucose at the retention time at 3.316, 3.983, 4.700, 5.850 and 7.183 minutes, respectively. Figure 26 (B); 5 g/L of rhamnose, 5 g/L xylose, 5 g/L arabinose, 5 g/L fructose, 5 g/L glucose and 5 g/L galactose at the retention time at 3.316, 4.000, 4.750, 5.900, 7.283 and 7.966 minutes, respectively.

2. Determination of sugars composition of tamarind pulp polysaccharide from tamarind pulps

Sugar composition analysis of acid hydrolyzates of tamarind pulp polysaccharides were analyzed by HPLC. HPLC chromatograms of the polysaccharide acid hydrolyzates were compared with that of standard monosaccharides. HPLC chromatograms of the polysaccharide acid hydrolyzates of sour tamarind, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K); and sweet tamarind, “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) cultivars are shown in Figures 27-30, respectively. Polysaccharide acid hydrolyzate of sour tamarind “Priao-Yak” (TI-PY/P) from Phetchabun showed the presence of rhamnose, xylose, arabinose and glucose/galactose. Polysaccharide acid hydrolyzate of sour tamarind “Priao” (TI-P/K) from Nakhon Ratchasima showed the presence of rhamnose, xylose, arabinose, fructose and glucose/galactose. Polysaccharides acid hydrolyzates of sweet tamarind “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima showed the presence of rhamnose, xylose, arabinose, fructose and glucose.

The presence of galacturonic acid along with neutral sugars such as rhamnose, xylose, arabinose, glucose and galactose in the acid hydrolyzates suggests that tamarind pulp polysaccharides of all sour and sweet tamarinds investigated are pectin-type polysaccharides.This result was resemble with the study of Osamu et al.(1997) who have found that pectin from citrus peels composes of galacturonic acid, rhamnose, mannose, xylose, arabinose, glucose and galactose. Therefore, polysaccharides extract from tamarind pulp of different tamarind cultivars and different cultivated area contained pectic polysaccharide

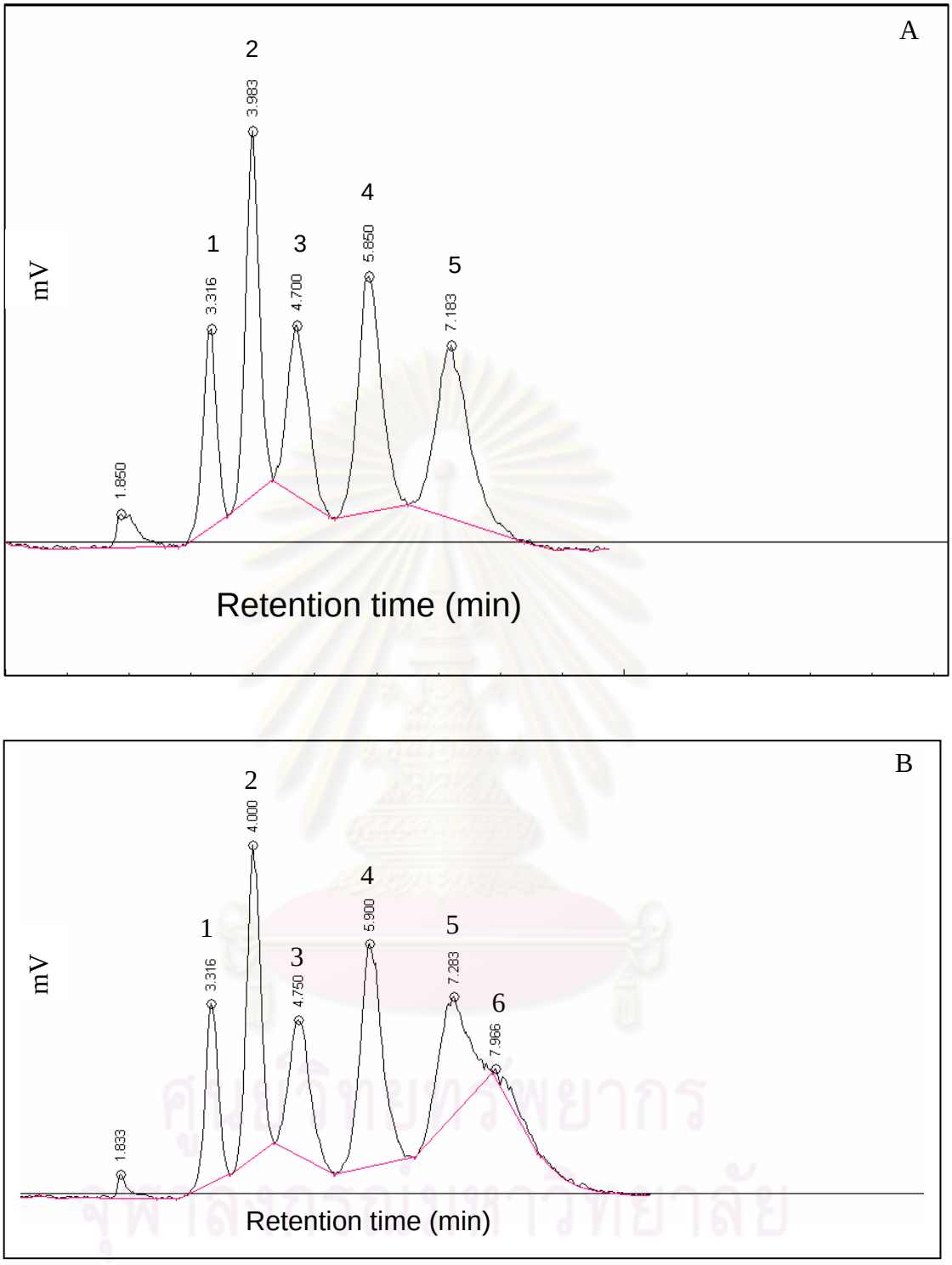


Figure 26. Chromatogram of 0.5% mixed-standard sugars (A) and (B).
Peak: 1=rhamnose, 2=xylose, 3=arabinose, 4=fructose, 5=glucose, 6=galactose

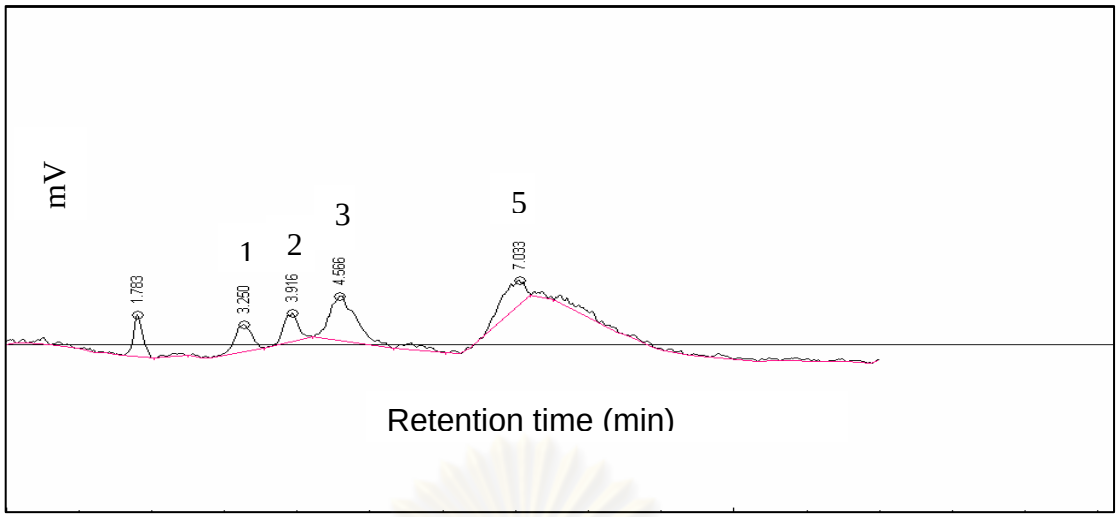


Figure 27. Chromatogram of acid hydrolyzate of 7.5% tamarind pulp polysaccharide of sour type *T.indica* "Preaw Yak" (TI-PY/P) from Phetchabun (P). Peaks: 1=rhamnose, 2=xylose, 3=arabinose, 5=glucose

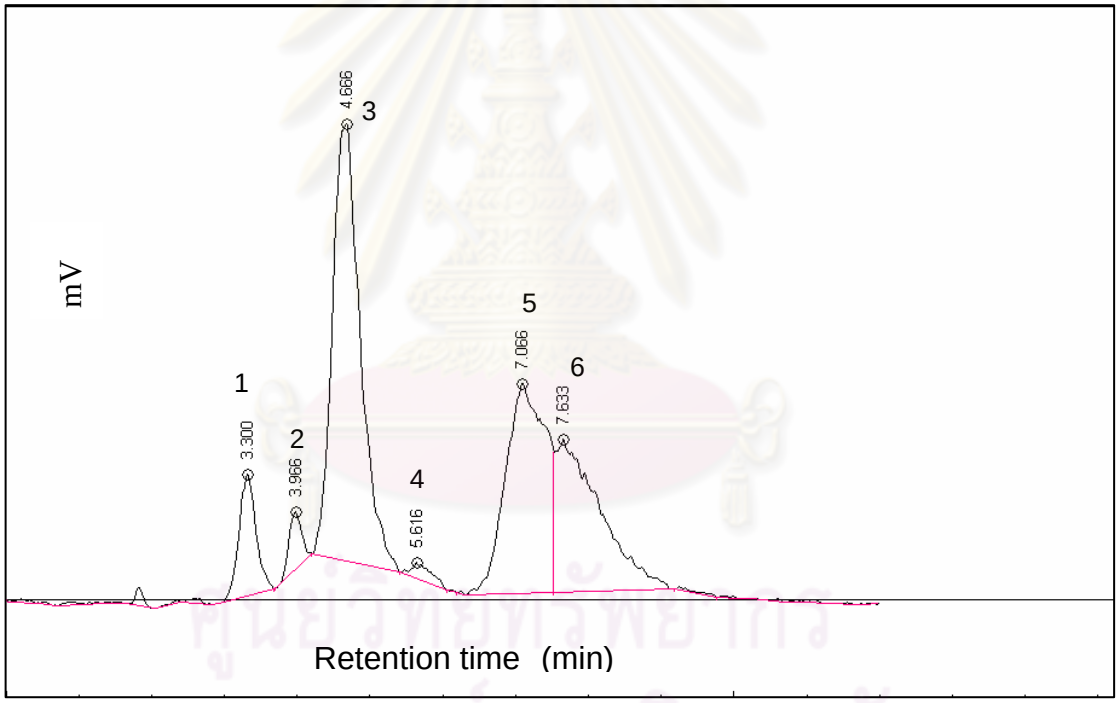


Figure 28. Chromatogram of acid hydrolyzate of 7.5% tamarind pulp polysaccharide of sour type *T.indica* "Preaw" (TI-P/K) from Nakhon Ratchasima (Khorat,K). Peaks: 1=rhamnose, 2=xylose, 3=arabinose, 4=fructose, 5=glucose, 6=galactose

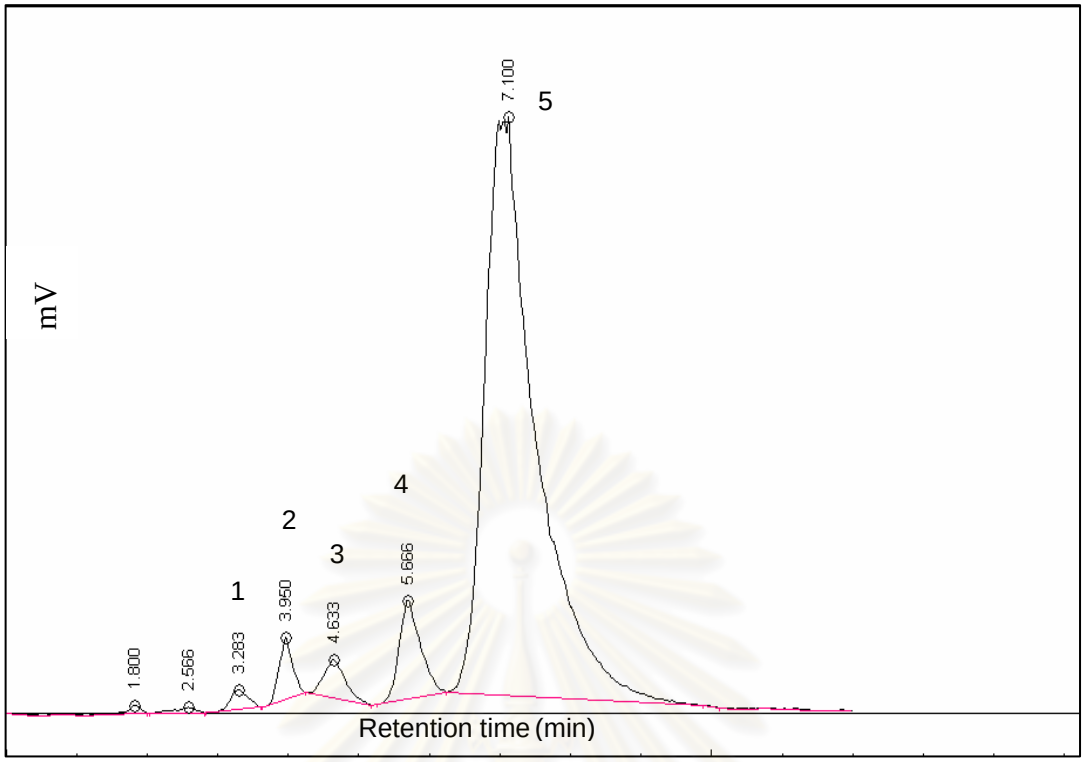


Figure 29. Chromatogram of acid hydrolyzate of 7.5% tamarind pulp polysaccharide of sweet type *T.indica* "Srichomphu" (TI-SP/K) from Nakhon Ratchasima (Khorat,K). Peaks: 1=rhamnose, 2=xylose, 3=arabinose, 4=fructose, 5=glucose/galactose

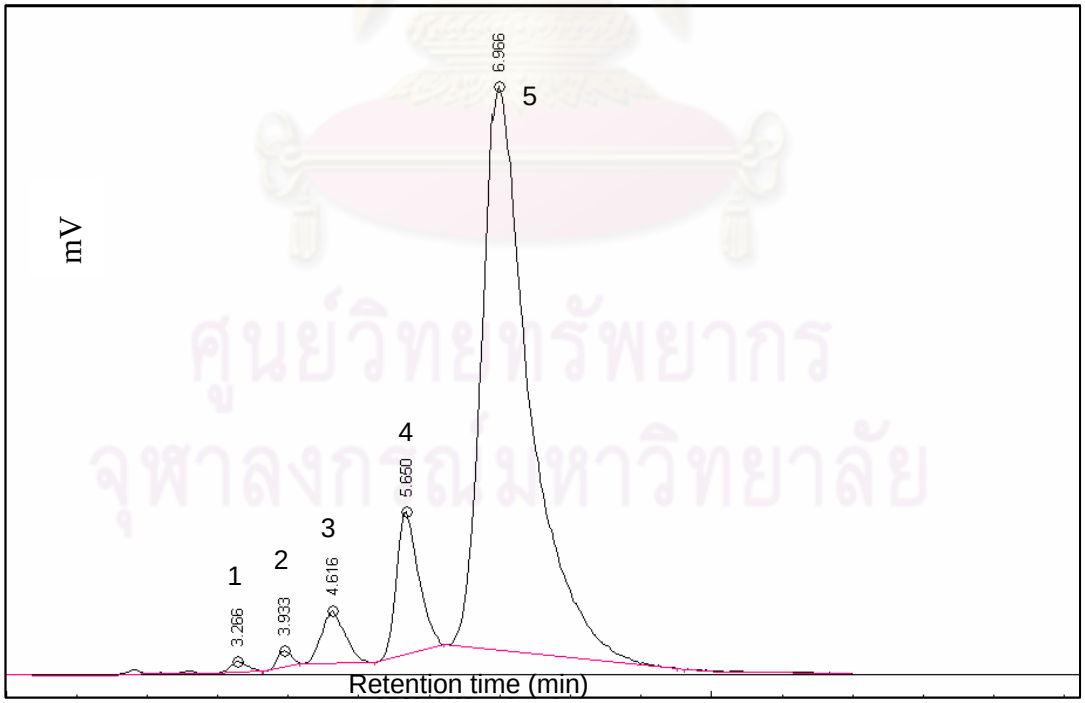


Figure 30. Chromatogram of acid hydrolyzate of 7.5% tamarind pulp polysaccharide of sweet type *T.indica* "Sithong-nak" (TI-STH/K) from Nakhon Ratchasima (Khorat,K). Peaks: 1=rhamnose, 2=xylose, 3=arabinose, 4=fructose, 5=glucose/galactose

Table 12. Sugar composition of the polysaccharide from tamarind pulp from Phetchabun (P) and Nakon Ratchasima (Khorat,K) provinces

T.indica Cultivars	Type of sugars (retention time, min)
Type “sour”	
Priao-yak (TI-PY/P)	rhamnose (3.250) ,xylose (3.916) ,arabinose (4.566), glucose (7.033), galactose(7.333)
Priao (TI-P/K)	rhamnose (3.300) ,xylose (3.966) ,arabinose (4.666), fructose (5.616) ,glucose (7.066), galactose(7.633)
Type “sweet”	
Srichomphu (TI-SP/K)	rhamnose (3.283) ,xylose (3.950) ,arabinose (4.633), fructose (5.666) ,glucose (7.100)
Sithong-nak (TI-STH/K)	rhamnose (3.266) ,xylose (3.933) ,arabinose (4.616), fructose (5.650) ,glucose (6.966)

5. Isolation of tamarind seed polysaccharides (TSP) from tamarind seed kernel

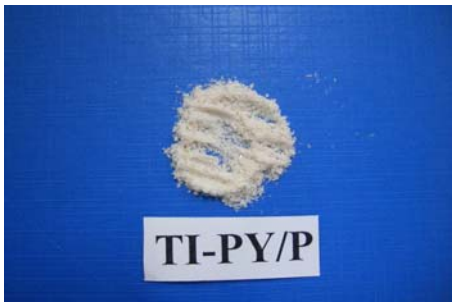
5.1 Isolation and yield of tamarind seed polysaccharides (TSP) from tamarind seed kernel

TSP was extracted from the tamarind kernel powder with hot water. An aqueous solution of tamarind kernel was viscous and polysaccharide solution was separated from the precipitate by centrifugation at 6800xg for 30 minutes. The whitish extract solution was odorless and TSP was precipitated by addition of 1.5 volumes of cold 95% ethanol. TSP was collected by filtration though fine nylon sieve and dried in hot air oven at 50°C. The polysaccharide obtained was a white solid material. After milling a whitish powder was obtained.

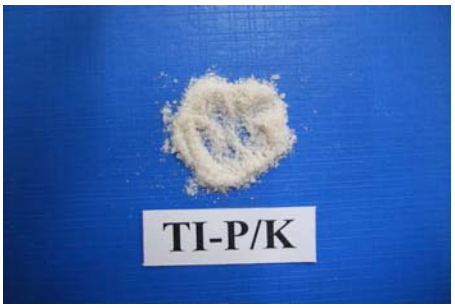
The Appearance of TSP isolated from tamarind kernel of different cultivars including sour type, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) and sweet type, “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) were shown in Figure 31. Table 13 illustrated the percentage yield and statistic analysis of tamarind seed polysaccharides yield. TSP yield of “Khantee” (TI-K/P) from Phetchabun gave significantly higher percent yield than that of “Priao-Yak” (TI-PY/P) from Phetchabun and “Priao” (TI-P/K), “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima ($P<0.05$). On the contrary TSP yield of “Priao-Yak” (TI-PY/P) from Phetchabun was not significant difference from that of “Priao” (TI-P/K) from Nakhon Ratchasima ($P>0.05$). Tamarind type sour gave lower TSP yield than those of type sweet. TSP yield of “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima was not significant difference from that “Srichomphu” (TI-SP/K) in the same province ($P>0.05$).

In this study, the result showed higher yield of TSP (48-61%) than the values reported by Suttananta (1986) by using the same method of extraction.

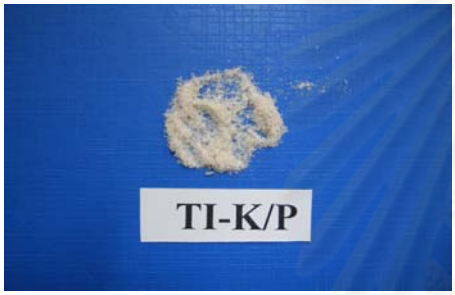
An aqueous dispersion of TSP from sour type, “Priao” (TI-P/K) and “Priao-Yak” (TI-PY/P) and sweet type, “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) were opaque white, viscous liquid.



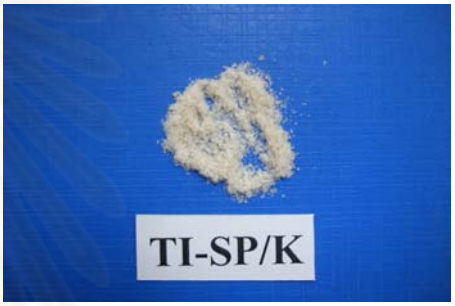
(a)



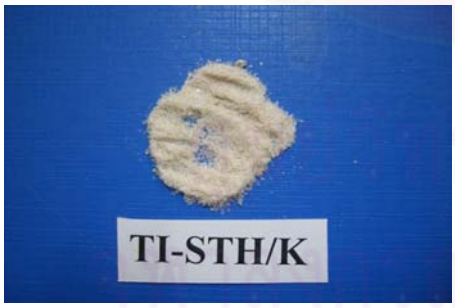
(b)



(c)



(d)



(e)

Figure 31. Appearance of Tamarind Seed Polysaccharides from tamarind kernel powder
(a) “Priao-Yak” (TI-PY/P) from Phetchabun (P)
(b) “Priao”(TI-P/K) from Nakhon Ratchasima (Khorat,K)
(c) “Khantee” (TI-K/P) from Phetchabun (P)
(d) “Srichomphu” (TI-SP/K) from Nakhon Ratchasima (Khorat,K)
(e) “Sithong-nak” (TI-STH/K) from Nakhon Ratchasima (Khorat,K)

Table 13. Appearance, viscosity and yield of the Tamarind Seed Polysaccharide (TSP) from tamarind kernel powder from Phetchabun (P) and Nakon Ratchasima (Khorat/K) provinces

TSP of *T.indica* Cultivars	Appearance of TSP powder	Viscosity of 2% TSP in water, cps (At shear rate 2840 1/s)	% yield of TSP (mean (SD))
Type "sour"			
Priao-yak (TI-PY/P)	creamy white powder	35.36	48.34[c] (0.89)
Priao (TI-P/K)	creamy white powder	106.12	48.43[c] (2.98)
Type "sweet"			
Khantee (TI-K/P)	creamy white powder	73.08	60.25[a] (0.50)
Srichomphu (TI-SP/K)	creamy white powder	45.07	58.09[ab] (1.37)
Sithong-nak (TI-STH/K)	creamy white powder	70.62	55.34[b] (1.85)

a,b,c show significant difference between cultivar at $P<0.05$

5.2 Determination of sugar composition of tamarind seed polysaccharides from tamarind kernel

5.2.1. Conditions of sugar analysis by HPLC method

The chromatographic conditions were the same method in Table13.

5.2.2 Determination of sugars composition of tamarind seed polysaccharides (TSP) from tamarind kernels

Analysis of sugar composition of acid hydrolyzates of tamarind seed polysaccharides were carried out by HPLC method. HPLC chromatograms of the polysaccharide acid hydrolyzates were compared with standard monosaccharides. Chromatograms of the polysaccharide acid hydrolyzates of sour tamarind, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K); and sweet tamarind, “Khantee” (TI-K/P) “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) cultivars are shown in Figures 32-36, Table 14 respectively.

From Figures 32-36 and Table 14, tamarind seed polysaccharide acid hydrolyzates of the sour and sweet tamarinds composed of peaks similar to standard xylose and glucose. The results indicated that glucose and xylose were the predominant monosaccharides of all tamarind seed polysaccharides of sour and sweet tamarinds.

The most outstanding structure of TSP is that its polysaccharide which composed of xylose and glucose as xyloglucan. In this experiment, the TSP hydrolyzate of sour tamarind, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K); and sweet tamarind, “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) contained xylose and glucose. Different laboratories have reported the wide ratio in xylose to glucose and galactose of tamarind seed polysaccharides. Iain and Edward (1984) investigated composition of TSP by X-ray diffraction. TSP contained glucose, xylose and galactose in the molar ratios of 4:3:1. Whereas Mary et al. (1991) suggested that the major polysaccharide in tamarind seed was a galactoxyloglucan for which the ratios galactose: xylose: glucose were 1:2.25:2.8 by the small angle X-ray diffraction. In addition, Savur (1959), Srivastava and Singh (1967) found that the acid hydrolysis of TSP contained glucose, xylose, galactose and arabinose in molar ratio of 8:2:4:1 but Marcos et al. (1992) found that acid hydrolysis of TSP composed of glucose, xylose and galactose in the molar ratios of 4:3.0-3.1:1.4.

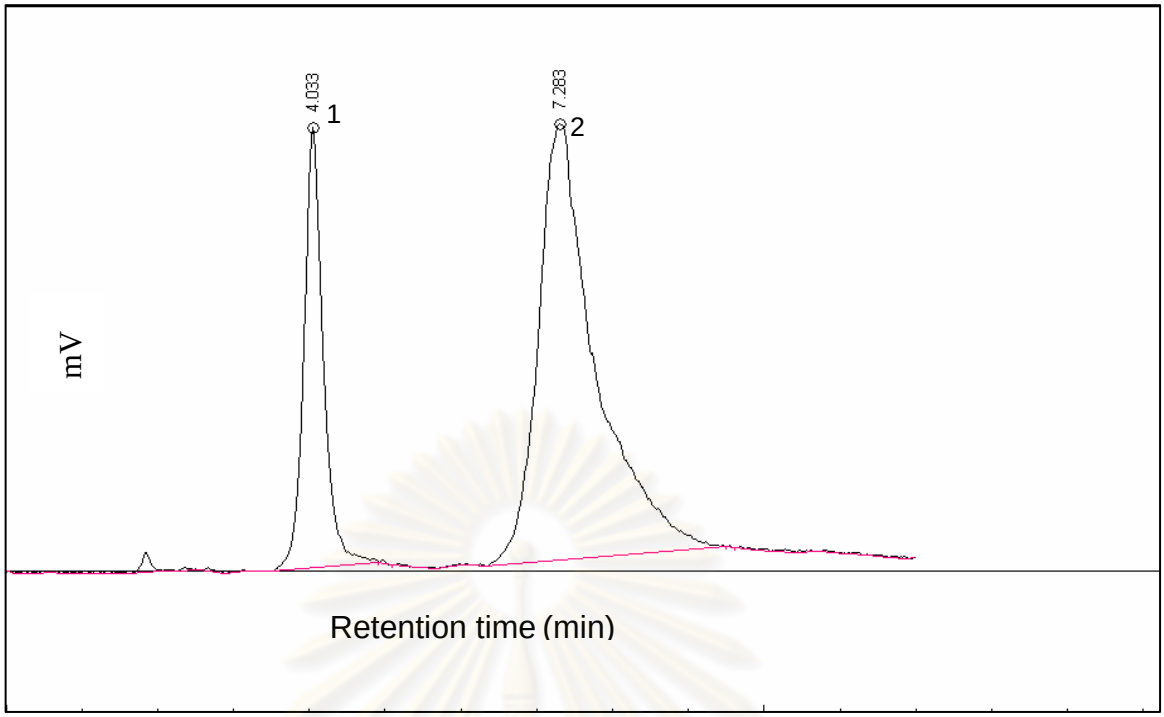


Figure 32. Chromatogram of acid hydrolyzate of 0.5% TSP of sour type *T.indica* "Preaw Yak" (TI-PY/P) from Phetchabun (P)
Peaks: 1=xylose, 2=glucose

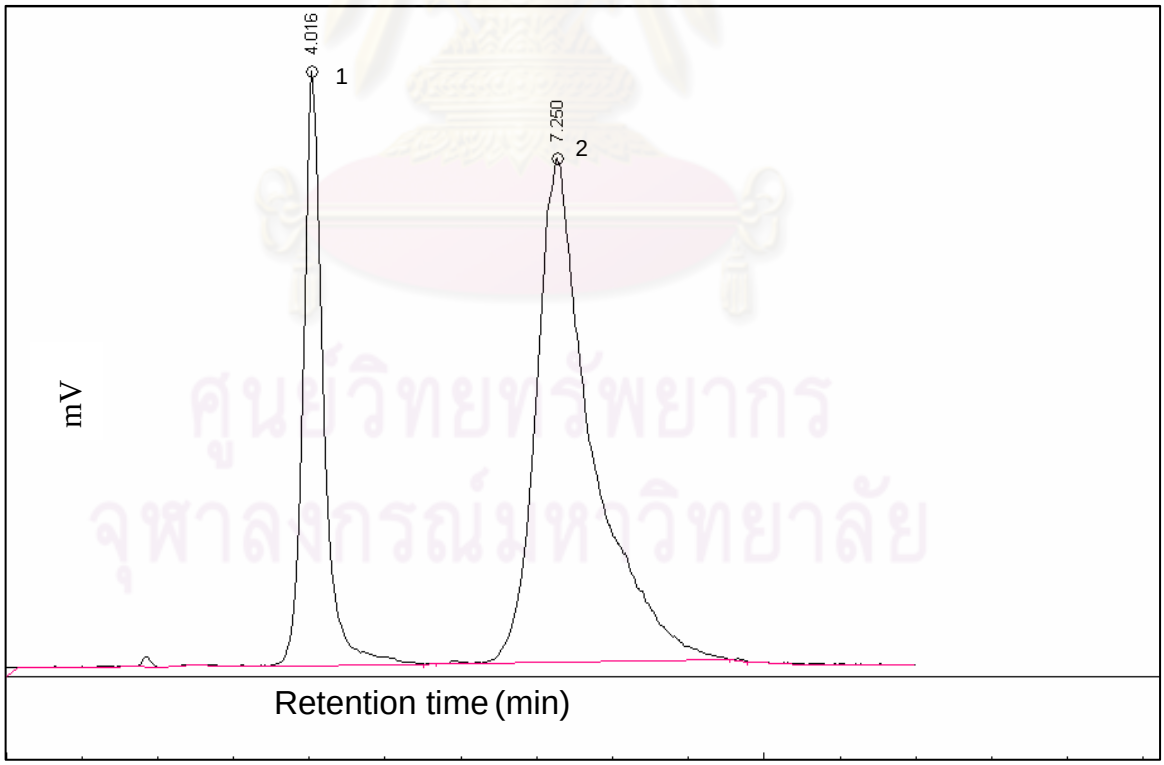


Figure 33. Chromatogram of acid hydrolyzate of 0.5% TSP of sour type *T.indica* "Preaw" (TI-P/K) from Nakhon Ratchasima (Khorat,K).
Peaks: 1=xylose, 2=glucose

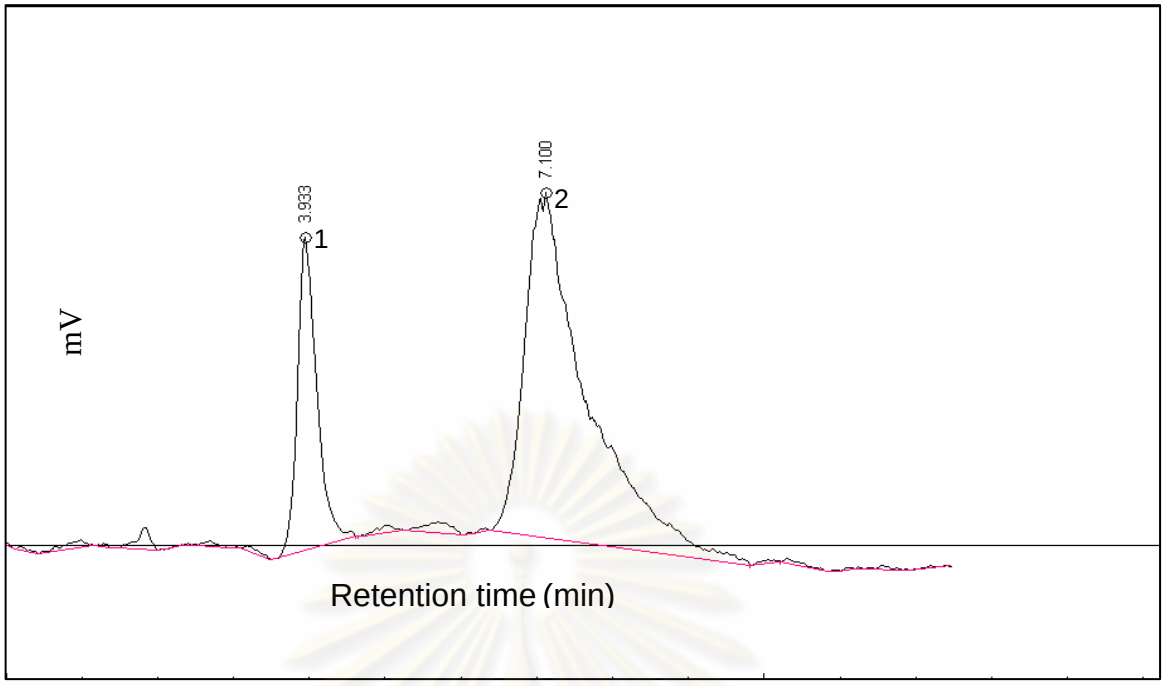


Figure 34. Chromatogram of acid hydrolyzate of 0.5% TSP of sweet type *T.indica* "Khantee" (TI-K/P) from Phetchabun (P).
Peaks: 1=xylose, 2=glucose

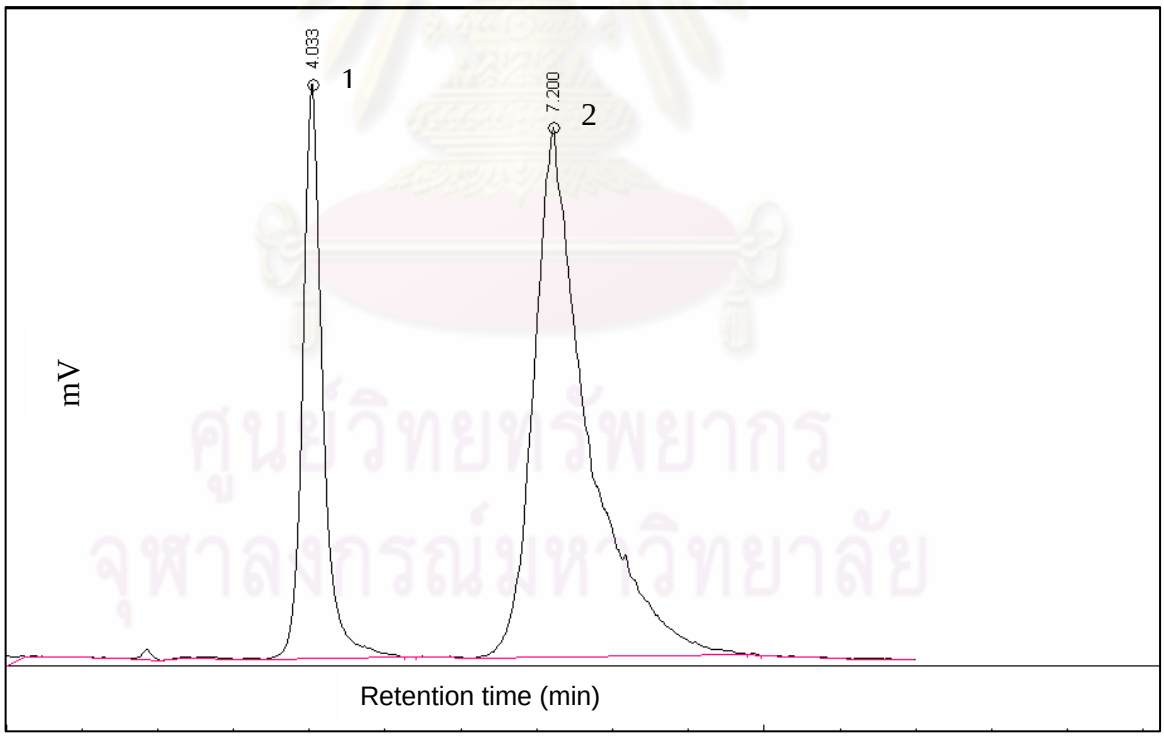


Figure 35. Chromatogram of acid hydrolyzate of 0.5% TSP of sweet type *T.indica* "Srichomphu" (TI-SP/K) from Nakhon Ratchasima (Khorat,K).
Peaks:1=xylose, 2=glucose

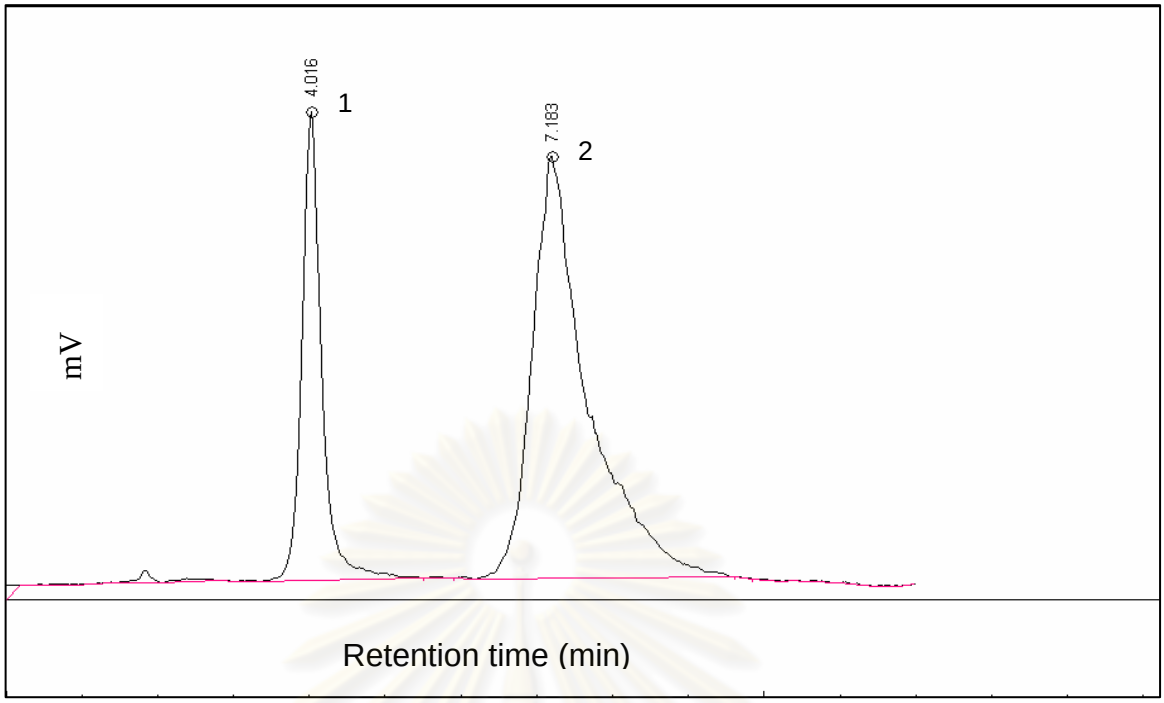


Figure 36. Chromatogram of acid hydrolyzate of 0.5% TSP of sweet type *T.indica* "Sithong-nak" (TI- STH/K) from Nakhon Ratchasima (Khorat,K). Peaks:1=xylose, 2=glucose

Table 14. Sugar compositions of the Tamarind Seed Polysaccharide (TSP) from tamarind kernel powder from Phetchabun (P) and Nakon ratchasima (Khorat,K) provinces

TSP of *T.indica* Cultivars	Type of sugars (retention time, min)
Type "sour"	
Priao-yak (TI-PY/P)	xylose (4.033) , glucose (7.283)
Priao (TI-P/K)	xylose (4.033) , glucose (7.250)
Type "sweet"	
Khantee (TI-K/P)	xylose (3.933) , glucose (7.100)
Srichomphu (TI-SP/K)	xylose (4.000) , glucose (7.183)
Sithong-nak (TI-STH/K)	xylose (4.100) , glucose (7.383)

In this experiment, TSP hydrolyzate of sour and sweet tamarind from different tamarind cultivars and different cultivated area composed of monosaccharides xylose and glucose after polysaccharide digestion with acid. Very low galactose might exist, since some TSP cultivars showed peaks of glucose with tailing. These findings agree with other reports that the major polysaccharide in tamarind seed composes of xyloglucan.

5.3 Rhelogy and viscosity of tamarind seed polysaccharides (TSP)

Flow curve of TSP of sour tamarind, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) and sweet tamarind, “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) are shown in Figure 37. At 2% concentration of TSP of sour and sweet tamarind exhibited a pseudoplastic flow and increasing shear rate resulted in decreasing viscosity.

The viscosity of 2% w/v TSP solution was scanned at shear rate from 0 to 6000 1/s by Rheometer using C35/1 Ti as the sensor. The shear rate at 2840 1/s was used to measure the viscosity of TSP in this study. Table 13 shows the viscosities of 2% TSP from different tamarind cultivars, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K), “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) were 35.36, 106.12, 73.08, 45.07 and 70.62 cPs, respectively.

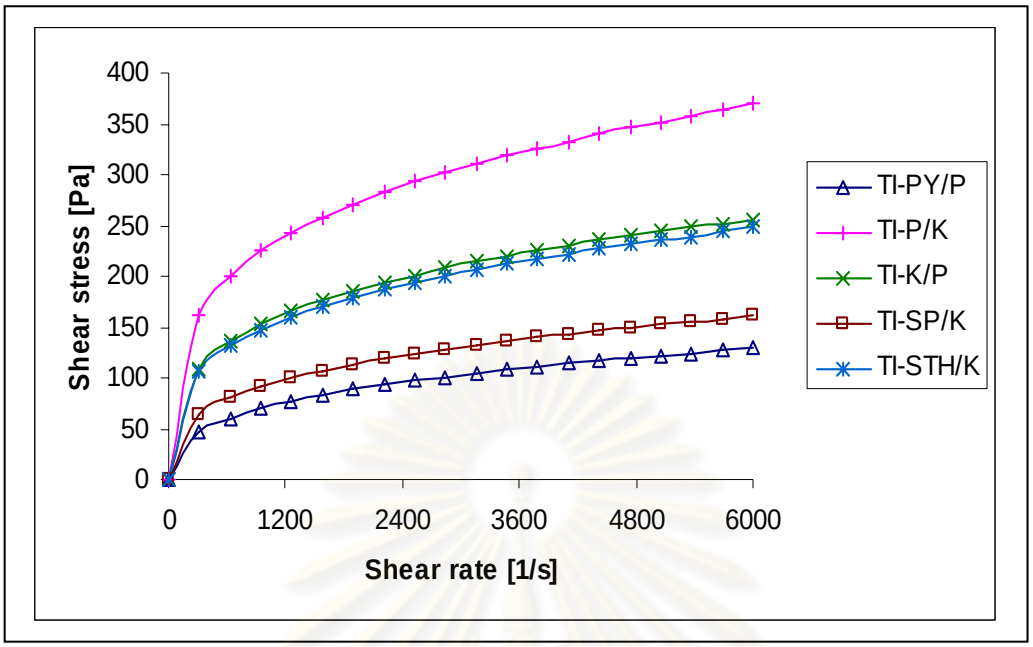


Figure 37. Flow curve at 2% Tamarind Seed Polysaccharide of tamarind cultivars, "Priao-Yak" (TI-PY/P), "Khantee" (TI-K/P) from Phetchabun province and "Priao" (TI-P/K), "Srichomphu" (TI-SP/K), "Sithong-nak" (TI-STH/K) from Nakhon Ratchasima (Khorat,K) province

6. Preparation of tamarind powder by spray drying technique

6.1 Physical Appearances

Formulations of tamarind powder were developed as shown in Table 15 and the other test formulations were also shown in appendix D. An appearance of tamarind powder products and moisture content are described in Table 16. The picture of the tamarind powder is shown in Figure 38. There was three satisfactory formulae produced fine powder product that easily dispersed in hot water.

Tamarind powder of formula 11, 1 L of tamarind mixture composed of TI-PY/P and TI-K/P each of 15 g/L, 1.35 g/L Fructose, 0.45 g/L NaCl, 25 g/L Maltodextrin and 0.3 g/L Silicon dioxide was prepared by spray dry, the product obtained was small particle, dry yellow powder, agglomerate and absorb moisture. The product easily dispersed in hot water.

Tamarind powder of formula 12, 1 L of tamarind mixture composed of TI-PY/P and TI-K/P each of 15 g/L, 1.35 g/L Fructose, 0.45 g/L NaCl, 25 g/L Maltodextrin, 5 g/L TSP and 0.3 g/L Silicon dioxide was prepared by spray dry, the product obtained was fine particle and dry light yellow powder. The product dispersed well in hot water.

Tamarind powder of formula 13, 1 L of tamarind mixture composed of TI-PY/P and TI-K/P each of 15 g/L, 1.35 g/L Fructose, 0.45 g/L NaCl, 25 g/L Maltodextrin, 5 g/L Pectin and 0.3 g/L Silicon dioxide was prepared by spray dry, the product obtained was fine particle and dry light yellow powder. The product dispersed well in hot water.

6.2 Quantitative determination of organic acids in tamarind powder

Table 17 show organic acids content in tamarind powder product of the three formulations. Figure 39-41 show chromatograms of tamarind powder of formula No.11-13. The analysis was done in triplicate. Low tartaric acid content in product No.13 could be due to its moisture content.

Table 15. Composition of ingredients in 1 L of tamarind mixture before spray-drying

Formula No.	Tamarind extracts* (g/L)	Fructose (g/L)	NaCl (g/L)	SiO_2 (g/L)	TSP (g/L)	Pectin (g/L)	Maltodextrin (g/L)
1	30	1.35	0.45	0.30	10	-	-
2	30	1.35	0.45	0.30	-	10	-
3	30	1.35	0.45	0.30	-	-	10
4	30	1.35	0.45	-	6	4	-
5	30	1.35	0.45	0.30	6	4	-
6	30	1.35	0.45	0.30	6	4	15
7	30	1.35	0.45	0.30	6	-	19
8	30	1.35	0.45	0.30	10	-	15
9	30	1.35	0.45	0.30	5	-	15
10	30	1.35	0.45	0.30	5	-	20
11	30	1.35	0.45	0.30	-	-	25
12	30	1.35	0.45	0.30	5	-	25
13	30	1.35	0.45	0.30	-	5	25

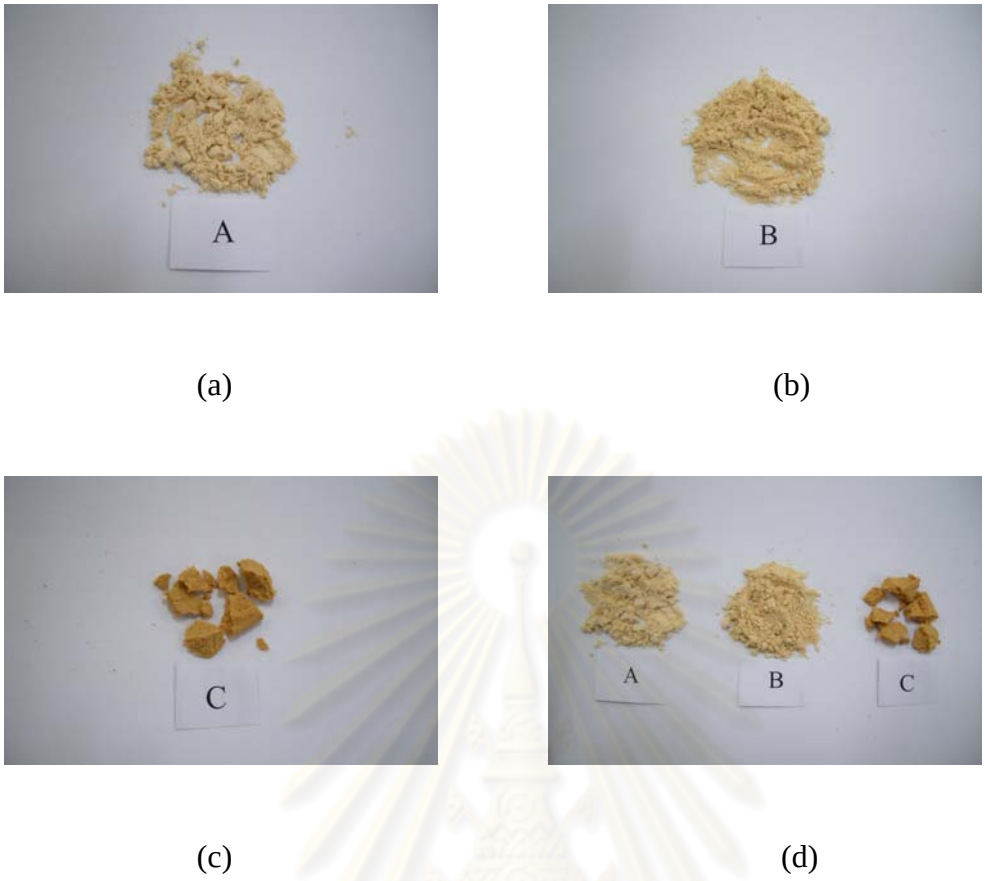
* Tamarind extract composed of TI-PY/P and TI-K/P each of 15 g/L

TSP (Formula No.6-10, 12) and pectin (Formula No.13) were autoclave at 121°C 30 minutes, pressure 0.1 MPa before mixed in tamarind mixture.

Table 16. Characters of tamarind powder products after spray drying

Product No.	Appearance tamarind powder product after spray drying	% yield (SD)	%moisture Content (SD)	Dispersibility of 10% product in hot water (time in min)
1	Dry yellow powder, small particle, agglomerate, absorb moisture	24.2	8.75	difficult (50)
2	Dry yellow powder, small particle, easily agglomerate, absorb moisture rapidly	42.25	9.89	easy (15)
3	Wet yellow powder	35.35	ND	easy (10)
4	Dry yellow powder, small particle, agglomerate, absorb moisture	17.16	8.65	difficult (40)
5	Dry yellow powder, small particle, agglomerate, absorb moisture	27.06	8.58	difficult (30)
6	Dry yellow powder, easily agglomerate, absorb moisture rapidly	45.10	10.97	difficult (20)
7	Dry yellow powder, small particle, agglomerate, absorb moisture	25.64	8.73	difficult (20)
8	Dry yellow powder, small particle, absorb moisture	33.33	9.08	difficult (30)
9	Dry yellow powder, small particle, easily agglomerate, absorb moisture rapidly	21.08 (0.52)	9.76 (0.10)	difficult (25)
10	Dry yellow powder, small particle, easily agglomerate, absorb moisture	40.20 (0.57)	8.92 (0.04)	difficult (20)
11	Dry yellow powder, small particle, easily agglomerate, absorb moisture	46.17 (0.99)	9.76 (0.08)	easy (5)
12	Dry light yellow powder, fine particle	46.35 (1.58)	8.05 (0.02)	easy (10)
13	Dry light yellow powder, fine particle	47.62 (1.67)	8.30 (0.20)	easy (10)

Product No.9-13 was done in triplicate, ND = not determined



(a) (b)

(c) (d)

Figure 38. Appearance of tamarind powder product by spray-drying technique
(a) Product No.12 (b) Product No.13 (c) Product No.11
(d) Comparison of tamarind powder of the 3 formulations

Table 17. Organic acids content in tamarind powder prepared by spray drying

Product No.	Organic acids in tamarind powder (mg/100g) mean (SD), N=3					
	OA	TA	SA	FA	L-MA	CA
11	288.75 (5.30)	15796.20 (100.32)	-	-	332.42 (4.15)	173.25 (8.49)
12	202.80 (0.65)	11931.65 (139.52)	-	-	225.25 (15.46)	162.42 (18.76)
13	311.40 (5.58)	9295.65 (3.75)	-	-	169.27 (5.64)	134.67 (1.73)

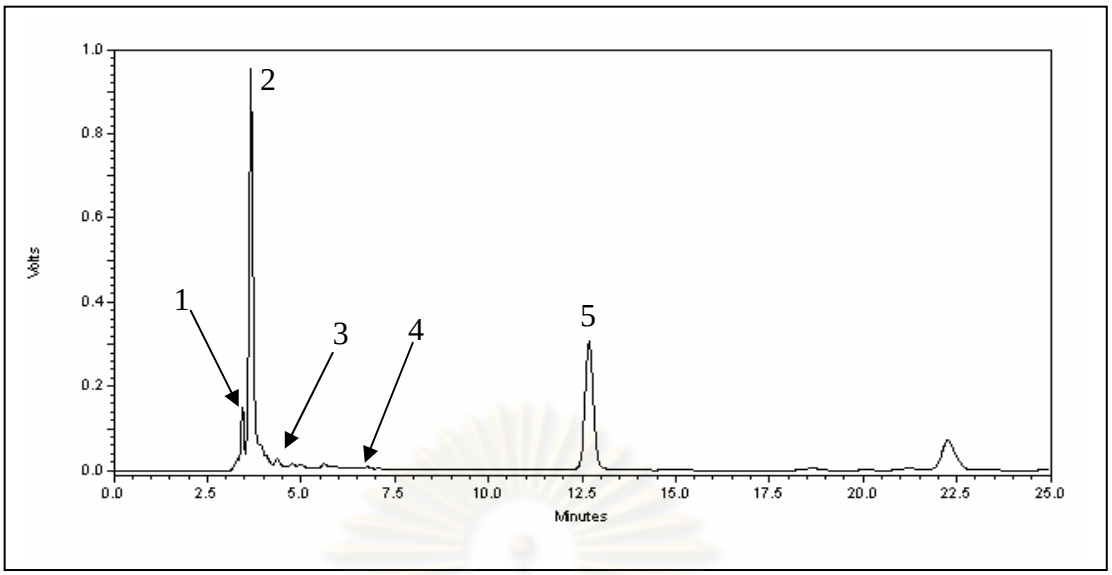


Figure 39. Chromatogram of organic acids in tamarind powder Formulation No.11 Peaks: 1 Oxalic acid (OA), 2 Tartartic acid (TA), 3 L-malic acid (L-MA), 4 Citric acid (CA) and 5 Gallic acid (GA)

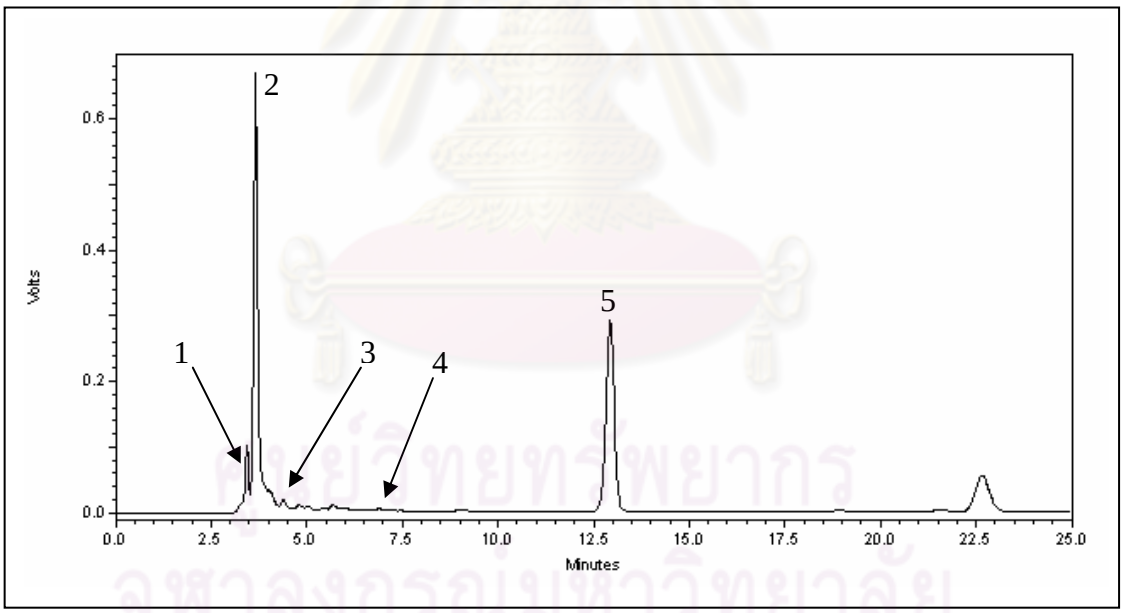


Figure 40. Chromatogram of organic acids in tamarind powder Formulation No.12 Peaks: 1 Oxalic acid (OA), 2 Tartartic acid (TA), 3 L-malic acid (L-MA), 4 Citric acid (CA) and 5 Gallic acid (GA)

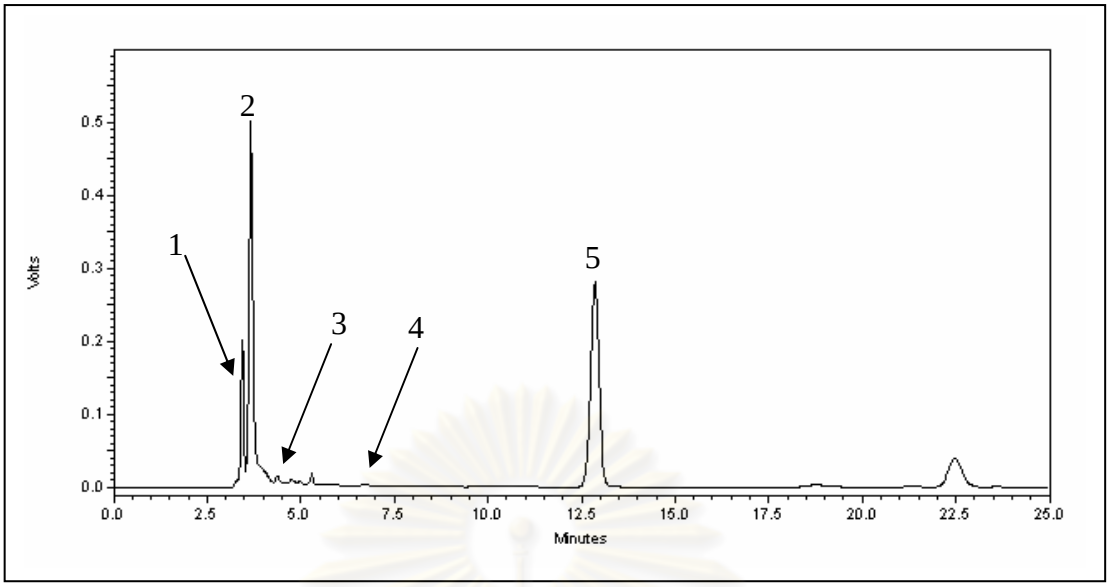
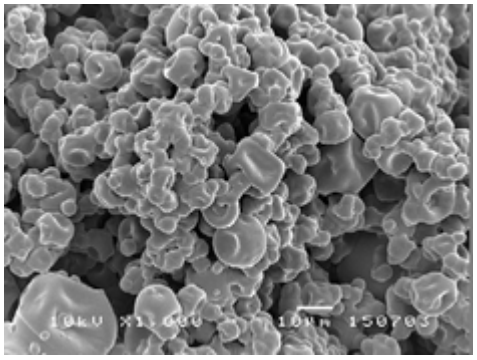


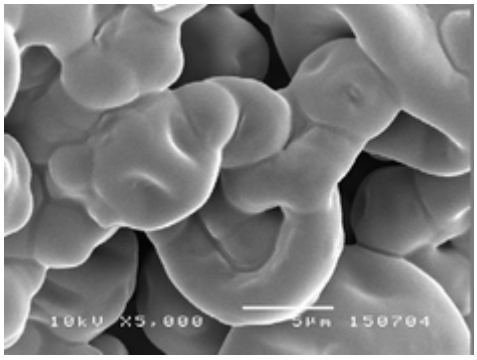
Figure 41. Chromatogram of organic acids in tamarind powder Formulation No.13 Peaks: 1 Oxalic acid (OA), 2 Tartartic acid (TA), 3 L-malic acid (L-MA), 4 Citric acid (CA) and 5 Gallic acid (GA)

7.3 Scanning electron microscopy

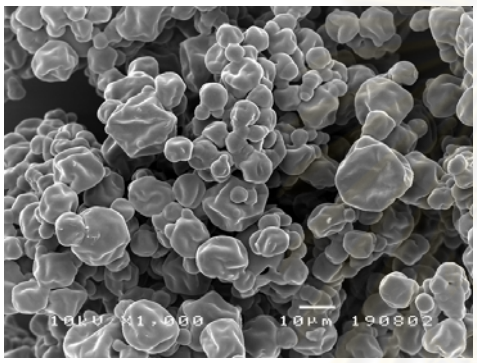
Scanning electron Microscopy was used to determine particle morphology of tamarind powder products. Scanning electron micrographs showing the outer surface of tamarind powder are illustrated in Figure 42.



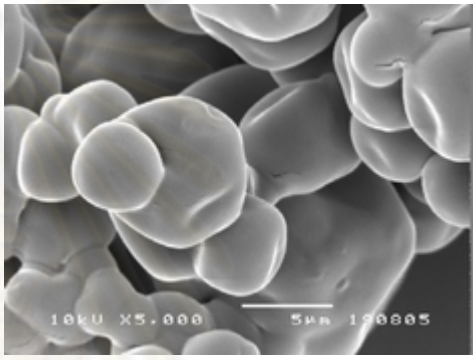
(A1)



(A2)



(B1)



(B2)

Figure 42. Scanning electron micrographs of tamarind powder products prepared by spray-drying
(A1) Product No.12 (x1000) (A2) Product No.12 (x5000)
(B1) Product No.13 (x1000) (B2) Product No.13 (x5000)

CHAPTER V

CONCLUSION

1. Quantitative determination of organic acids in tamarind extracted by High Performance Liquid Chromatography (HPLC)

- Analytical method development for quantitative determination of organic acids in aqueous extracts of tamarind pulps by HPLC was successful and the chromatographic condition was as follow: C18 column under isocratic elution was developed with 0.5%(w/v) $(NH_4)H_2PO_4$ buffer pH 2.6 flow rate 1 ml/min, UV detector at 210 nm.

- Under the condition described, the mixture of standard acids (oxalic acid, tartaric acid, L- malic acid, citric acid, fumaric acid, succinic acid and gallic acid) could be well resolved with symmetrical peak. The resolution values of peak organic acids were in the ranges 1.64-4.54.

- The organic acid contents in tamarind cultivars type “sour” and “sweet” in tamarind pulp extracts from the different growing areas, Phetchabun (P) and Nakhon Ratchasima (Khorat/K) province were quantitatively determined.

- Tartaric acid contents of sour tamarind “Priao-Yak” (TI-PY/P) were significantly higher than other sour and sweet tamarinds. The amount of tartaric acid was significant difference between cultivars.

- Oxalic acid, L-malic acid, and fumaric acid contents showed the highest value in sweet tamarind “Sithong-nak” (TI-STH/K).

- Sour tamarind contained lower amount of L-malic acid but higher amount of tartaric acid than those of sweet tamarind.

2. Isolation of tamarind pulp polysaccharide

- The total yield of tamarind pulp polysaccharide isolated from tamarind pulps of different cultivars including sour type, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K); and sweet type, “Srichomphu” (TI-SP/K) and “Sithong-nak”(TI-STH/K) was 1.74±0.03%, 2.44±0.16%, 2.75±0.20% and 1.98±0.30% (w/w) of dried tamarind pulps, respectively. Tamarind pulp polysaccharides extracted from different cultivars and different cultivated area composed of pectic polysaccharide according to their profiles of FT-IR spectra, which were similar to the standard pectin from citrus fruits. Galacturonic acid in tamarind pulp polysaccharide was also confirmed by HPLC-RID technique.

- The composition of neutral sugars in tamarind pulp polysaccharide of sour tamarind cultivars composed of rhamnose, xylose, arabinose, glucose and galactose while sweet tamarind composed of rhamnose, xylose, arabinose, fructose and glucose.

3. Determination of tamarind seed polysaccharide (TSP) from tamarind seed kernels

- The total yield of tamarind seed polysaccharide isolated from tamarind seed kernel of different cultivars including sour type, “Priao-Yak” (TI-PY/P) and “Priao” (TI-P/K) and sweet type, “Khantee” (TI-K/P), “Srichomphu” (TI-SP/K) and “Sithong-nak” (TI-STH/K) was 48.34±0.89%, 48.43±2.98%, 60.25±0.50%, 58.09±1.37% and 55.34±1.85% (w/w) of tamarind kernel powder, respectively.

- At 2% w/v concentration of TSP of sour and sweet tamarind exhibited a pseudoplastic flow behavior, increasing shear rate resulted in decreasing viscosity.

- The composition of sugars in tamarind seed polysaccharide of all tamarind cultivars composed of xylose and glucose.

4. Preparation of tamarind powder by spray drying technique

- Formula of tamarind powder was developed, the product of formula composed of TI-PY/P and TI-K/P each of 15 g/L, 1.35 g/L Fructose, 0.45 g/L NaCl, 25 g/L Maltodextrin, 5 g/L TSP and 0.3 g/L Silicon dioxide in 1 L DI water was successfully prepared by spray drying, the product obtained was fine particle, light yellow dry powder. Moisture content was 8.05±0.02 %. Tamarind powder (10 g) was dispersed in 100 mL hot water in 10 minutes.

- Tamarind powder can be prepared by spray drying technique using TSP together with maltodextrin as carriers.

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APPENDICES

Appendix A HPLC chromatogram of standard organic acid

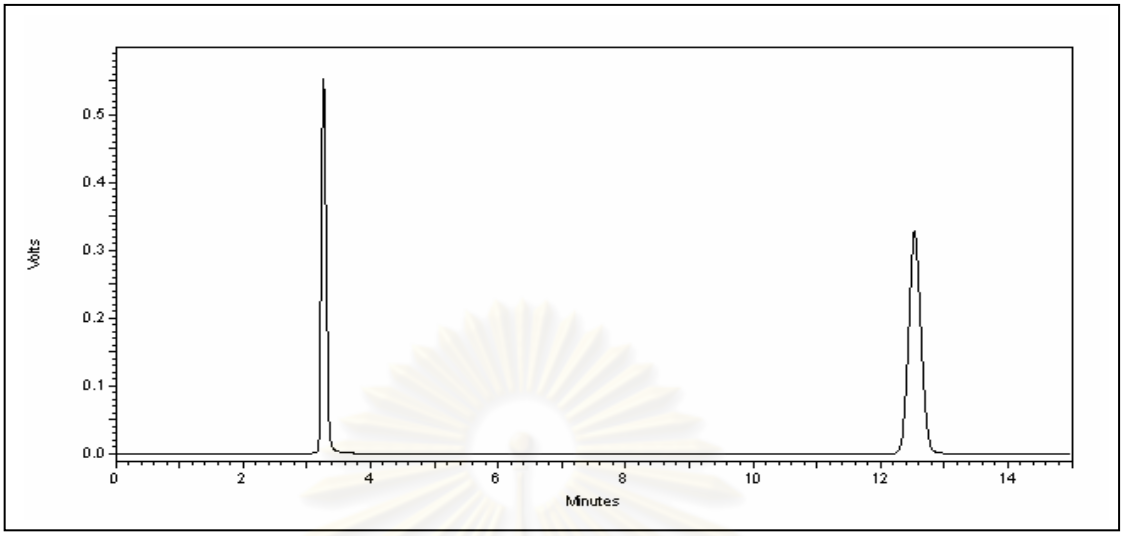

Figure A1. Chromatogram of standard oxalic acid (OA)

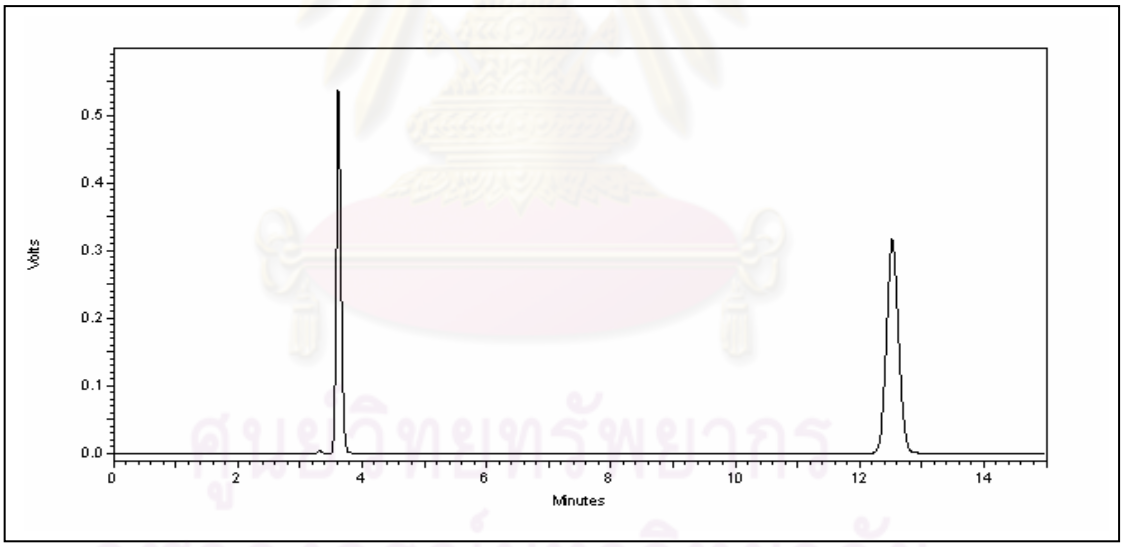

Figure A2. Chromatogram of standard tartaric acid (TA)

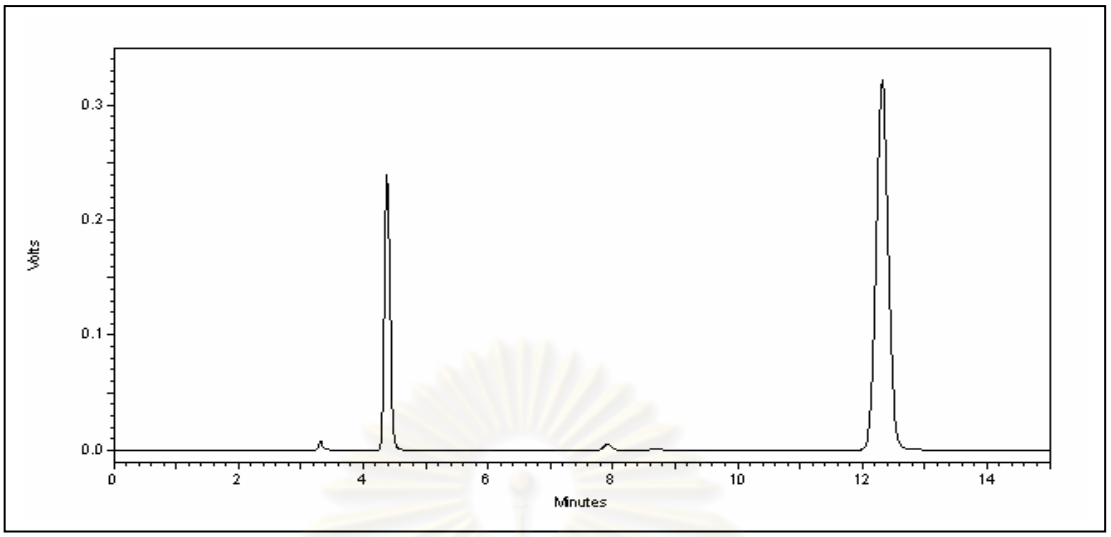

Figure A3. Chromatogram of standard L-malic acid (L-MA)

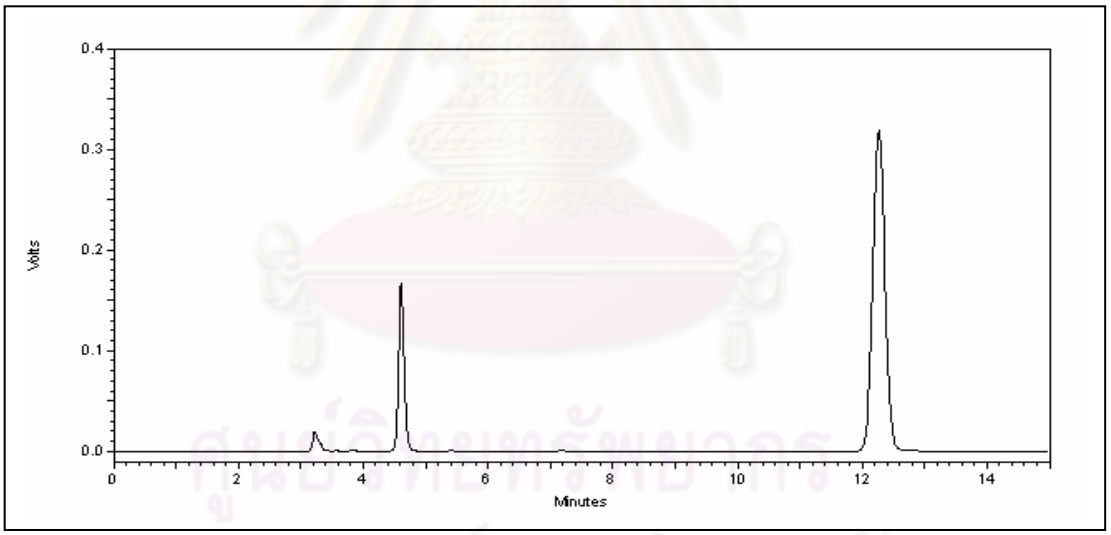

Figure A4. Chromatogram of standard Ascorbic acid (AA)

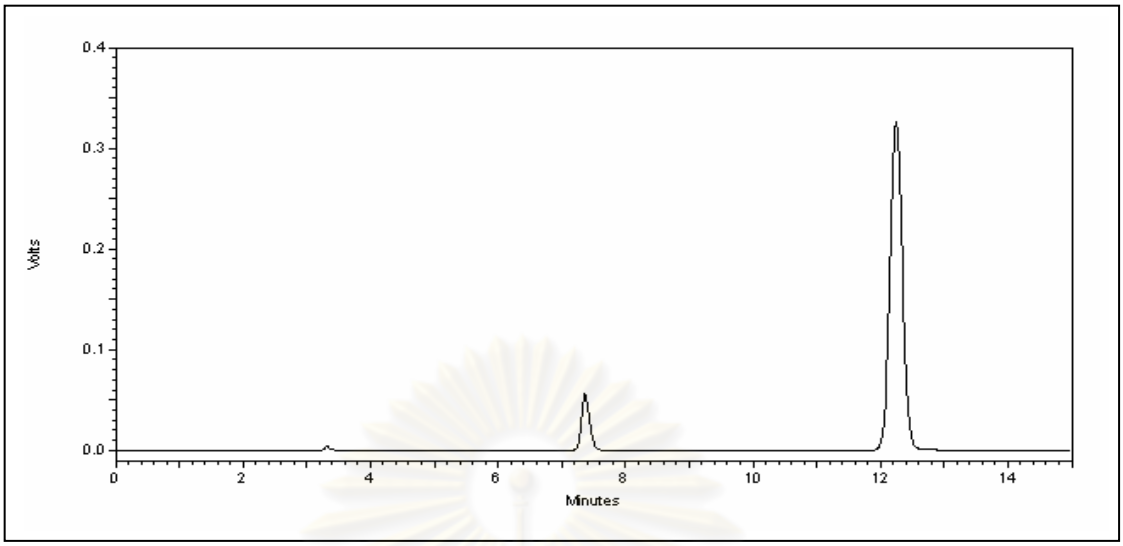

Figure A5. Chromatogram of standard citric acid (CA)

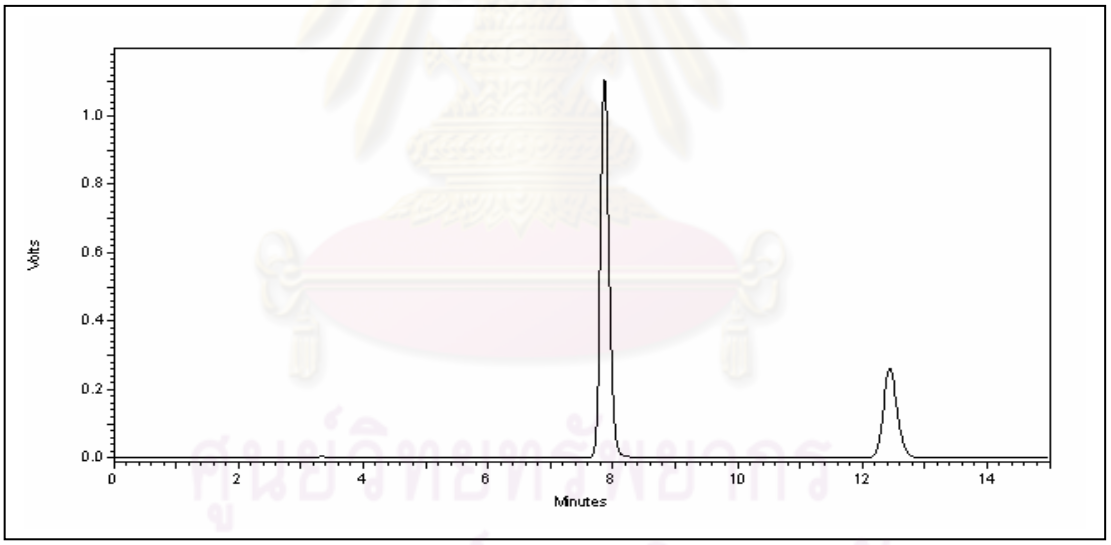

Figure A6. Chromatogram of standard fumaric acid (FA)

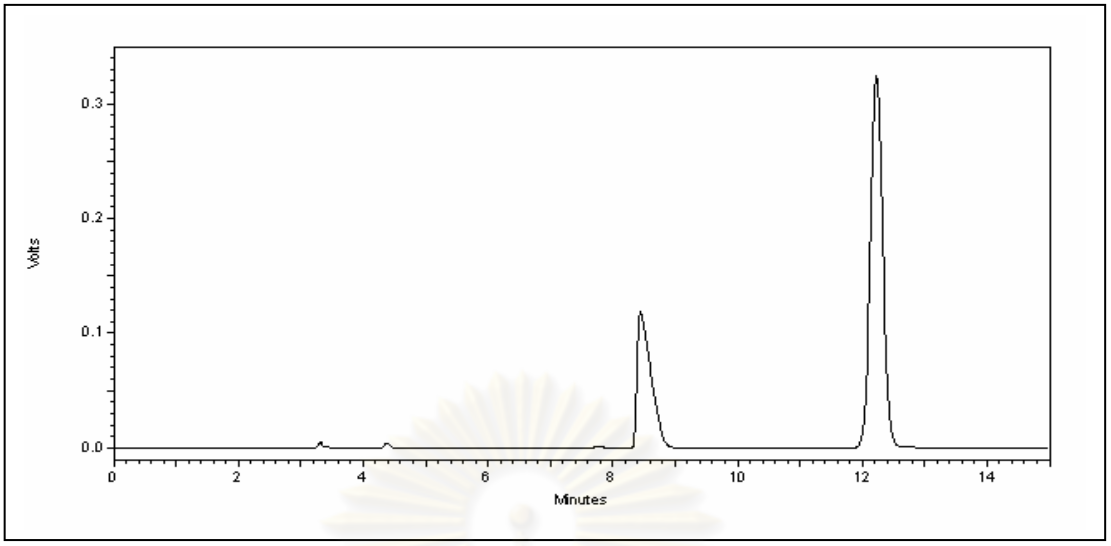

Figure A7. Chromatogram of standard succinic acid (SA)

Appendix B Standard curve of organic acid

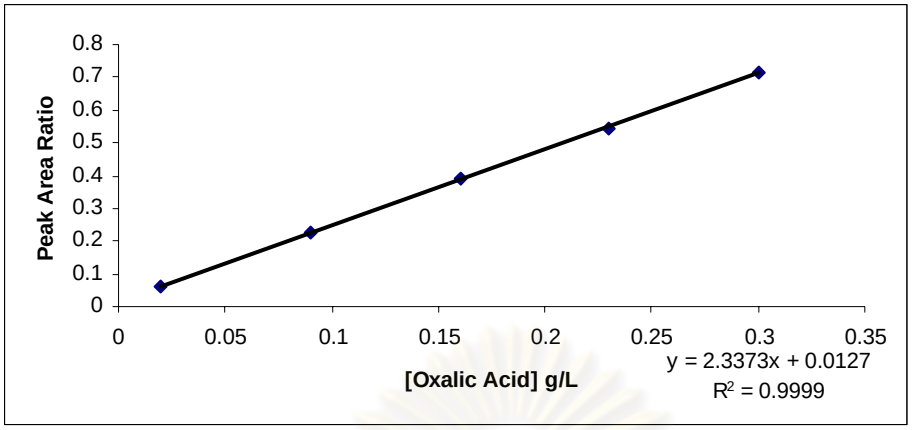

Figure B1. Standard curve of oxalic acid

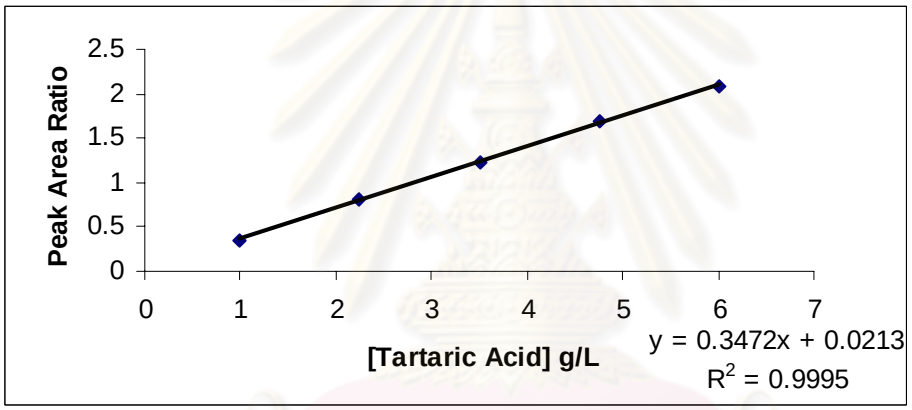

Figure B2. Standard curve of tartaric acid

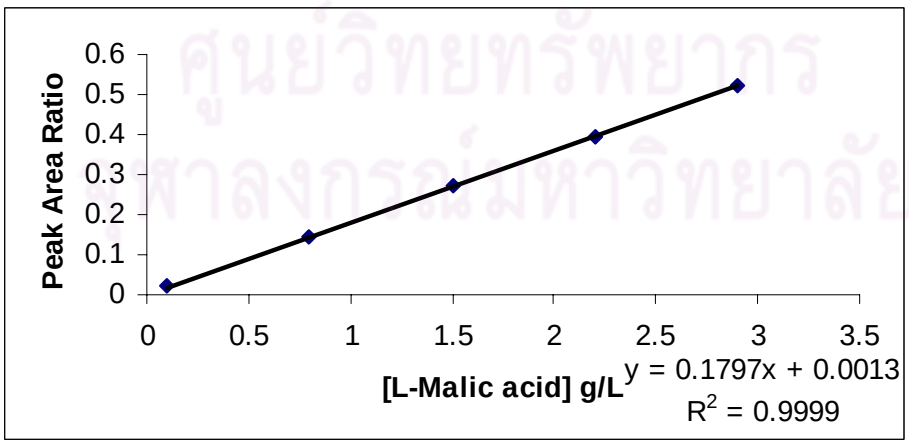

Figure B3. Standard curve of L-malic acid

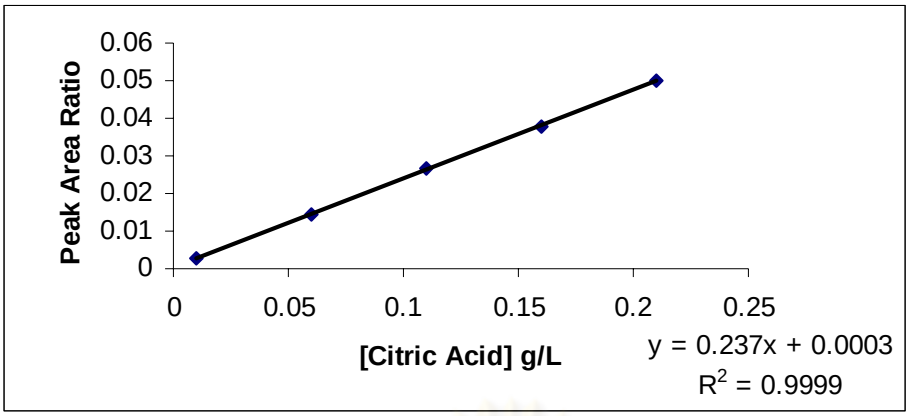

Figure B4. Standard curve of citric acid

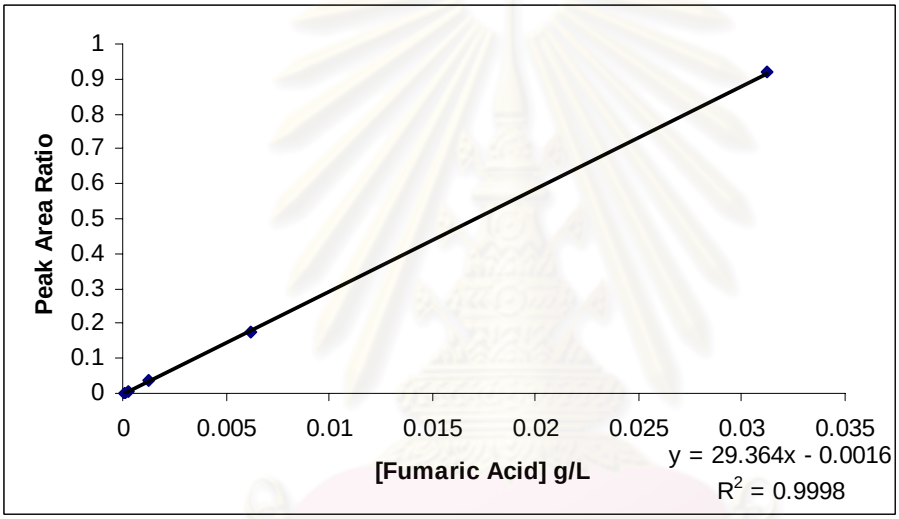

Figure B5. Standard curve of fumaric acid

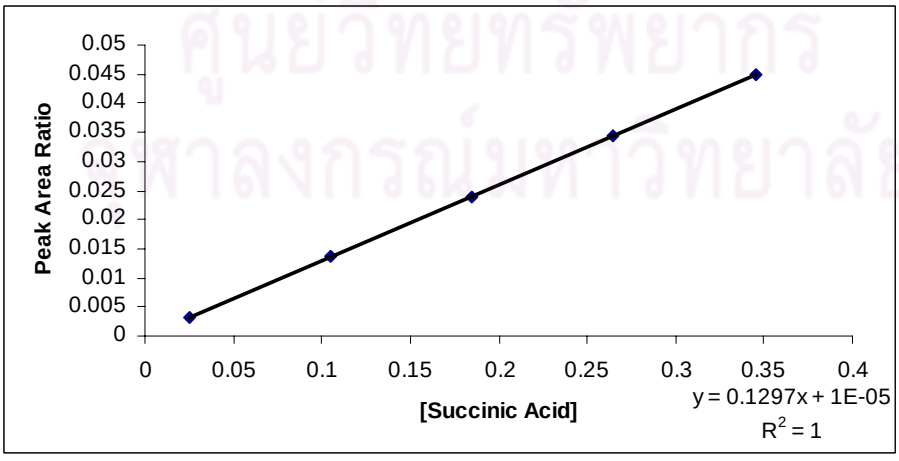

Figure B6. Standard curve of succinic acid

Appendix C HPLC Chromatogram of standard sugar (ELSD)

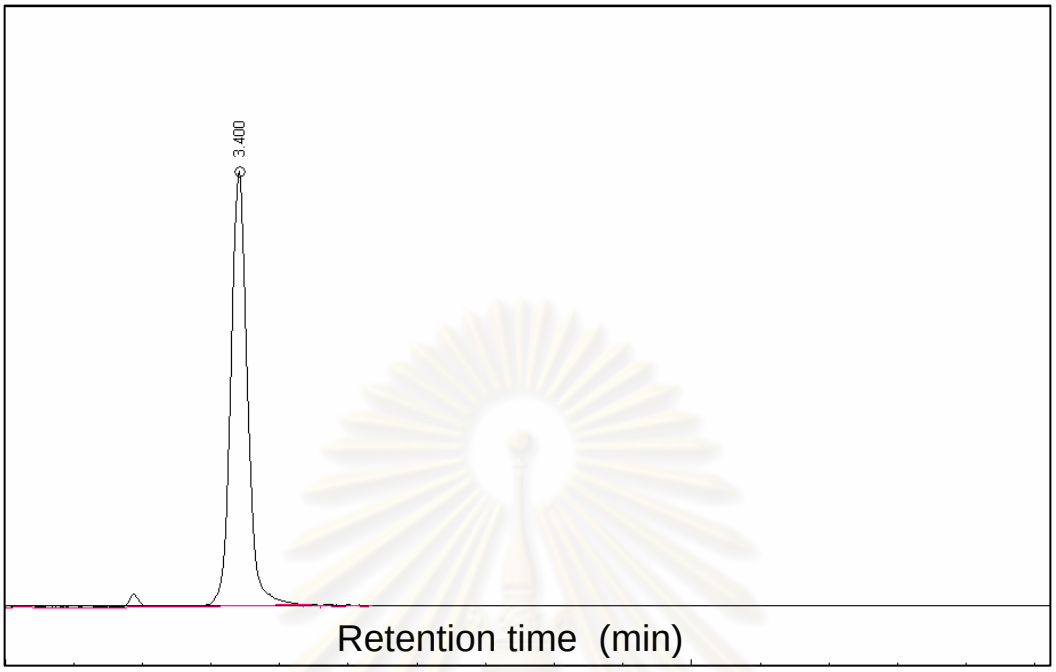

Figure C1. Chromatogram of 0.5% Rhamnose

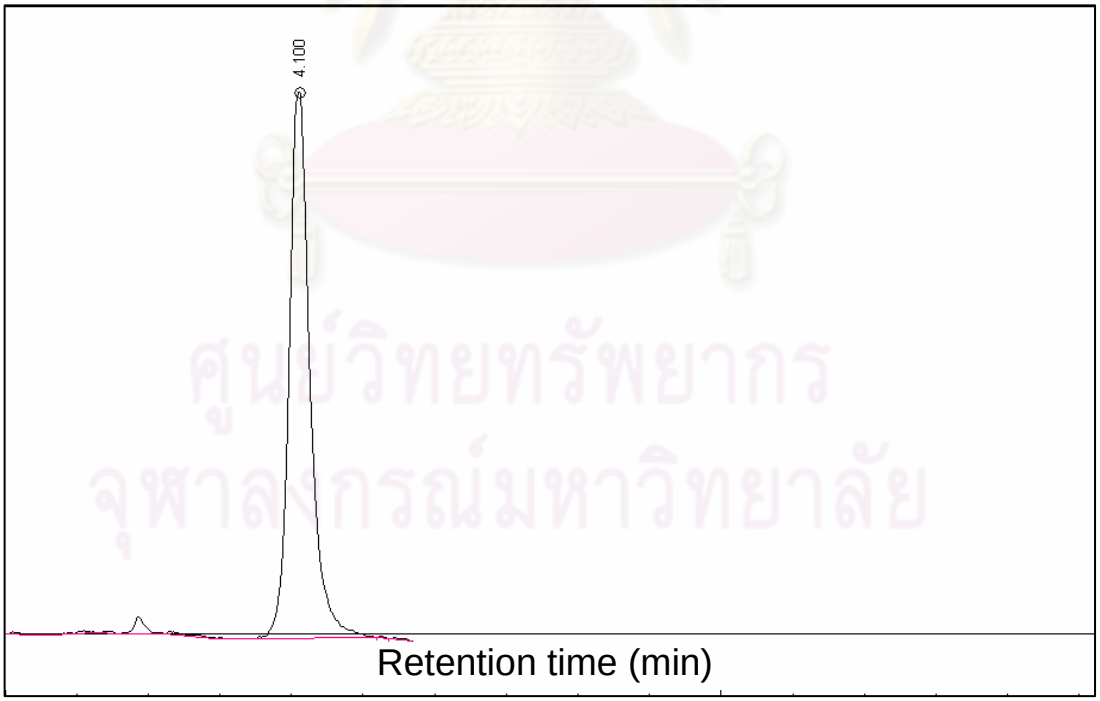

Figure C2. Chromatogram of 0.5% Xylose

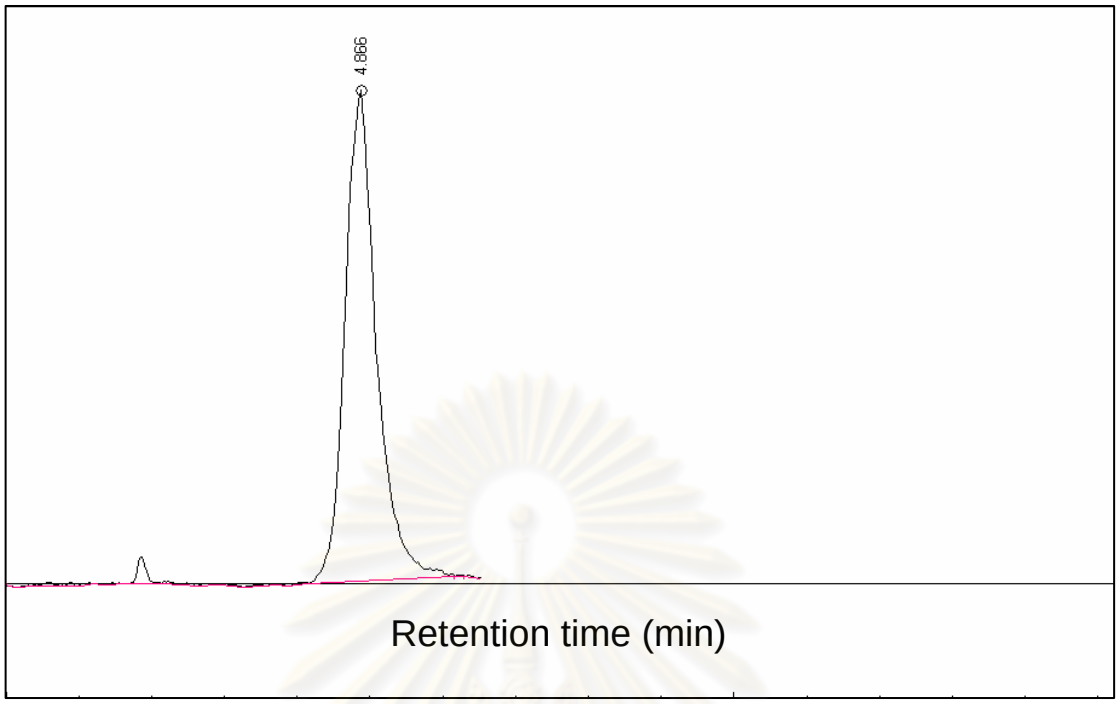

Figure C3. Chromatogram of 0.5% Arabinose

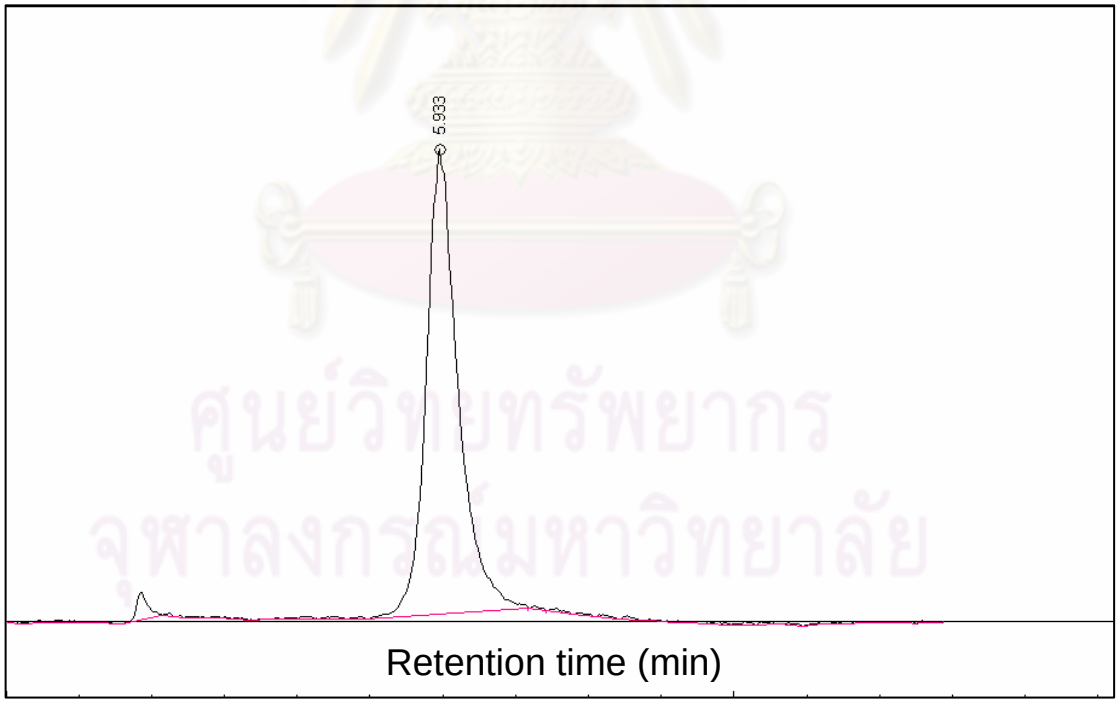

Figure C4. Chromatogram of 0.5% Fructose

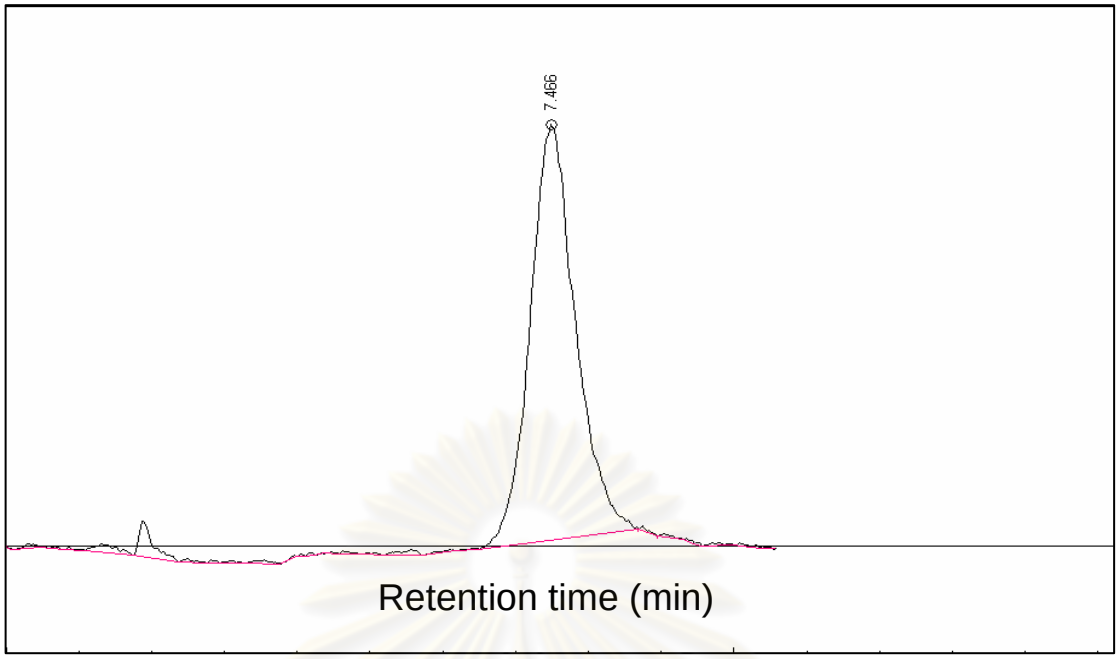

Figure C5. Chromatogram of 0.5% Glucose

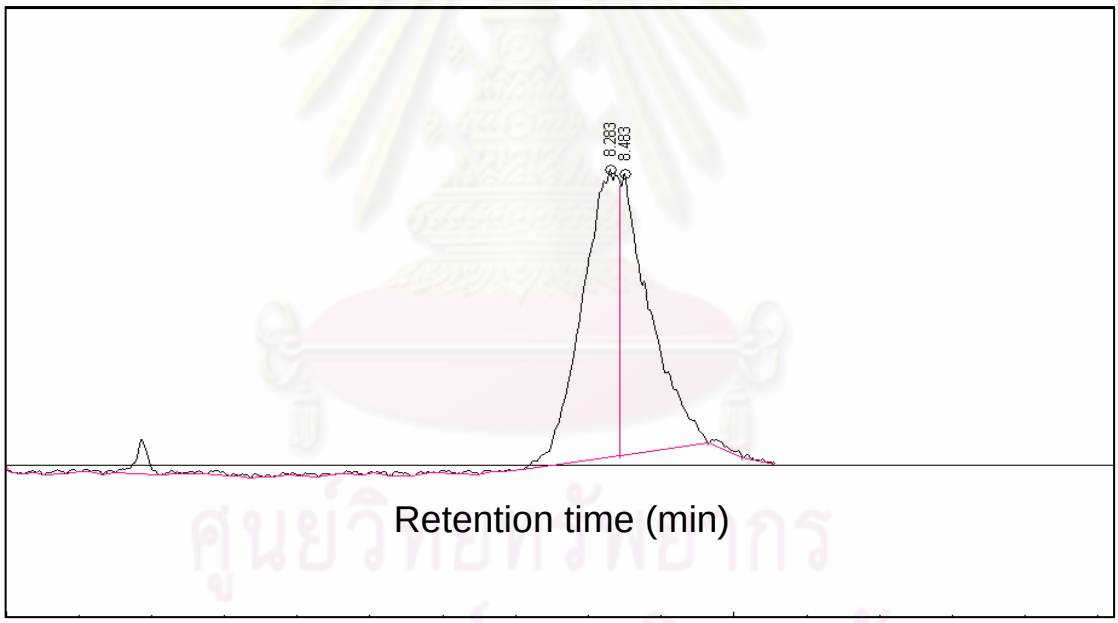

Figure C6. Chromatogram of 0.5% Galactose

Appendix D

Table D1. Formulation of tamarind powder and appearance of tamarind powder products by spray drying technique

Product No.	Formula	Appearance tamarind powder product after spray drying	%moisture content	Solubility in 10% in hot water (min)
1	TI-PY/P 150 g/L + 5 g/L TSP	Wet yellow powder	ND	40
2	TI-PY/P 150 g/L + 10 g/L TSP	Wet yellow powder	ND	50
3	TI-PY/P 60 g/L + 10 g/L TSP	Wet yellow powder	ND	50
4	TI-PY/P 50 g/L + 10 g/L TSP	Wet yellow powder	ND	50
5.	TI-PY/P 50 g/L + 10 g/L Maltodextrin	Wet yellow powder	ND	50
6.	TI-PY/P 40 g/L + 10 g/L TSP	Yellow powder, easily agglomerate, absorb moisture rapidly	ND	50
7.	TI-PY/P 40 g/L + 6 g/L TSP + 0.25 g/L Silicon dioxide	Wet yellow powder	ND	50
8.	TI-PY/P 40 g/L + 10 g/L TSP+ 0.25 g/L Silicon dioxide	Yellow powder, easily agglomerate, absorb moisture rapidly	ND	50
9.	TI-PY/P 40 g/L + 10 g/L TSP+ 0.50 g/L Silicon dioxide	Yellow powder, easily agglomerate, absorb moisture rapidly	ND	40
10.	TI-PY/P 30 g/L + 10 g/L TSP	Dry yellow powder, small particle, agglomerate	8.15	40
11.	TI-K/P 30 g/L + 5 g/L TSP + 10 g/L Maltodextrin	Wet yellow powder	ND	20
12.	TI-K/P 30 g/L + 8 g/L TSP + 7 g/L Maltodextrin	Wet yellow powder	ND	30
13.	TI-K/P 30 g/L + 5 g/L TSP + 5 g/L Maltodextrin + 0.3 g/L Silicon dioxide	Wet yellow powder	ND	25

Table D1. Formulation of tamarind powder and appearance of tamarind powder products by spray drying technique (Cont's)

Product No.	Formula	Appearance tamarind powder product after spray drying	%moisture content	Solubility in 10% in hot water (min)
15	TI-PY/P 15g/L, TI-K/P 15 g/L* + 1.35 g/L Fructose + 0.45 g/L NaCl + 6 g/L TSP+ 4 g/L Pectin	Dry yellow powder, small particle, easily agglomerate, absorb moisture	8.35	25
16.	TI-PY/P 15g/L, TI-K/P 15 g/L + 1.35 g/L Fructose + 0.45 g/L NaCl + 8 g/L TSP*+ 2 g/L Pectin + 0.3 g/L Silicon dioxide	Yellow powder, small particle, easily agglomerate, absorb moisture	8.45	35
17.	TI-PY/P 15g/L, TI-K/P 15 g/L + 1.35 g/L Fructose + 0.45 g/L NaCl + 5 g/L TSP*+ 6 g/L Pectin + 0.3 g/L Silicon dioxide	Yellow powder, small particle, easily agglomerate, absorb moisture rapidly	9.68	25

Tamarind extracts, TSP and *pectin were autoclaved 121°C 30 minutes, before mixed in tamarind mixture.

ND = not determined

VITA

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