กลไกการกำจัดสิ่งสกปรกบนผ้าฝ้ายด้วยเอนไซม์เพกทิเนส โพรทิเอส ไลเปสและเซลลูเลส

นางสาวพรทิพย์ แซ่เบ๊

MECHANISM OF ENZYMATIC SCOURING ON COTTON FABRIC USING
PECTINASE, PROTEASE, LIPASE AND CELLULASE

Ms. Porntip Sae-be

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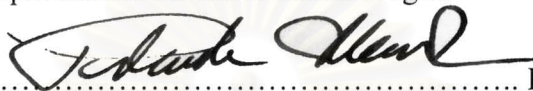Faculty of Science
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By   Ms. Porntip Sae-be

Field of Study   Materials Science

Thesis Advisor   Assistant Professor Usa Sangwatanaroj, Ph.D.

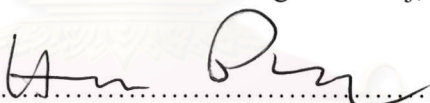Thesis Co-advisor   Associate Professor Hunsa Punnapayak, Ph.D.

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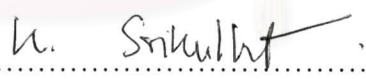
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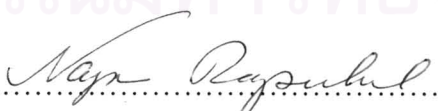
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.... Member

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พรทิพย์ แซ่เบ๊ : กลไกการกำจัดสิ่งสกปรกบนผ้าฝ้ายด้วยเอนไซม์เพกทิเนส โพรทิเอส ไลเปส และเซลลูเลส. (MECHANISM OF ENZYMATIC SCOURING ON COTTON FABRIC USING PECTINASE, PROTEASE, LIPASE AND CELLULASE) อ. ที่ปรึกษา : ผศ.ดร. อุษา แสงวัฒนาโรจน์, อ. ที่ปรึกษา ร่วม : รศ.ดร.หรรษา ปุณณะพยัคฆ์ 156 หน้า.

งานวิจัยนี้แสดงกลไกการกำจัดสิ่งสกปรกบนผ้าฝ้ายด้วยเอนไซม์ทางการค้า 4 ชนิด คือ เพกทิเนส โพรทิเอส ไลเปส และเซลลูเลส โดยอาศัยผลของการวิเคราะห์หาผลิตภัณฑ์ที่เกิดขึ้นจากการไฮโดรไลซิสผ้าฝ้ายด้วยเอนไซม์ และการวิเคราะห์ผ้าหลังผ่านกระบวนการกำจัดสิ่งสกปรกแบบขั้นตอนเดียวด้วยเอนไซม์เพกทิเนส และกระบวนการกำจัดสิ่งสกปรกแบบสองขั้นตอนด้วยเอนไซม์ไลเปสแล้วตามด้วยเซลลูเลส เอนไซม์โพรทิเอสแล้วตามด้วยเซลลูเลส และเอนไซม์ผสมไลเปส/โพรทิเอสแล้วตามด้วยเซลลูเลส ซึ่งการวิเคราะห์หาผลิตภัณฑ์ที่ได้จากการไฮโดรไลซิสประกอบด้วยการหาปริมาณน้ำตาลรีดิวซิงและกรดกาแลคทูโรนิกที่เกิดขึ้นในสารละลายโดยการวิเคราะห์ด้วยเทคนิคอัลตราไวโอเลต - วิสิเบิลสเปกโทรสโกปี การหาชนิดและปริมาณของกรดอะมิโนที่เกิดขึ้นในสารละลายโดยการวิเคราะห์ด้วยเทคนิคไฮเพอร์ฟอร์มานซ์ลิควิดโครมาโทกราฟี และการหาชนิดและปริมาณของกรดไขมันบนผ้าโดยการวิเคราะห์ด้วยเทคนิคแก๊สโครมาโทกราฟี นอกจากนี้มีการวิเคราะห์และทดสอบผ้าหลังผ่านกระบวนการกำจัดสิ่งสกปรก

จากผลของการวิเคราะห์ปริมาณน้ำตาลรีดิวซิงและกรดกาแลคทูโรนิกพบว่า กระบวนการกำจัดสิ่งสกปรกบนผ้าฝ้ายด้วยเอนไซม์เพกทิเนสก่อให้เกิดปริมาณน้ำตาลรีดิวซิงและกรดกาแลกทูโรนิกเป็น 18 เท่าของการกำจัดสิ่งสกปรกสองด้วยเอนไซม์ชนิดอื่นๆ โดยอัตราเร็วของการเกิดน้ำตาลรีดิวซิงและกรดกาแลกทูโรนิกระหว่างกระบวนการกำจัดสิ่งสกปรกมีแนวโน้มลดลงเมื่อเวลาของการกำจัดสิ่งสกปรกเพิ่มขึ้น และมีความสัมพันธ์กันตามสมการกำลังสอง รูปแบบของการเกิดน้ำตาลรีดิวซิงและกรดกาแลกทูโรนิกในกระบวนการกำจัดสิ่งสกปรกนี้สามารถอธิบายได้ด้วยสมการทางจลศาสตร์ของ Ghose-Walseth

เมื่อทำการวิเคราะห์หาปริมาณกรดอะมิโน 17 ชนิดที่เกิดขึ้นในสารละลาย หลังผ่านกระบวนการกำจัดสิ่งสกปรกพบว่า กระบวนการกำจัดสิ่งสกปรกด้วยโพรทิเอส ไลเปส และเซลลูเลสมีปริมาณกรดอะมิโนมากกว่ากระบวนการกำจัดสิ่งสกปรกด้วยเพกทิเนสอยู่ 5 เท่า และเมื่อทำการวิเคราะห์หาปริมาณกรดไขมัน 18 ชนิด (C_8–C_{24}) บนผ้าพบว่า กรดไขมัน 3 ชนิดที่มีปริมาณสูงสุดบนผ้าดิบและผ้าที่ผ่านกระบวนการกำจัดสิ่งสกปรก คือ กรดพลามิติก กรดสเตอริก และกรดบีฮีนิค นอกจากนี้ผ้าที่ผ่านกระบวนการกำจัดสิ่งสกปรกด้วยเอนไซม์ชนิดต่างๆ ถูกนำมาทดสอบหาปริมาณโปรตีน ทดสอบหาสารประกอบที่สามารถละลายน้ำและไม่ละลายน้ำ (ปริมาณไขมัน) และทดสอบหาปริมาณสารประกอบที่มีประจุลบ (รวมทั้งเพกทินและอื่นๆ) พบว่าปริมาณสารต่างๆ เหล่านี้บนผ้ามีความแตกต่างกันบ้างเมื่อมีการใช้เอนไซม์ในการกำจัดสิ่งสกปรกที่แตกต่างกัน แต่การกำจัดสิ่งสกปรกทุกวิธีสามารถทำให้ผ้าดูดซึมน้ำได้ดี เนื่องจากปริมาณไขมันบนผ้าได้ถูกกำจัดออกเกือบหมด และการกำจัดสิ่งสกปรกไม่ส่งผลเสียต่อสมบัติโดยรวมของผ้าฝ้าย เช่น ความแข็งแรงและปริมาณผลึก การใช้เอนไซม์ในการกำจัดสิ่งสกปรกนี้สามารถใช้เอนไซม์เพียงชนิดเดียว หรือใช้เอนไซม์หลายชนิดร่วมกันได้อย่างมีประสิทธิภาพ ถึงแม้ว่าเอนไซม์แต่ละชนิดจะมีผลต่อการกำจัดสิ่งสกปรกบนผ้าแตกต่างกัน แต่ก็มีประสิทธิภาพในการกำจัดสิ่งสกปรกเหมือนกัน โดยพบว่า เพกทิเนสและเซลลูเลสสามารถไฮโดรไลซ์ทั้งเพกทินและเซลลูโลสได้โดยตรง โพรทิเอสสามารถไฮโดรไลซ์โปรตีนแล้วก่อให้เกิดการไฮโดรไลซ์เพกทินและเซลลูโลสทางอ้อม ในขณะที่ไลเปสจะไม่ไฮโดรไลซ์เพกทินและเซลลูโลสเลย เอนไซม์ทั้ง 4 ชนิดที่ใช้ในงานวิจัยนี้สามารถไฮโดรไลซ์โปรตีนและไขมันน้ำมันบนผ้าฝ้ายได้ทั้งทางตรงและทางอ้อม

ภาควิชา วัสดุศาสตร์   ลายมือชื่อนิสิต.................. พรทิพย์ แซ่เบ๊ ....
สาขาวิชา วัสดุศาสตร์   ลายมือชื่ออาจารย์ที่ปรึกษา...............................
ปีการศึกษา 2549   ลายมือชื่ออาจารย์ที่ปรึกษาร่วม...............................

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PORNTIP SAE-BE : MECHANISM OF ENZYMATIC SCOURING ON COTTON FABRIC USING PECTINASE, PROTEASE, LIPASE AND CELLULASE. THESIS ADVISOR : ASST. PROF. USA SANGWATANAROJ, Ph.D., THESIS COADVISOR : ASSOC. PROF. HUNSA PUNNAPAYAK, Ph.D., 156 pp.
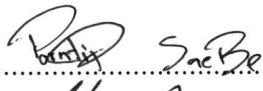
This research revealed the mechanism of enzymatic scouring of cotton fabric using 4 commercial enzymes: pectinase, lipase, protease, and cellulase. The mechanism was established based on results from the analyses of the hydrolyzed products and the scoured fabrics after scouring using a one-step process with pectinase and a two-step process with either lipase then cellulase, protease then cellulase, or lipase/protease then cellulase. Three analytical techniques consisting of UV-Vis spectrophotometry, HPLC and GC were used to determine the amounts of reducing sugars, galacturonic acid, amino acids, and fatty acids from the hydrolyses of cellulose, pectins, proteins, and waxes/fats respectively, and other tests were performed on scoured fabrics.

UV-Vis spectrophotometric analysis indicated that the pectinase scouring process produced approximately 18 fold higher amounts of reducing sugars and galacturonic acid than any of the two-step scouring processes. The production rate of reducing sugars and galacturonic acid from most of the scouring processes showed a decrease with an increase in time in a quadratic relationship. The kinetic study of these two hydrolyzed products suggested that Ghose-Walseth kinetic system could be used to explain the production of reducing sugars and galacturonic acid from these enzymatic scouring processes. HPLC analysis revealed that the lipase/protease/cellulase scouring processes produced approximately 5 fold higher amounts of 17 amino acids than the pectinase scouring process. GC analysis for 18 fatty acids (C_8–C_{24}) revealed that three major fatty acids; palmitic acid, stearic acid, and behenic acid were found on both the scoured and the unscoured fabrics. Scoured fabrics were tested for content of proteins, extractable components, waxes, and anionic components including pectins and some differences among the fabric scoured with different enzyme combinations were found. After all scouring processes, fabrics showed adequate water absorbency because only a residual content of waxes was left. All enzymatic scouring processes did not affect the bulk property of the fabric such as the physical strength and the crystalline. All enzymes could be effectively used to scour cotton either alone or in combination. Although these enzymes showed various actions on cotton impurities, they all performed effective scouring. It was found that pectinase and cellulase could hydrolyze both pectins and cellulose directly, protease could act on them indirectly through the protein hydrolysis while lipase did not act on them at all. All four enzymes could act either directly or indirectly on proteins and waxes/fats.

Department Materials Science   Student's signature...

Field of study Materials Science   Advisor's signature...

Academic year 2006   Co-advisor's signature...

Acknowledgement

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CHAPTER I

INTRODUCTION

Cotton contains impurities (4-12% by weight) in the form of waxes, proteins, pectins, ash, and miscellaneous substances such as pigments and hemicellulose. The waxes consist primarily of a variety of long chain (C_{15} to C_{33}) alcohols, acids, and hydrocarbons. The pectin is mainly methyl ester of poly-D-galacturonic acid [1]. It is necessary to remove these impurities from cotton by scouring in order to obtain good wettability for the subsequent bleaching and dyeing processes.

Enzymatic scouring is another alternative for cleaning the fabric before coloring and finishing because it enables the textile industry to reduce the energy and the waste treatment costs, to reduce the environmental impact of the overall process and to improve the quality and functionality of the final products. Enzymes are non-toxic and they allow mild conditions of temperature and pH.

A previous work [2] has shown the effects on various cotton fabrics scoured with alkaline and enzymes such as pectinase, lipase, protease, and cellulase either alone or in various combinations. All scoured fabrics similarly showed adequate absorbency of water and dye solutions. Both alkaline scouring and enzymatic scouring successfully removed the anionic components, including pectins, from fabrics. Some of the lipase/protease/cellulase scoured fabrics even showed lower amounts of anionic components (including pectins) when compared to pectinase and alkaline scoured fabrics. This was partly due to the loss of fiber by cellulase which contained the anionic components.

The aim of this work was to establish the mechanism of enzymatic scouring of cotton fabric using a one-step process with pectinase and a two-step process with either lipase then cellulase, protease then cellulase, or lipase/protease then cellulase. UV-Vis spectrophotometric and HPLC analyses of the scouring solutions were made for hydrolyzed products released from the cotton fabric during scouring such as galacturonic acid from pectins, reducing sugars from cellulose, and amino acids from proteins. The progress of the scouring reaction was also monitored and kinetically studied. The residual impurities on the scoured cotton fabric were determined

including some fabric properties. Data obtained from this work can help to better understanding the structure of cotton impurities.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Cotton Fiber

The cotton fibers of commerce are seed hairs of plants belonging to the genus *Gossypium*, family *Malvaceae*. Cotton is a single cell fiber and develops from the epidermis of the seed. An elongation period continues for 17 - 25 days after flowering. Cotton fiber has a fibrillar structure as illustrated in Figure 2.1 [3, 4]. The outermost layer of the fiber is the thin waxy cuticle, which protects the fiber from its environment. Beneath this layer is the primary wall of the fiber cell, which is composed of fine threads of cellulose laid down during growth and spiraled round the longitudinal fiber axis at an angle of about 70° [5]. Winding layer is the very first layer of the secondary thickening and differs somewhat in structure from either the primary wall or the remainder of the secondary wall. Secondary wall consists of concentric layers of cellulose, which constitute the main portion of cotton fiber. Lumen is the central cavity or canal of the fiber and lumen appears to be more resistant to certain reagent than secondary wall layer. It is highly irregular in both size and shape and often contains solid dried matter, largely nitrogenous in compositions.
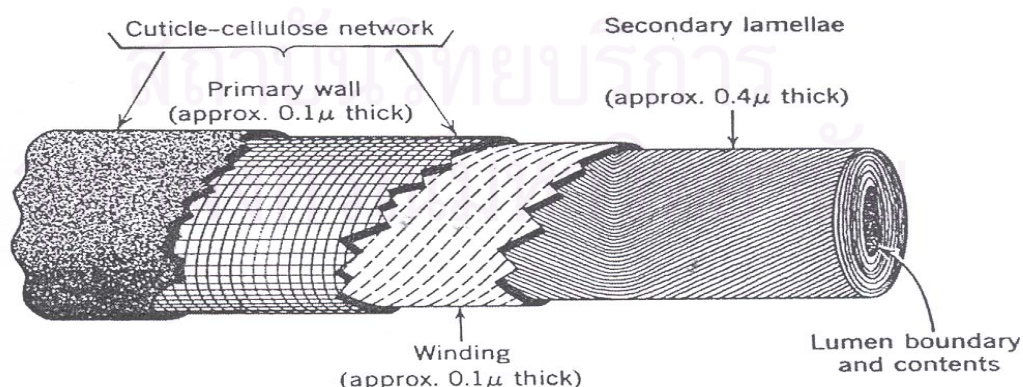
Figure 2.1 Schematic diagram of cotton fiber structure [3]

2.2   Structure of Cotton fiber

Cotton fiber has a fibrillar structure and it consists of a primary wall, a secondary wall and a lumen. In addition to cellulose, cotton also contains noncellulosic components. The noncellulosic components found in mature cotton fibers are located in the cuticle and the primary cell wall. The surface layers, which contain lipids, waxes, pectin, organic acids, protein/nitrogenous substances, noncellulosic polysaccharides and other unidentified substances, constitute approximately 10% of the total fiber weight [6 - 7]. The chemical composition of a mature cotton fiber is presented in Table 2.1. The secondary wall constitutes the bulk of a mature fiber and consists almost entirely of fibrils of cellulose arranged spirally around the fiber axis, the direction of the spiral reversing many times along a single fiber.

Cotton cellulose consists of crystalline fibrils varying in complexity and length and connected by less organized amorphous regions with an average ratio of about two-thirds crystalline and one-third non-crystalline material, depending on the method of determination [8]. In the crystalline part, the cellobiose units are closely packed to form cellulose I in native cellulose fibers. In cellulose I the chain molecules are parallel to each other. Thus, the basis for helical structure for cellulose I is preferably extended to the structure of cellulose.

Table 2.1 Chemical composition of cotton fiber and primary wall [9].

Constituents	Composition of a fiber (%)			Composition of the cuticle (%)
	Typical	Low	High	
Cellulose	94.0	88.0	96.0	
Protein*	1.3	1.1	1.9	30.4
Pectic substances	0.9	0.7	1.2	19.6
Wax	0.6	0.4	1.0	17.4
Mineral matters	1.2	0.7	1.6	6.5
Maleic, citric, and other organic acids	0.8	0.5	1.0	-
Total sugars	0.3	-	-	-
Cutin	-	-	-	8.7

* Calculated by multiplying the nitrogen content by 6.25

The cellulose content of the raw fiber (cotton that has only been subjected to ginning and mechanical cleaning) ranges from 88 to 96%, but this may reach as high as 99% after scouring and bleaching. The secondary wall, or secondary layers, is nearly pure cellulose. The other substances are almost entirely confined to the primary and the protoplasmic residue in the lumen. The primary wall contains mostly cellulose. Waxes, pectin, some of the ash, and a part of the nitrogenous material are contained in and on this thin wall. The pigment, the rest of the protein, the other ash, sugar, organic acids, etc., are found in the residue of the lumen. The waxes are distributed throughout the primary wall in such a way that lint cotton is resistant to wetting [3].

2.2.1 Cellulose

Cotton consists of practically pure cellulose. The chemical structure of cellulose is simple, consisting of anhydroglucose unit (cellobiose unit) joined by β-1, 4-glucosidic bonds to form linear polymeric chains shown in Figure 2.2. The chain length, or degree of polymerization (DP) of cotton cellulose molecule represents the number of anhydroglucose units connected together to form the chain molecule. DP of cotton may be as high as 14,000. The molecular weight (MW) of cotton usually lies in the range of 50,000-1,500,000 depending on the source of the cellulose. The individual chains adhere to each other along their lengths by hydrogen bonding and van der Waals forces. The physical properties of the cotton fiber as a textile material, as well as its chemical behavior and reactivity, are determined by arrangements of the cellulose molecules with respect to each other and to the fiber axis.
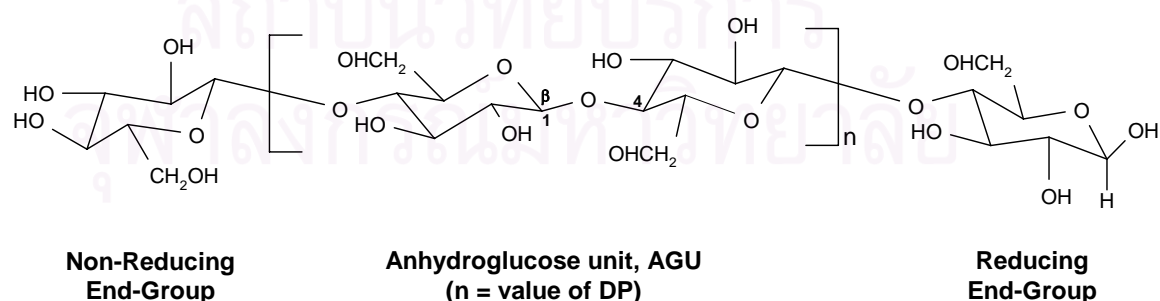
Figure 2.2 Molecular structure of cellulose.

2.2.2 Pectins

Pectic substances are acid polysaccharides of high molecular weight that are widespread in the plant kingdom. The size, charge density, charge distribution, and degree of substitution of pectin molecules may be changed biologically or chemically [10]. The definitions of pectic substances are shown in Table 2.2. A schematic survey of the interrelationship of pectic substances is presented in Figure 2.3.
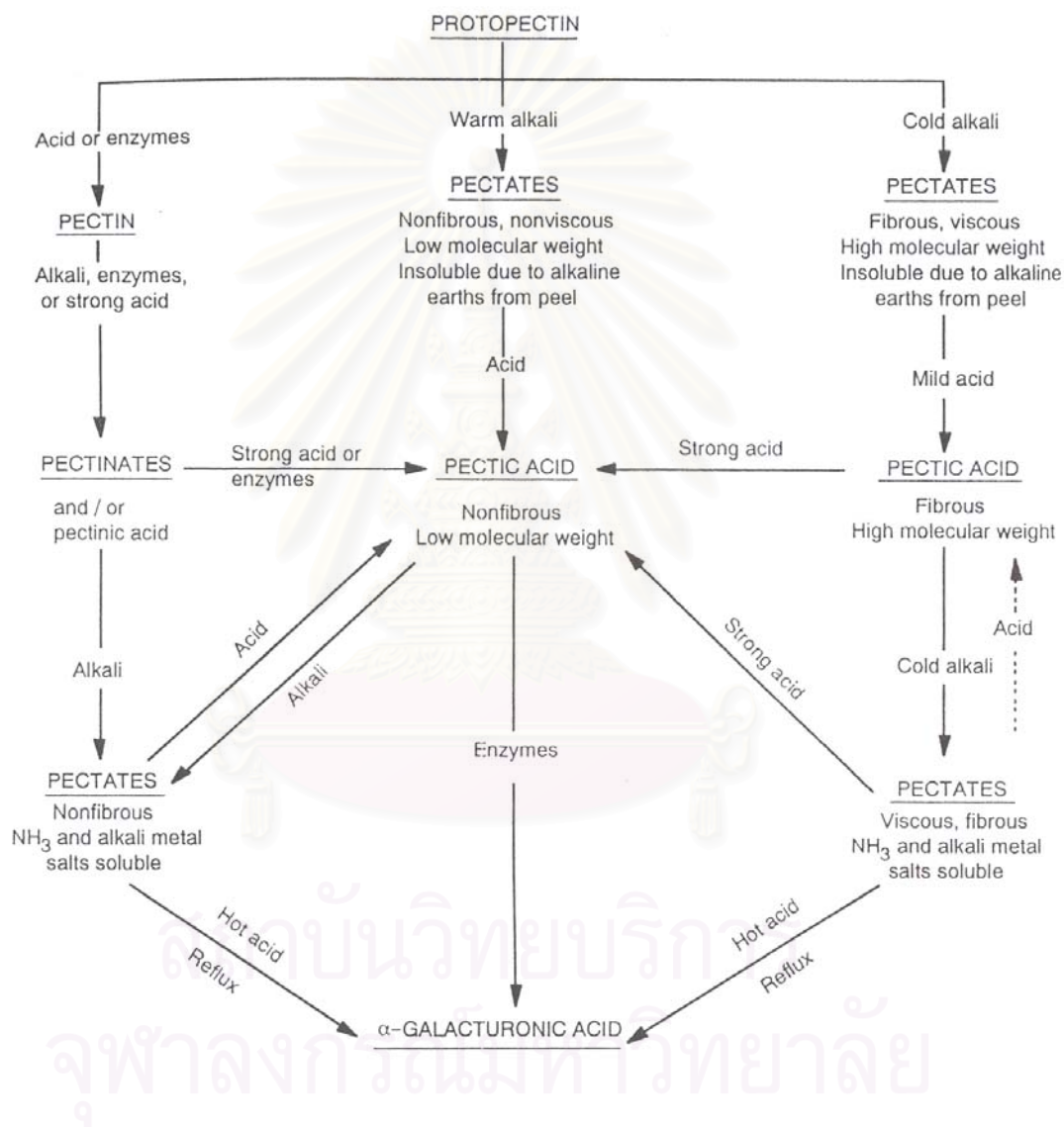
Figure 2.3 Interrelationship of the pectic substances [11].

Table 2.2 Nomenclature for pectic substances [10]

Pectic substances	Definition
Pectic acid (pectate)	Polygalacturonic acid
Pectin (pectinate)	Pectic acid partially esterified with methanol and containing some neutral sugars
Protopectin	Pectic substance fixed in the plant tissue

Among the noncellulosic contents of the cotton fiber, pectin is one of the most prominent. According to the best available estimate, the pectin content of the mature fiber is about 0.6 to 1.2% depending on the method of determination. Chemically, pectins are high molecular weight carbohydrates with a chain structure similar to cellulose. The chief constituent of complex group of substances is polygalacturonic acid in which a considerable proportion of the carboxyl groups have been methylated. The unesterified acid (known as pectic acids) is shown in Figure 2.4. The acid consists of a chain of α-1, 4-linked D-galacturonic acid units. Cellulose breaks down into glucose, but pectins decompose to give galactose, several pentoses, polygalacturonic acid and methyl alcohol.
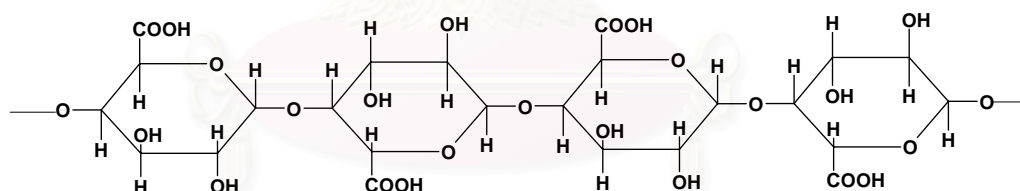
Figure 2.4 Chain of α-D-Galacturonic acid units in pectic acid.

Pectins appear to be localized in the primary wall and may be stained with ruthenium red or a basic dye such as methylene blue. The stains are not specific to pectins since the adsorption of these colored materials is characteristic of carboxyl and other acid groups. These materials do not appear to contribute to the strength of fibers since their removal, for example by boiling in sodium hydroxide solution (1%) for half an hour in the absence of air, does not change the strength or fluidity [12].

2.2.3 Waxes

The material extracted from cotton fiber with chloroform, carbon tetrachloride, or other organic solvents is usually referred to as wax. The wax acts as a water-resistant protective coating on the raw cotton fiber. Raw cotton will float for days on a water surface, while cotton dewaxed by heating with dilute sodium hydroxide or by extraction with organic solvents will sink in a few minutes. The wax is nearly all located in the primary wall of the fiber. Whether it is a purely mechanical coating outside the primary wall, or whether some of its constituents are chemically combined with the pectins, cellulose, or proteins in the primary wall is unknown [3]. Analyses of cotton wax [13] have indicated that it contains all the even-numbered, carbon primary alcohols, and that the alcohol present in the largest is n-triacontanol ($C_{30}H_{61}OH$). It also contains all the even-numbered, high molecular weight ester; e.g., ceryl oleate, montanyl triacontanoate, montanol, 1-triacontanol, β-sitosterol and $C_{26}H_{52}O$ [14]. Other components were fatty acids such as palmitic acid, stearic acid, and oleic acid.

2.2.4 Proteins

The fiber contains a small percentage of nitrogen. Little is known of the detailed composition of the nitrogen containing compound but, although not all of the nitrogen is present as protein, it is generally assumed that a mixture of pure proteins will contain 16% nitrogen. Thus, the protein content of a cotton fiber is obtained by multiplying the determined nitrogen by the factor 6.25 = (100/16) [15]. Most of the protein is found in the lumen of the fiber [16].

Proteins are molecules composed of many amino acid joined together by peptide linkages. The peptide linkage is a covalent bond formed between the nitrogen of one amino acid and the carbon of the carboxyl group of another amino acid; a molecule of water is eliminated in the reaction shown in Figure 2.5.

A protein may contain several hundred amino acid moieties linked together into a very complex molecule. Amino acids are organic substances containing two characteristic functional groups: the amino ($-NH_2$) group and the carboxyl (-COOH) group. The amino and the acid groups and various organic components are attached to

a single carbon (the α carbon). These various organic components (designated as *R* groups) are provided in Table 2.3 [17].
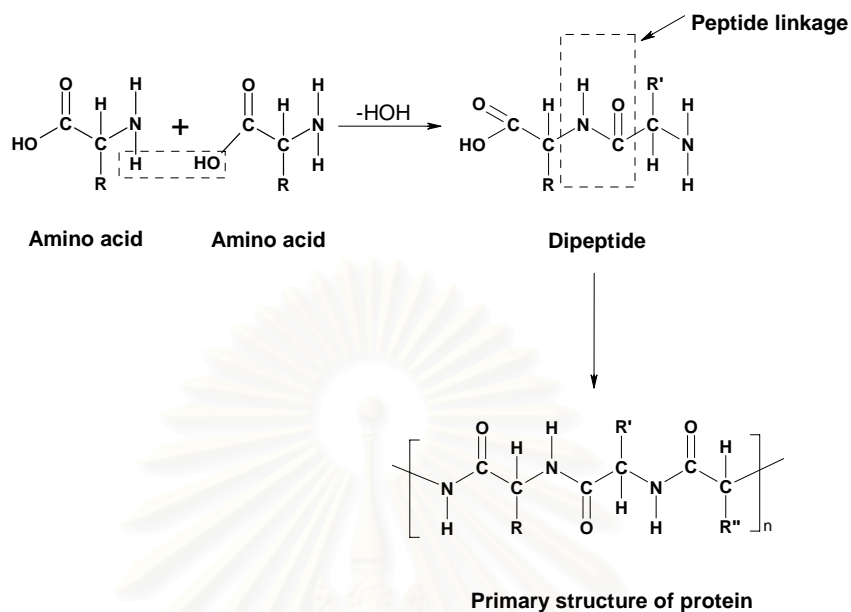
Figure 2.5 The production of protein.

Table 2.3 The structure of the naturally occurring amino acid found in proteins [17].

It has been assumed that the protein material in cotton is the residual dead protoplasm left in the lumen after the cell dies when the boll open. The nitrogen content of the raw cotton is approximately 0.3% and can be converted to protein, using of the usual factor of 6.25, to get a protein content of 1.875%. Tripp [18] found nitrogenous material in primary wall isolated from mature cotton, to the extent of 2% nitrogen which calculates to approximately 14% protein in the composition of the outer skin of the fiber. Kier boiling of cotton reduces the nitrogen content about one-tenth of the original value; the nitrogen content of the scoured cotton is about 0.035% [3].

Little is known about the composition and properties of the protein of cotton fiber, or about the nature of other nitrogenous constituents of the fiber. It is likely that part of the nitrogen in the fiber is non-protein. In Catlett's [19] invertigation of the lumen residue, analysis showed approximately 4.5% nitrogen. The solid residue could be removed from lumen by digestion with the proteolytic enzymes trypsin or papain. Amino acid determination by paper chromatography disclosed no free amino acid in the lumen residue, but in a hydrolyzed sample of the material, the presence of glutamic and aspartic acids, valine, ananine, and probably serine and arginine were indicated [3].

2.2.5 Coloring matter

Coloring matter presents in small quantities and situated in the cortical cells beneath the epidermis, fragments of which remain attached to the fiber bundles after scotching [12]. Cotton's color may vary from gray to yellowish in according to types of microorganisms, growth patterns within a season, as well as fungi and soil. In addition to color variations due to these factors, raw cotton fiber typically is not homogeneous in appearance because it contains extraneous matter [20]. Chemically, the colored materials are chlorophyll, xanthophylls and carotene together with modification of these substances. The colored materials are usually associated with complex compounds such as tannin [12]. When waxes and nitrogen impurities have been removed, cotton still has a yellowish or brown discoloration. This is caused by the natural coloring matter, which can only be removed effectively by oxidizing agent in bleaching step.

2.2.6 Ash

The composition of the ash is given in Table 2.4. The quantity of ash may be decreased by 85% by boiling the cotton in water; however, most of the calcium, iron and aluminum remain [12].

Table 2.4 Composition of ash of cotton fiber [12].

Substance	Percentage	Substance	Percentage	Substance	Percentage
K_2O	34	Al_2O_3	2	CO_2	20
CaO	11	SiO_2	5	Cu	Trace
MgO	6	SO_3	4	Mn	Trace
Na_2O	7	P_2O_5	5		
Fe_2O_3	2	Cl	4		

2.3 Enzymes [21]

Enzymes are biological catalysts. They increase the rate of chemical reactions taking place within living cells without themselves suffering from any overall change. The reactants of enzyme-catalyzed reaction are called substrates, and enzymes have a high degree of specificity, typically acting on a particular substrate or substrates to produce a particular product or products.

Organic molecule (COENZYME)

Inactive protein (APOENZYME) + COFACTOR

ENZYME ◄—————— (HOLOENZYME) ——————►

Active protein   Metal ion
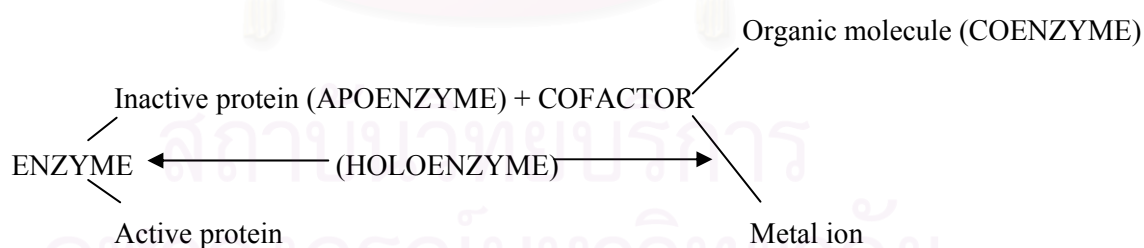
Figure 2.6   Enzyme composition.

All enzymes are proteins. However, absence of a non-protein component called a cofactor, many enzyme proteins lack catalytic activity. When this is the case, the inactive protein component of an enzyme is termed the apoenzyme, and the active enzyme, including cofactor, the holoenzyme. The cofactor may be an organic molecule, when it is known as a coenzyme, or it may be a metal ion. Some enzymes

bind cofactors more tightly than others. When a cofactor is bound so tightly that it is difficult to remove without damaging the enzyme it is sometimes called a prosthetic group as illustrated in Figure 2.6. Both the protein and cofactor components may be directly involved in the catalytic processes taking place [21].

2.3.1 Classification of enzymes [21]

The enzyme commission divided enzymes into six main classes, on the basis of the total reaction catalyzed. Each enzyme was assigned a code number, consisting of four elements, separated by dots. The first digit shows to which of the main classes the enzyme belongs, as follows:

First digit	Enzyme class	Type of reaction catalyzed
1	Oxidoreductases	Oxidation/reduction reactions
2	Transferases	Transfer of an atom or group between two molecules (excluding reactions in other classes)
3	Hydrolases	Hydrolysis reactions
4	Lyases	Removal of a group from substrate (not by hydrolysis)
5	Isomerases	Isomerization reactions
6	Ligases	The synthetic joining of two molecules, coupled with the breakdown of pyrophosphate bond in a nucleoside triphosphate

The second and the third digit in the code further describe the kind of reaction being catalyzed. There is no general rule, because the meanings of these digits are defined separately for each of the main classes [21].

Main Class 3: Hydrolases

These enzymes catalyze hydrolytic reaction of the form:

$$A\text{-}X + H_2O \Leftrightarrow X\text{-}OH + HA$$

They are classified according to the type of bond hydrolyzed. For example:

Second digit	Bond hydrolyzed
1	Ester
2	Glycosidic (linking carbohydrate units)
4	Peptide,
5	C-N bonds other than peptides

The third digit further describes the type of bond hydrolyzed. Thus,

E.C 3.1.1   enzymes are carboxylic ester hydrolases,

E.C 3.1.2   enzymes are thiol ester hydrolases,

E.C 3.1.3   enzymes are phosphoric monoester hydrolases,

E.C 3.1.4   enzymes are phosphoric diester hydrolases,

2.3.2   Enzymatic catalysis

Enzymes are catalysts because they speed up the rate of chemical reactions but remain unaltered themselves. Enzymes do not change the position of equilibrium of a reaction, because they affect both the forward and reverse reactions. However, if a system is allowed to come to equilibrium, they hasten that process. The catalyst acts by reducing the energy of activation. The catalyst, or part of it, combines with the reactants to form a different transition-state from that involved in the uncatalyzed reaction; one which is more stable and, therefore, of lower energy as illustrated in Figure 2.7 [21].

Enzyme + Substrate ⇌ Enzyme-Substrate Complex ⟶ Product

  (initial state)   (intermediate or transition state)   (final state)

Figure 2.7 The effects of a catalyst [21].

Enzyme catalysis involves formation of a transition state between a substrate and an enzyme accelerating the conversion of a substrate into a product. In this reaction, the substrate must fit precisely into the active site of the enzyme. Since enzymes are highly specific catalysts, it can be expected that the formation of the enzyme-substrate complex or the binding of the substrate in the active site will require only little energy. Consequently, enzymes are very effective catalysts, enhancing reaction up to 10,000 fold more than the most effective chemical catalysts [17]:

2.3.3 Mechanism for enzyme action

An enzyme has a quite specific three-dimensional shape. This shape and other factors, such as the location of the active site on the enzyme, control the specificity of the molecule. An enzyme is absorbed onto a given substrate surface in "lock-and-key" fashion [22]. At the surface of the substrate, the enzyme serves to accelerate the reaction of the substrate and the environment before converting into products. Since enzymes are catalysts, they themselves are not changed by the reaction that the substrate undergoes. After the reaction has taken place, the enzyme is released to be reabsorbed onto another substrate surface. The process continues until the enzyme is poisoned by a chemical bogie or inactivated by extremes of temperature, pH, or other negative conditions in the processing environment.

2.3.4 Enzyme activity

Enzymes are proteins specialized to catalyze (biological) reactions. They are among the most remarkable bioactive molecules known because of their extraordinary specificity (concerning both substrate and reaction) and catalytic power, which are superior to those of many synthetic catalysts. In general, a chemical reaction takes place when a reactant can pass the transition state wherein the reactant possesses enough energy to form or to break chemical bonds. The difference in energy between the initial and transition state of the reactant is lowered when the reaction is catalyzed by an enzyme, owing to a transient combination with the reactant. In this way, an enzyme accelerates a chemical reaction by lowering the energy of activation.

The enzymes are specific to a limited number of substrates. Especially, the supplier often guarantees the amount of active enzyme activity measured on a defined substrate using a fixed set of parameter. Enzyme activity is expressed as unit where one unit is defined as the amount of enzyme transforming one mole of substrate per second under standard conditions of temperature, pH and substrate concentration. If a preparation contains pure enzyme, the activity measured per milligram of protein is called the specific activity. Each enzyme originating from a different (microorganism) sources exhibits its own specific activity, but activities measured under different circumstances and using different substrates, not being the substrate in the application itself, cannot be directly compared with each other and cannot be liked directly to a certain performance level.

2.3.5 Enzyme kinetics

2.3.5.1 Homogenous system

Kinetics is the study of reaction rates measured by the change in quantity of reactants with time. Chemical kinetics is ruled by the law of mass action. This raw states that the rate of reaction is proportional to the product of the activities of reactants (A, B) considering the stoichiometric constants (a, b) of each reactant.

$$aA + bB \rightarrow P(product)$$
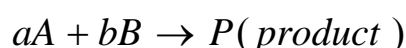
$$v = k[A]^a \times [B]^b \qquad \text{(Equation 2.1)}$$

For practical process, activity can be replaced by concentration measured in molarities. The order of the reaction is a for reactant A and b for reactant B and of the general order, $a + b$. The rate of the reaction is v.

$$A \quad \rightarrow \quad P$$
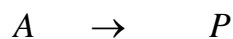
Can be described as:

$$v = -\frac{d[A]}{dt} = +\frac{d[P]}{dt} = k[A] \qquad \text{(Equation 2.2)}$$

Where k is a rate constant and $[A]$ and $[P]$ are the concentrations of reactant A and the product P at the time t. $-\frac{d[A]}{dt}$ and $+\frac{d[P]}{dt}$ describe the rate of decrease of A and increase of P, respectively. The rate of reaction at various times can be found by taking tangents in a plot of concentration change versus time and calculating their gradients. The reaction orders for each reactant are experimentally determined by measuring the initial reaction rates at different initial concentrations of this reactant.

These rules can be also applied to enzymatic reactions. Enzyme-catalyzed reactions occurring in homogeneous media where both the substrate(s) and the enzyme are in solution show a general tend: the initial rates are first order at low substrate concentrations and zero order at high substrate concentrations (Figure 2.8).
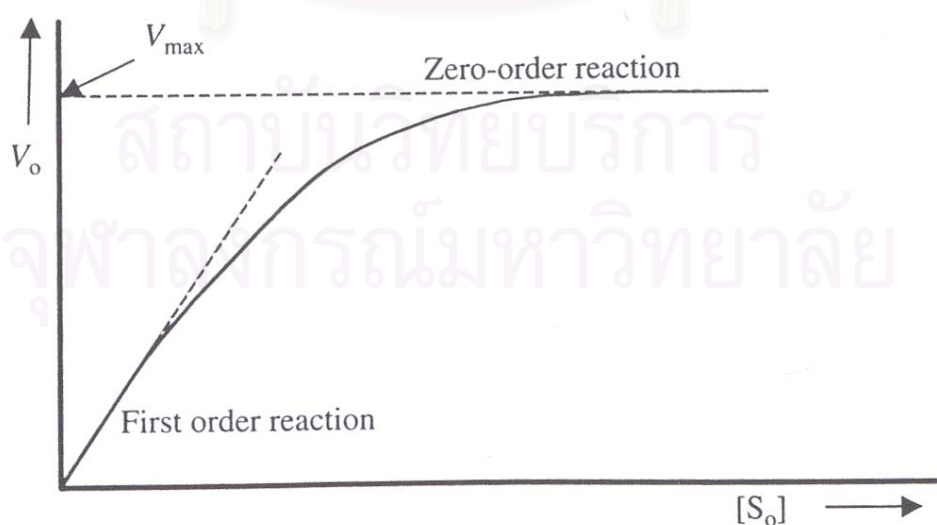
Figure 2.8 Typical initial rates-substrate dependence [17].

This behavior can be explained by the formation of an enzyme-substrate complex:

$$E + S \xleftrightarrow[k_b]{k_a} ES \xrightarrow{k_c} E + P$$

(initial state)   (intermediate state)   (final state)

During the reaction all the enzyme is usually present in the form of the enzyme-substrate complex ES if the concentration of the enzyme is much lower than the concentration of the substrate. A quasi-steady state for the enzyme-substrate complex can be assumed:

$$-\frac{d[ES]}{dt} = k_a[E][S] - k_c[ES] - k_b[ES] = 0 \qquad \text{(Equation 2.3)}$$

Using the mass balance for the enzyme in free or associated form:

$$[E] = [E_0] - [ES] \qquad \text{(Equation 2.4)}$$

From the equation:

$$k_a[E][S] = (k_b + k_c)[ES] \qquad \text{(Equation 2.5)}$$

The concentration of ES can be determined to give:

$$k_a[E_0][S] - k_a[ES][S] = (k_b + k_c)[ES] \qquad \text{(Equation 2.6)}$$

or

$$[ES] = \frac{[E_0][S]}{k_m + [S]} \qquad \text{(Equation 2.7)}$$

where :

$$k_m = \frac{k_b + k_c}{k_a} \qquad \text{(Equation 2.8)}$$

With the reaction rate for the dissociation of the enzyme-substrate complex and formation of the product:

$$\nu = k_c[ES] \qquad \text{(Equation 2.9)}$$

The result is Michaelis-Menten equation:

$$v = \frac{k_c[E_0][S]}{k_m + [S]}$$ (Equation 2.10)

For the maximum reaction rate:

$$v_{max} = k_c[E_0]$$ (Equation 2.11)

We obtain:

$$v = \frac{v_{max}[S]}{k_m + [S]}$$ (Equation 2.12)

k_m gives the substrate concentration $[S_0]$ at v_0 as $1/2 v_{max} \cdot k_m$ is also called the Michaelis-Menten constant.

For an enzymatic reaction, k_c is also called the turnover number k_{cat}, which represents the maximum number of substrate molecules that can be converted by a unit of time. In more complex enzymatic reaction involving several steps and various intermediates following Michaelis-Menten kinetics k_{cat} can be seen as a function of several individual reaction rates.

2.3.5.2 Heterogeneous system

In heterogeneous systems at least the catalyst or one of the reactants or products is present in a different phase from the others. An example of the application of an insoluble enzyme used to convert soluble substrates related to textile processing is the application of immobilized enzymes such as cellulase or pectinase for scouring process. Heterogeneous systems consist of soluble enzymes and insoluble substrates in the form of textile materials or their components. Classical examples of heterogeneous enzymatic catalysis are the enzymatic hydrolysis of insoluble polymer like wool or silk by protease and cotton or regenerated fiber by cellulase. Most carbohydrolases such as cellulase, pectinase, and amylase are known to have substrate binding domains. These enzymes have been designed by nature with a special peptide binding to the substrate which is the driving force of the soluble enzyme in attacking an insoluble substrate. It is believed that substrate binding domains increase the

concentration of the enzyme nearby the substrate and that they are essential for efficient enzymatic hydrolysis of insoluble polymers.

Often there is a limitation in terms of accessibility of the insoluble substrate to the soluble enzyme. The enzyme can only access the outer parts of the substrate at the liquid-solid interface while inner parts are only accessible when the outer parts are removed. Interesting, the synergistic action between several cellulase components during hydrolysis of crystalline cellulose has only been observed at lower concentration, i.e. when there was no competition between the different cellulase components for the hydrolytic substrates sites [23]. These facts are of particular importance for industrial applications using soluble enzyme for the modification of insoluble substrate, since sometimes very high enzyme concentration are used. It is most likely that in soluble enzymes-insoluble substrate systems, enzymes are saturating the few available substrates sites. It is obvious that classical Michealis-Menten kinetics cannot be applied to these systems because of the simple fact that a solid concentration cannot be determined.

The cellulase-catalyzed heterogeneous reaction is characterized by an insoluble reactant (cellulose) and a soluble reactant (enzymes). The structural features of cellulose and the mode of enzyme action influence the rate of reaction. It is a slow process as cellulose of biological origin has a highly resistant crystalline structure and the number of active enzyme binding sites available is limited. Over year several different kinetic and mechanism have been proposed for the action of cellulose to glucose.

In Michealis-Menten kinetics, saturation of the enzyme by the substrate is verified, but in soluble enzyme-insoluble substrate systems it is the substrate that is saturated by enzyme, therefore a relationship has been suggested [24] between v_0 and $[E_0]$.

$$v_0 = \frac{v_{max}[E_0]}{k_e + [E_0]}$$

(Equation 2.13)

In a similar fashion to classical kinetics (equation 2.12), interchanging $[S_0]$ by $[E_0]$, the parameters would have similar significance but could not be interchanged, since enzyme concentration could barely be expressed in molar units. Empirically these expressions have been verified and the estimated parameters are of prime

importance to characterize different enzymes systems and different process conditions [25].

The Ghose-Walseth kinetic empirical equation applied to the degradation reaction of cellulose materials such as cotton, lyocell, ramie and linen fabrics expressed in equation 2.14 [26].

$$P = kt^{m}$$ (Equation 2.14)

Where P was product concentration at time t (µg/ml), indicating the extension of enzymatic degradation, t was the enzymatic degradation time (min), k was the kinetic constant, and m was the characteristic parameter of the substrate-enzyme system.

2.3.6 Enzyme inhibition

Inhibition of the rate of enzyme-catalyzed reactions involves specific interaction of agent (inhibitors) with catalytic or regulatory sites on the enzyme substrate intermediate. There are three types of reversible inhibition including competitive, non-competitive, and uncompetitive inhibition.

Competitive inhibitors usually have a structure similar to the substrate and they bind in competition with the actual substrate at the substrate binding site without being transformed. At high substrate concentration v_{max} remains unchanged while higher κ_m values result.

A non-competitive inhibitor dose not influence the binding of the substrate but it prevents the enzyme-substrate complex from dissociating. In this case v_{max} is reduced while κ_m remains the same.

The non-competitive inhibitor can bind both to the free enzyme and to the enzyme-substrate complex while so-called uncompetitive inhibitor can only react with the enzyme-substrate complex changing both κ_m and v_{max}. The rate equations for the different types of inhibition based on dissociation constants κ_i of the enzyme (E) - inhibitor (I) complexes are presented in equations 2.15 - 2.17.

Competitive $$v_0 = \frac{v_{max} \cdot [S_0]}{\kappa_m (1 + \frac{[I]}{\kappa_i}) + [S_0]}$$ (Equation 2.15)

Non-competitive $\qquad v_0 = \dfrac{v_{max} \cdot [S_0]}{(1 + \dfrac{[I]}{\kappa_i}) + (\kappa_m + [S_0])}$ $\qquad$ (Equation 2.16)

Uncompetitive $\qquad v_0 = \dfrac{v_{max} \cdot [S_0]}{\kappa_m + [S_0](1 + \dfrac{[I]}{\kappa_i})}$ $\qquad$ (Equation 2.17)

Both strong non-covalent binding (binding constants of $> 10^{-10}$ M) and covalent binding of inhibitor to the enzyme can lead to irreversible inhibition. A time-dependent decrease of the enzyme activity is characteristic of irreversible inhibition [17].

2.3.7 Kinetic model of enzymatic hydrolysis of cellulose

A kinetic model has been devised to describe the behavior of enzymatic hydrolysis of cellulose. To develop such a model, the following assumptions have been made [27].

1. Some of the enzymes present within the solutions adsorb instantly at the cellulose surface and no appreciable reaction occurs during this process [28].

2. Only those enzymes that adsorbed initially are able to catalyze the reaction.

3. Enzymes adsorb solely at the cellulose external surface and no internal diffusion occurs due to large molecular sizes of enzymes [29].

4. The number of sites at the cellulose surface which may be occupied by the enzymes is proportional to the total surface area of cellulose particles [30].

5. Transfer of enzymes from the bulk solution to cellulose surface is rapid in comparison with the chemical reaction.

6. The activity of enzymes, adsorbed at the cellulose surface, decreases with time. Finally, a permanent and inactive enzyme–substrate complex is formed [31].

7. Inhibition of product occurs during the reaction which affects negatively the enzyme activity.

8. The cellulose particles shrink with the progress of reaction.

The major distinction between the present model and those available in the literature is the last assumption that forms a physical picture for the progress of reaction and provides a frame within which a realistic kinetic model could be formulated. The kinetic of cellulose hydrolysis may be viewed to compose of two basic stages. At the initial stage of the reaction, the enzymes adsorb rapidly at the external surface of cellulose, occupying some of the sites available at the substrate surface. With the progress of reaction, the cellulose particles shrink and consequently the number of vacant sites is decreased. This will continue until no site remains vacant and the whole substrate surface is covered with the active and inactive enzyme–cellulose and product inhibited complexes. At the second stage of reaction, due to the shrinkage of the particles, some of the enzymes, attached to the substrate surface will be released and return to the solution. This process continues until the total substrate surface is completely covered by the permanent inactive cellulose–enzyme and product inhibited complexes. As the result of such a phenomenon, despite that the cellulose particles are not fully hydrolyzed and some active enzymes are still present in the solution, the reaction is ceased (Figure 2.9).

End of adsorption process   End of first stage of reaction   Termination of reaction
(start of reaction)
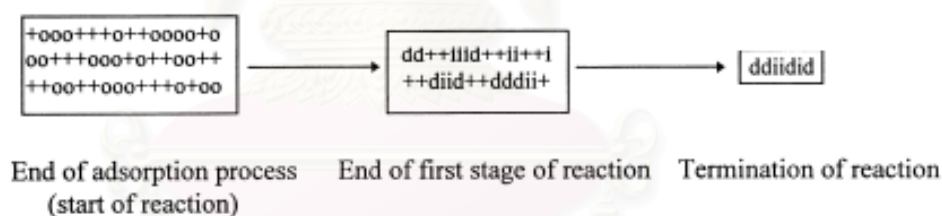
Fig. 2.9 This mode is a graphical representation. Inactive cellulose–enzyme complexes is denoted by 'd', active complexes by '+' , free active sites at the cellulose external surface by 'o' and product inhibited complex by 'i' [27].

2.4 Pretreatment processes

Pretreatment processes of cotton and other fibers are necessary for removing impurities from the fibers and for improving their aesthetic appearance and processability prior to dyeing, printing and/or mechanical and chemical finishing.

The need for good pretreatment has long been appreciated, but the developments taken place in dyeing processes, particularly continuous pad dyeing,

have accentuated the importance of the correct pretreatment of cotton. Over long continuous runs, the fabric must be evenly treated to have excellent absorbency, low residual size and wax content, whiteness appropriate to the color to be dyed and minimal fiber degradation. This technical standard must be met against economic constraints relating to the cost of chemical, labor, power and water.

Among these pretreatment processes, scouring is one of the most important process for all kinds of fibers. Without this step, the fibers will not absorb water, dyes and chemical adequately. In general, scouring is conducted at a high temperature in an aqueous solution containing detergent, chelating agent, and alkali. Some solvents can be used for scouring as well, and the process was called "Solvent Scouring".

2.5 Cotton scouring

Scouring is a purifying treatment of textiles. The objective of scouring is to reduce the amount of impurities sufficiently to obtain level and reproducible results in dyeing and finishing operations. Before cotton fabric can be dyed and finished, it has to be treated in order to make it hydrophilic and to remove the primary wall. In conventional cotton scouring processes high temperatures (90-100°C) and high concentration of NaOH (approx.10 mol/L) are used to remove the primary wall (pectin, protein, and organic acid) and hydrophobic components from cuticle (waxes and fats) in a non-specific way to make the fiber hydrophilic. Although alkaline scouring is effective and the cost of sodium hydroxide is low, the process is costly because it consumes large quantities of energy, water, and auxiliary agents. The risks of environmental contamination and depletion of natural resources is also serious. The strict pH and temperature requirements for alkaline scouring are damaging to many other fibers, which limits the blending of cotton. Another disadvantage is that a harsh hand develops due to the removal of the lubricating wax from the cotton fiber. More time and water are needed to rinse NaOH from the cotton substrate. Scouring cotton with enzymes is one of the areas where considerable research effort has been expanded resulting in the release of a commercial product. The major advantages feasible with this product seem to be saving in water and energy consumption, since the process is carried out at milder pH values and at lower temperatures when compared with conventional scouring processes [32, 33].

2.6 Enzyme for scouring process

The quality of life on earth is inextricably linked to the overall quality of the environment. The increasing awareness of the environment we live in is bringing about a more intensive search for alternative cleaner technologies. Currently, there are two fundamental pollution-related problems. The disposal of the large quantities of wastes are continually being produced, and the removal of toxic compounds that have been accumulating at dump sites in the soils and in water systems over the last few decades [34]. Biotechnology is an essential tool to deal with this problem because it can provide new approaches for understanding, managing, preserving, and restoring the environment, transforming pollutants into benign substances, generating biodegradable materials from renewable sources, and developing environmentally safe manufacturing and disposal processes [35].

Utilization of highly specific enzymes for various textile-processing applications is becoming increasingly popular because of their ability to replace harsh organic/inorganic chemicals currently used by the textile industry. Applications of biotechnology to textile wet processing are an example of more environmentally compatible processes. Enzymatic processes have been developed for wet processing of textile goods in wide-ranging operations from cleaning preparations to finishing processes. This is advantageous in the sense that they do not cause any physical chemical damage to cellulosic materials [36, 37].

The possibility of replacing alkaline scouring with the enzymatic decomposition of cotton impurities using various types of enzyme—cellulase, pectinase, lipases and proteases under different applications have been studied [6, 38, 39]. Typical temperatures for enzymatic treatments are about 40 – 50°C, which offer a significant decrease in energy consumption compared with conventional alkaline scouring. Also the wastewater effluent from enzymatic treatments is readily biodegradable and does not pose an environmental threat [40]. The potential advantages that make enzymatic scouring commercially attractive include more readily treatable wastewater, energy saving, and better compatibility with other processes, machinery, and materials [41].

2.6.1 Cellulases

In nature cellulose is degraded by both fungi and bacteria. These organisms produce cellulase that specifically degrade cellulose, yielding shorter chain cellulose polymers and glucose which are metabolized by these organisms. Typically, the fungal and some bacterial cellulolytic enzyme systems consist of several enzymes acting at the ends (exoglucanases, also called cellobiohydrolases) or in the middle (endoglucanases) of the cellulose chains [42] According to current knowledge, its cellulolytic system is composed of two cellobiohydrolases (CBHI and CBHII) and at least six endoglucanases (EGI, EGII, EGIII, EGIV, EGV and EGVI) and two β-glucosidases [43 – 45]. There are at least three major types of hydrolytic enzymes involved in the degradation of native cellulose to glucose.

1. Endoglucanases (endo-1,4-β-D-glucan-4-glucanohydrolase, EC3.2.1.4) mainly hydrolyze internal bonds in the random cellulose polymer, producing new chain ends as a result. A rapid decrease in chain length and a slow increase in reducing groups take place [46].

2. Exoglucanases present as cellobiohydrolases (exo-1,4-β-D-glucan-4-cellobiohydrolase, EC 3.2.1.91), initiate the hydrolysis at the chain ends, and do not produce significant amounts of new chain ends on the cellulose surface [47]. CBHII splits cellobiose from the non-reducing and CBH I from the reducing ends of cellulose chains [48, 49]. They are very active on swollen and partially degraded amorphous cellulose. They are not very active against crystalline cellulose but exhibit highly cooperative synergistic action in the presence of endoglucanases.

3. β-glucosidases or cellobiases (EC 3.2.1.21) complete the hydrolysis process by catalyzing the hydrolysis of cellobiose and other water soluble cellodextrins to glucose (Figure 2.10). They have maximum activity toward cellobiose and their action on cellodextrins decreases as chain length increases.
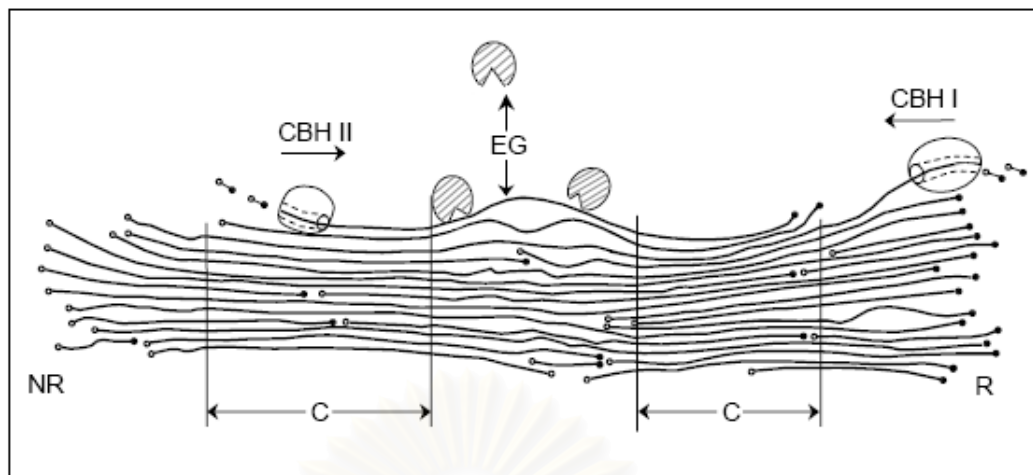
Figure 2.10 Mechanism for enzymatic hydrolysis of cellulose [50]. C defines the crystalline regions. The filled circles, denoted R, represent the reducing ends and the open circles, denoted NR, represent the non-reducing ends.

The efficiency of enzymatic hydrolysis of cellulosic material depends not only on the presence of all cellulase component but also more importantly on the appropriate proportional ration of the various components. Efficient overall hydrolysis of crystalline cellulose by cellulase requires the synergistic action of both EGs and CBHs [51]. Maximum synergism is usually obtained with a large amount of exo-enzyme and a minor amount of endo-enzyme [52 – 53]. It is also known that the degree of synergy is dependent on the substrate used [54].

The comparison of cellulase mixtures or individual cellulase poses special problems related to the multicomponent nature of the enzymes and their different efficacies on different cellulose structures. A wide range of different model substrates and many different assays have been developed and used to demonstrate the specific action of cellulase.

Cellulase activities measurement can be divided into two groups: one type using soluble substrate and the other using insoluble substrate such as cotton, dyed Avicel or filter paper. When using soluble substrate, such as carboxymethyl cellulose (CMC) or hydroxyethyl cellulose (HEC), the amount of reducing sugars liberated or the reduction in viscosity as a function of time can be measured. These methods are interpreted as endoglucanase activity measurements because the presence of side groups in CMC and HEC appears to prevent the action of most CBH enzymes

produced by fungal systems on these substrates [42]. EGI and EGII, the major endoglucanases, exhibit marked activity on β-glucan and soluble cellulose derivatives, such as HEC [55] and they are therefore considered to be true endoglucanases [52]. For the determination of total cellulolytic activities of cellulase mixtures or their complex synergistic action, more heterogeneous substrates such as filter paper, Avicel and cotton can be used.

Cellulase activities can be measured towards insoluble cellulose in the form of filter paper, or microcrystalline cellulose eventually swollen in phosphoric acid. Reducing sugars released can be monitored by, for example, the Nelson-Somogyi method [17, 56]. In the commercial mixture, the values obtained with this method reveal the hydrolysis rate cause by the synergistic action of EG and CBH activities.

Cellulase enzymes also employ an acid-base mechanism for the hydrolysis of their substrates. The catalytic activity and stability are generally dependent of the presence of metallic ions. Hydrolysis of cellulose is catalyzed via the β-β retaining mechanism or via the β-α inverting mechanism. When evaluating cellulase designed for the textile industry, the comparisons should not be based on assays run on soluble substrate, but on the effect on a relevant cotton fabric [17, 49]. Although the main priority of textile chemists is to identify the activities which deliver certain finishing effects, it is also essential to understand the mechanism of hydrolysis and how mechanical action affects the liberation of reaction products.

Cellulase mechanism

The enzyme mechanism of cellulase attack on cellulose is extremely complicated. Synergism between the different components in the cellulase system has been documented but detailed explanation of their mechanism and kinetics is not completely understood. The most widely proposed mechanism of hydrolysis of cellulose can be conveniently divided into the following stages [57].

1. Transfer of enzyme molecules from the aqueous phase to the surface of cellulose molecules.

2. Adsorption of the enzyme molecules onto the surface of cellulose resulting in the formation of an enzyme-substrate (E-S) complex.

3. Transfer of molecules of water to the active sites of the E-S complex.

4. Surface reaction between water and cellulose catalyzed by E-S complex.

5. Transfer of the products of reaction (cellobiose and glucose) to the aqueous phase.

2.6.2 Pectic enzymes

Basically, there are three types of pectic enzymes consisting of pectinesterase (esterases), pectinase (hydrolases) and protopectinase (lyases), shown in Table 2.5. The modes of action of the different types of pectic enzymes are illustrated in Figure 2.11. However, no distinction is made between endo-enzyme and exo-enzyme [10]. Several pectinase that have pectate liase activity are commercially available and can be used in cotton bioscouring [58 – 60], depend on differing in substrate preference, reaction mechanism, and action pattern [61].
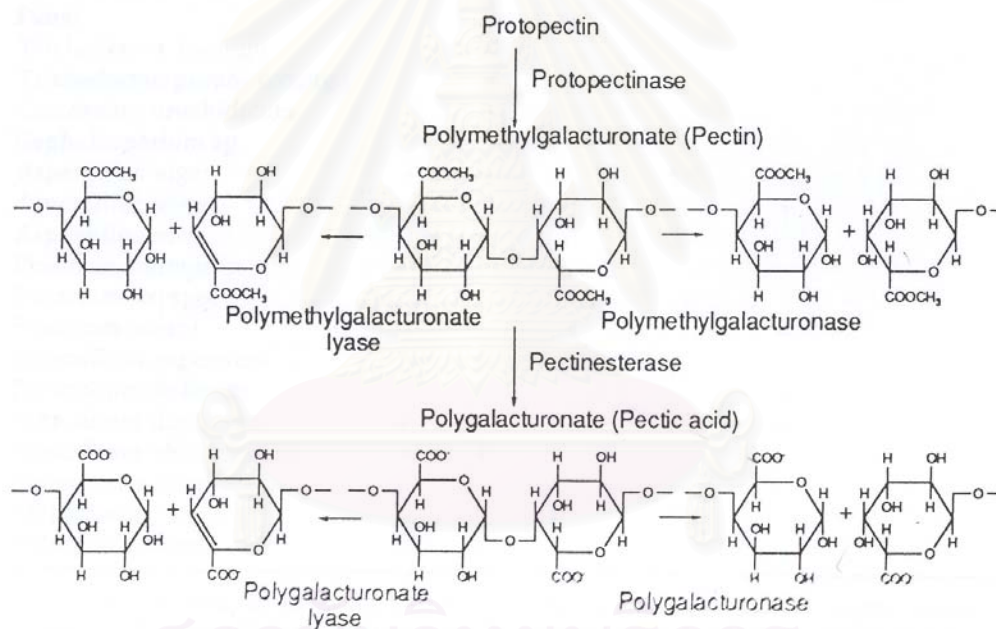
Figure 2.11 Mode of action of pectic enzymes [10].

Table 2.5 Classification of pectic enzymes [10].

Pectinesterase (PE)

(Pectin methylhydrolase, EC 3.1.1.11) catalyzes deesterification of the methoxyl group of pectin forming pectic acid.

Depolymerizing enzymes

Enzymes hydrolyzing glycosidic linkages:

Polymethylgalacturonase (PMG)

Endo-PMG, causes random cleavage of α-1,4-glycosidic linkages of pectin, preferentially highly esterified pectin.

Exo-PMG, causes sequential cleavage of α-1,4-glycosidic linkages of pectin from the non-reducing end of the pectin chain.

Polygalacturonase (PG)

Endo-PG [EC 3.2.1.15, poly (1,4-α-D-galacturonide) glycanohydrolase], catalyzes random hydrolysis of α-1,4-glycosidic linkages in pectic acid (polygalacturonic acid).

Exo-PG, [EC 3.2.1.67, poly (1,4-α-D-galacturonide) galacturonohydrolase], catalyzes hydrolysis in a sequential fashion of α-1,4-glycosidic linkages in pectic acid.

Enzymes cleaving α-1,4-glycosidic linkages by transelimination which results in galacturonide with an unsaturated bond between C_4 and C_5 at the non-reducing end of the galacturonic acid formed.

Polymethylgalacturonate lyase (PMGL)

Endo-PMGL [EC 4.2.2.10, poly (methoxygalacturonide) lyase], catalyzes random cleavage of α-1,4-glycosidic linkages in pectin.

Exo-PMGL, catalyzes stepwise breakdown of pectin by transeliminative cleavage.

Polygalacturonate lyase (PGL)

Endo-PGL [EC 4.2.2.2, poly (1,4-α-D-galacturonide) lyase], catalyzes random cleavage of α-1,4-glycosidic linkages in pectic acid by transelimination.

Exo-PGL [EC 4.2.2.9, poly (1,4-α-D-galacturonide) exolyase], catalyzes sequential cleavage of α-1,4-glycosidic linkages in pectic acid by transelimination.

Protopectinase

The enzymes solubilizes protopectin forming highly polymerized soluble pectin.

Polygalacturonases (PG) or pectinase enzymes are produced by numerous fungi and bacteria. They are a multi-component consisted of endopolygalacturonases (Endo-PG), exopolygalacturonases (Exo-PG), oligogalacturonases (OG), and polymethylgalacturonases (PMG). In general, by the action of polygalacturonase, pectic acid is broken down into mono-, di-, and trigalacturonic acid. These end products may be produced by a "single chain multiple attack" mechanism, in which case they can be detected rapidly, or by a "multi-chain attack" mechanism, where the mono-, di-, and trimers accumulate only after further hydrolysis of the initial depolymerization products (higher oligogalacturonates) [61].

Endo-PG is specific for pectic acid. If the degree of methoxylation increases, the rate and extent of hydrolysis decreases and the rate of splitting of the glycosidic bonds also decrease with the shortening of the substrate chain. Furthermore, free carboxyl groups seem to be necessary for catalytic activity and endo-PG is optimally active at a rather low pH (4.0 to 6.0) and at a temperature of 30 - 40°C.

Exo-PG is composed of exo-PG1 and exo-PG2. Exo-PG1 is produced by fungi, also called Fungal exo-PG (e.g. from *Coniothyrium diplodiella*) that produce monogalacturonic acid as the main end product and have pH optima from 4.0 to 6.0. Exo-PG2 is produced by bacterial (e.g. from *Erwinia arodeae* or *Selenomonas ruminantium*) that produce digalacturonic acid as the main end product. Both enzymes degrade pectic acid from non-reducing end chain (Figure 2.12).

Pectin-degradation enzymes have received much interest for their use in the pretreatment of textile fabrics ("bioscouring") prior to dyeing. The removal of pectin components from the cotton cell wall is claimed to improve hydrophilic property fiber, to facilitate dye penetration to contribute to substantial water saving when compared to the traditional alkaline scouring process.
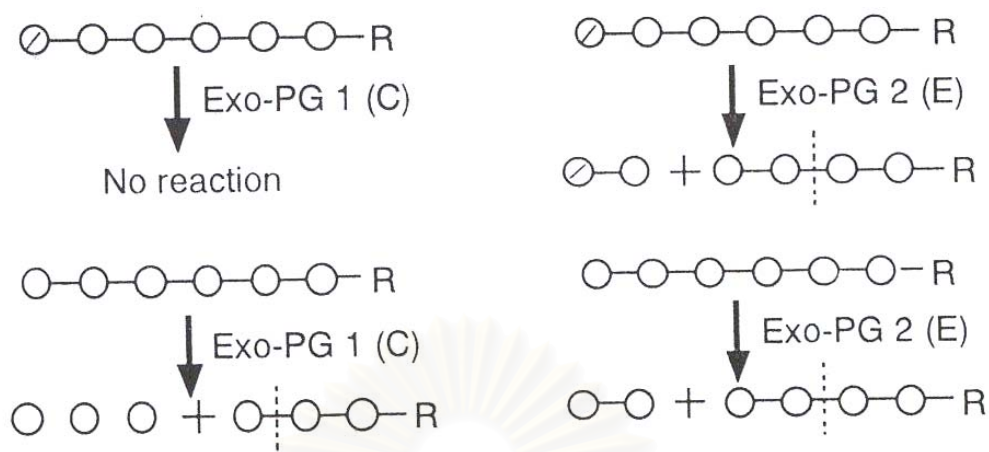
Figure 2.12 Action of exo-PG of *Coniothyrium diplodiella* (C) and *Erwinia arodeae* (E) on saturated pectic acid [62]. ∅, unsaturated galacturonic acid unit.

The determination of endo- and exopolygalacturonase activity towards pectin can be followed by the formation of reducing sugars. Since the enzyme catalyzes the depolymerization of a soluble substrate, an alternative assay method measures the decrease in viscosity. To distinguish between endo and exo enzymes, chromatographic techniques that identify short chain oligosaccharides formed or viscosity methods can be used.

2.6.3 Proteases

Proteases are important components of detergents formulations for removing protein stains (egg, blood etc.) from textiles. Additionally, proteases have a useful potential in silk and wool processing. Proteases or, more correctly, peptidases hydrolyze peptide bonds in soluble and insoluble peptides and form the group EC 3.4.X.X. of hydrolases. Peptidases can be divided into endopeptidases (proteinases) and exopeptidases (peptidases), which cleave peptide bonds within the protein or release amino acids sequentially from either the N- or C- terminus, respectively. Proteinases act on the interior peptide bonds of proteins and peptides, and include pepsin, trypsin, and chymotrypsin from animal and papaine from papaya. Peptidases act on peptide bonds adjacent to the free amino acid or carboxyl group [16, 17].

Protease activities can be measured towards proteins such as casein or hemoglobin by following the release of hydrolysis products colorimetric. Other more specific substrates are used if the hydrolysis of a certain peptide bond is targeted.

2.6.4 Lipases

Esterases are produced by numerous bacteria and fungi. Esterases have been suggested as useful components of detergent formulations to remove lipid-based stains from textiles while some esterases have been claimed to hydrolyze polyester. Esterases hydrolyze ester bonds and their classification is based on the type of ester bond hydrolyzed. Lipase is the kind of esterases.

Esterase with application in textile processing include carboxylesterases (EC 3.1.1.1) which hydrolyze carboxylic esters yielding the corresponding alcohol and carboxyl anion, arylesterases (EC 3.1.1.2) which hydrolyze phenyl acetate to phenol and acetate and triacylglycerolesterases (EC 3.1.1.3) which hydrolyze triacylglycerol giving a diacyl glycerol and fatty acid anion. The latter enzymes are better known as lipases.

Lipases catalyze the hydrolysis of fats at the interface between the insoluble substrate and the aqueous phase in which the enzymes are soluble. Lipases attack the ester bond in these fats, regenerate water soluble glycerol and water insoluble fatty acids, and convert to water soluble salts by the addition of alkali. Lipase cleaves triglycerides, hydrolyzes into 2-monoglycerides in step II and hydrolyzes to free glycerol and fatty acid in step III. The hydrolysis reaction is shown in Figure 2.15.

Lipid hydrolysis depends on different parameters such as pH, temperature, water content, and the phase boundary area. The optimum pH of most lipases lie between 7.5 – 9.0 [63].

Lipase activity was normally determined by colormetric method bases in the activity in cleavage of p-nitrophenyl palmitate (pNPP) at pH 8.0 [64].
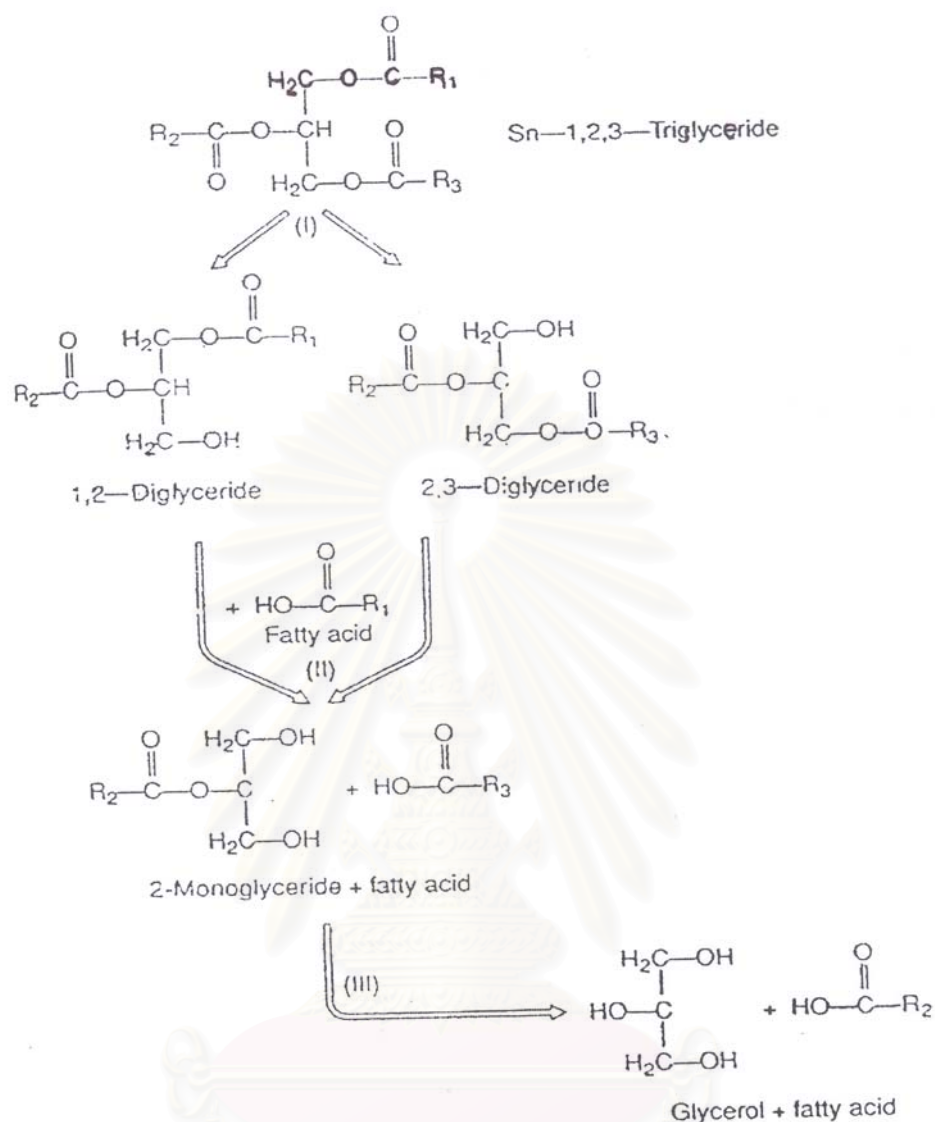
Figure 2.13 Reaction steps of lipase in triglyceride hydrolysis [63]

This review is specifically devoted to the use of enzymes to achieve scouring goals, in addition to establishing empirical kinetic equation and examining the change of degradation productions in scouring solutions.

In 1997-1998, Li and Hardin [9, 65] studied the influence of pectinase and cellulase enzymes on structural changes of cotton surfaces during scouring observed from staining tests and microscopy observations. Pectinases could destroy the cuticle structure by digesting the inner layer pectins in the cuticle and cellulases could destroy the cuticle structure by digesting the primary wall of cellulose immediately

under the cuticle. In addition, they also studied factors that effected the scouring efficiency such as rate of agitation and selection of surfactant and enzymes [65]. They concluded that nonionic surfactants were compatible with enzymes because they did not interfere with the three-dimensional structure of enzymes. Mechanical agitation could increase apparent enzyme activity and efficiency in scouring, but should be cautious when using mechanical agitation with high shear forces because enzymes could be denatured.

In 1998, Hsieh and Hartzell [6] investigated four enzymes containing pectinase, cellulase, protease and lipase for their effectiveness in improving the water absorbency and retention properties of cotton fabrics. Cellulase was the only enzyme to produce detectable improvements in water wettability of cotton. It was able to gain access to cellulose on removing the hydrophobic noncellulosic component from the fabric surface. But when they combined cellulase with pectinase, both water contact angle and water retention values fall within the range of commercially scoured fabrics. They also used pectinase combined with 100°C water pretreatment that resulted in the same wetting properties as cellulase treatment.

In 1998, Cavaco-Paulo [66] investigated that the effects on fabric caused by cellulase enzymes always result from a process in which strong mechanical action affecting the fabric is provided. Possible mechanisms for cooperation between cellulase activities and mechanical agitation could be:

1. EG randomly cleaves the accessible cellulose chains.

2. Mechanical action on the fibers will raise these broken ends, creating microfibrils and consequently more sites in the fibers for EG attack.

3. (Lack of CBHs) since mechanical agitation desorbs EGs rapidly, the endowise action will proceed in other randomly accessible points of the fiber. A shorter process will result in a fiber surface with raised microfibrils and several microfibrils in the liquor, whereas a longer process will result in the complete transformation of the fibers and the fabric structure into a mass of microfibrillar material.

4. (Presence of CBHs) CBH starts the hydrolysis on the more accessible raised broken chains, leaving localized under chains for attack by EGs. The CBH enzymes seem to focus the action of EG. The mechanical action will raise material

from the holes formed, helping the enzymes in a possible 'cutting' process. When the fibers are weak, mechanical action will completely break them. However, more experimental evidence is needed to explain this mechanical cutting off of fibers and microfibrils.

In 1998, Sawada *et al.* [67] studied bioscouring of cotton using pectinase enzyme, together with multiple mixed surfactants and D-limonene as scouring assistants. The effective of the bioscouring was evaluated by means of water absorbency test, weight loss measurement of cotton substrate and analyses of the amounts of pectic substance and cotton wax remaining in the cotton after scouring. The result showed that the scouring using pectinase improved water absorbency but much less wax removed than by alkaline scouring process. The improved water absorbency of the scoured cotton reverted to that of the original following a thermal process, indicating that the thermal process caused wax that had remained inside the bioscouring cotton to migrate to the surface. They found that addition of small amounts of nonionic surfactant in the scouring solution greatly enhanced the effectiveness of the removal of cotton wax without inhibiting the activity of pectinase enzyme. The characteristics of cotton scoured by pectinase-surfactant were equivalent to or better than those of cotton produced by the conventional alkaline process.

In 1999, Hsieh and Cram [16] studied the effect of proteolytic enzymes on improving the water wettability and absorbency of cotton fabrics. Proteases were used on greige cotton fabric pretreated twice in 100°C water for 2 seconds. Reactions with four of the protease significantly improved the wettability of cotton fabrics, and the resulting wetting behavior was similar or superior to alkaline scoured cotton. Several other proteases also improved cotton wettability. The optimized reaction condition for the most effective proteases afforded a range of moderate scouring conditions, including neutral to acid (pH 4) media, low temperature (25 to 45°C), and short reaction times (10 to 30 min). The low temperature of these protease reactions also led to fewer changes in fabric thickness and porosity than in the alkaline scoured cotton fabrics.

In 2000, Buchert and Pere [38] studied scoured cotton fiber and fabric using pectinase, protease, and lipase enzymes and studied the scouring using techniques of HPLC, ESCA and others. They found that pectin and protein could be removed from the fibers in the enzymatic treatments, whereas the waxes were less susceptible to

enzymatic action. Removal of pectin resulted in lower amounts of waxes on the cotton surface and subsequently improved water absorbency of the fabric, while the removal of protein improved the fabric whiteness.

In 1999-2000, Bushle-Diller *et al.* [68, 69] studied bioscouring of cotton using pectinase alone and combination with lipase, cellulase, and xylanase. They concluded that the water absorbency of textile material was improved when pectinase was used in combination with lipase and cellulase. In 2001 [70] their work involved a combination of all three preparatory processes including desizing, scouring and bleaching. They used glucose wastes from the desizing bath to react with the glucose oxidase enzyme in order to produce hydrogen peroxide for the bleaching step. They found that the whiteness of the enzymatically-bleached goods was closed to those of fabrics bleached conventionally with hydrogen peroxide.

In 2001, Sarkar and Etters [71] studied a preliminary kinetic of the enzymatic hydrolysis of raw cotton fibers using a commercial cellulase mixture. The reaction was executed at different flow rates of the reaction mixture through a reactor. Product formation time curves, soluble protein adsorption, rate of hydrolysis, and percentage of degree of hydrolysis during the course of hydrolysis were measured. They found that the hydrolysis rate decreased drastically during the initial hydrolysis period. Probable factors causing the reduction in the hydrolysis rate were elucidated. The results of this study may be useful in optimizing enzymatic processing of cellulosic substrates.

In 2001, Tzanova *et. al.* [72] attempted to introduce the bio-processes in the conventional scouring and bleaching preparation of cotton. The scouring with two types of pectinases, acting under acidic and alkaline conditions respectively, was as efficient as the chemical process in terms of obtained adequate water absorbency of the fabrics. The necessity of surfactants application in scouring was outlined. Bleaching of the fabrics was performed with hydrogen peroxide, which was enzymatically produced by glucose oxidase during oxidation of glucose. The aeration plays an important role in the enhancement of the enzyme reaction, so that the quantity of generated peroxide is sufficient to overcome the stabilizing effect of the glucose and protein in the subsequent bleaching. A closed-loop process reusing starch containing desizing baths in a single step scouring/bleaching operation with enzyme-generated peroxide was performed.

In 2001, Lin and Hsieh [73] investigated that proteases were effective scouring agents when directly applied on greige cotton fabrics without the boiling water pretreatment. Direct reactions with three proteases consisting of trypsin, chymotrypsin, and subtilisin on greige cotton fabrics showed that all three enzymes improve fabric wettability to a level similar to alkaline scouring under mild condition (45 - 55°C, pH 7). The reaction conditions required to achieve optimal fabric wettability (cos θ > 0.6) were 5 g/l and 45°C for trypsin and 5 g/l and 55°C for subtilisin. Chymotrypsin was effective under several reaction conditions such as 1 g/l and 55°C, 2 g/l and 45°C, and 5 g/l and 35°C. Most reactions took 30 min, and room temperature water rinses replace the post-reaction buffer rinses. Compared to protease scouring of boiling water pretreated cotton, direct protease scouring of greige cotton fabrics requires increased concentration (subtilisin), higher temperature (subtilisin and chymotrypsin), or longer time (trypsin and chymotrypsin) to achieve similar to water wettability and absorption properties. The most distinct outcome of direct protease scouring of greige cotton fabrics in comparison to boiling water preteated and protease scoured and alkaline scoured cotton, were the resulting fabric characteristics, consisting of less lateral shrinkage, no change in surface friction, easier to shear, and more resilient to compression and bending.

In 2003, Sangwatanaroj, Choonukulpong, and Ueda [2] studied the effects on various cotton fabrics scoured with alkaline and enzymes such as pectinase, lipase, protease, and cellulase either alone or in various combinations. All scoured fabrics similarly showed adequate absorbency of water and dye solutions. Both alkaline scouring and enzymatic scouring successfully removed the anionic components, including pectins, from fabrics. Some of the lipase/protease/cellulase scoured fabrics even showed lower amounts of anionic components (including pectins) when compared to pectinase and alkaline scoured fabrics. This was partly due to the loss of fiber by cellulase which contained the anionic components.

In 2004, Calafell and Garriga [74] studied the individual effects and the interaction between the process parameters, temperature, pH, and surfactant concentration, in the bioscouring of cotton fibers with an acid pectinase. This study indicated that increasing the temperature does not increase the total percentage of bioscouring but accelerates the rate of the process. The pH and surfactant seem to be determinant for the optimal enzyme performance under the studied conditions. The

enzyme–substrate ratio has been analyzed and a kinetic study at different enzyme concentrations has been carried out.

In 2004, Sarkar and Etters [75] studied the empirical equation adequately described the hydrolysis of cotton fibers, and the hydrolysis of non-textile cellulosic substrates. In addition, useful mechanistic information providing insight into the structural nature of the cellulose-cellulase system was obtained. Such information included the rate of the reaction, the sterical and diffusional features of the system, and probable inhibitory effects of excess substrate and products of reaction on enzymatic hydrolysis. Theoretical analysis of enzymatic reactions using such simple empirical equations will hopefully pave the way for efficient kinetic studies. As more enzymes are introduced in the wet-processing of cotton, there is a need for quick but reliable data to judge the effectiveness of the enzyme-substrate system. Modeling using empirical equation fills the requirement admirably.

In 2004, Karapinar and Sariisik [76] studied the effect of pectinase, protease and cellulase on scouring using different treatment times, either in the baths containing one enzyme or different enzyme combinations, in order to evaluate the effects of these enzymes on 100% cotton fabric's wettability and absorbency. At the end of the enzymatic and alkaline scouring, the wettability and absorbency properties of the garments were evaluated in terms of wettability, CIE*L, WI values and pectin analysis by Ruthenium Red dyeing. In the result of treatment time, 30 minutes of treatment time was in most cases insufficient for effective scouring when compared with reaction times of 60 and 90 minutes, whereas the evaluation test results obtained after 60 and 90 minutes were statistically the same. In the wettability, the mixture of cellulase and pectinase and the combination of cellulase, pectinase, and protease gave better results than other enzymatic combinations. Most of the enzymatic scouring did not give better results than conventional alkaline scouring; however, the most similar results to alkaline scouring by means of wettability and pectin removal were achieved with the mixture of cellulase and pectinase and the combination of cellulase, pectinase, and protease.

In 2005, Mitchell et al. [77] investigated the surface chemical composition of raw unscoured cotton by X-ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS). The complementary surface analytical XPS and ToF-SIMS techniques have confirmed the presence of a complex

mixture of ''wax-type'' material on the surface of 'raw' cotton fibers. This surface contamination has been shown to consist of mainly long chain fatty acids with associated fatty alcohols and alkanes. In addition there is evidence of triglycerides and esters also being present in the surface cotton wax. The effect of scouring and bleaching is to remove the majority of these surface species but still a residual, tenacious layer remains.

In 2006, Valle *et al*. [78] studied the effect of cotton scouring using enzymes produced in *Sclerotium rolfsii*. Enzyme production was induced by glucose, glucose–pectin or cellulose, and in all cases the enzyme activity corresponded to polygalacturonase activity. The weight loss after bioscouring was more efficient with enzymes induced by pectin because these have a specific activity for removing pectin content from the cotton fiber. The effectiveness of the enzymatic scouring was equivalent or better than that achieved by the conventional alkaline process.

In 2006, Wang *et al.* [26] studied the kinetic of the degradation of pectins in cotton by an alkaline pectinase 'Bioprep 3000L'. The experimant was performed and the influences of initial pectinase concentration and treatment time on bioscouring were evaluated quantitatively. The results showed that although the degradation products increased as pectinase concentration grew higher at same incubation time, the growth multiples of the maximum degradation rate which was used as the starting degradation rate were less than those of initial enzyme concentration. The degradation kinetics of pectins in cotton fibers with a pectinase could be described by modified Ghose–Walseth kinetic empirical equations which had been previously applied to the degradation reaction of cellulose.

The aim of this work was to establish the mechanism of enzymatic scouring of cotton fabric using a one-step process with pectinase and a two-step process with either lipase then cellulase, protease then cellulase, or lipase/protease then cellulase. UV-Vis spectrophotometric and HPLC analyses of the scouring solutions were made for hydrolyzed products released from the cotton fabric during scouring such as galacturonic acid from pectins, reducing sugars from cellulose, and amino acids from proteins. The progress of the scouring reaction was also monitored and kinetically studied. The residual impurities on the scoured cotton fabric were determined including some fabric properties. Data obtained from this work can help to better understanding the structure of cotton impurities.

CHAPTER III

EXPERIMENTAL

Chapter 3 describes the materials used in this research (consisting of enzymes, fabric, and chemicals), and the methods of the enzymatic scouring of cotton and the enzyme activity determination. Analyses of the scouring solutions at various scouring time periods and analyses of fabrics before and after scouring are also shown in this chapter with an objective to study the kinetics of the enzymatic scouring processes.

3.1 Materials

3.1.1 Fabric:

The fabric used in this work was purchased from a local knitting plant. It was a greige cotton knitted fabric weighing 1.2 g/100 cm^2 with a single jersey construction and a yarn count of 50/1.

3.1.2 Enzymes:

Five different enzymes were used in this work. Details are shown in Table 3.1.

Table 3.1 Enzymes used in this work.

Enzyme	EC number	Source	Activity (units/g)	Manufacturer
pectinase	EC 3.2.1.15	*Aspergillus niger*	2,400	Tokyo Chemical Industry, Japan
lipase*	EC 3.1.1.3	*Porcine pancreas*	20	Tokyo Chemical Industry, Japan
protease	EC 3.4.23.6	*Aspergillus oryzae*	14,000	Tokyo Chemical Industry, Japan
cellulase	EC 3.2.1.4	*Aspergillus niger*	25,000	Tokyo Chemical Industry, Japan
α-amylase	EC 3.2.1.1	*Bacillus Lichen*	20,000	Profiltex, France

* Lipase contained residual amounts of amylase and protease.

3.1.3 Chemicals:

All chemicals used in this work were reagent grade chemicals.

Table 3.2 Chemicals used in this work.

Chemical	Manufacturer
Monosaccharide standards	
D-glucose and galacturonic acid	Fluka, Switzerland
Amino acid standards	
alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionin, phenylalanine, proline, serine, threonine, tyrosine, valine, and α-amino butyric acid.	Fluka, Switzerland
Fatty acid standards	
arachidic acid, behenic acid, caprylic acid, capric acid, cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA), heptadecanoic acid, heneicosanoic acid, lauric acid, lignoceric acid, linoleic acid, myristic acid, ρ-nitrophenol, oleic acid, palmitic acid, pentadecanoic acid, stearic acid, tricosanoic acid, tridecanoic acid, undecanoic acid, and methyl ester.	Supecol, USA
Protein standard	
bovine serum albumin (BSA)	Fluka, Switzerland
Enzyme substrate	
casein, polygalacturonic acid, p-nitrophenyl palmitate (pNPP), and Whatman no.1 filter paper	Fluka, Switzerland
Nelson Reagent	
ammonium molybdate and disodium hydrogen arsenate heptahydrate	Fluka, Switzerland
anhydrous sodium carbonate	Sigma-Aldrich, Germany

Table 3.2 (continued)

Chemical	Manufacturer
concentrated sulphuric acid	Merck, Germany
cupric sulphate pentahydrate	BDH, England
anhydrous sodium sulphate, sodium hydrogen carbonate, and sodium potassium tartrate	APS Ajax Finechem, Australia
Lowry reagent	
anhydrous sodium carbonate	Sigma-Aldrich, Germany
cupric sulphate pentahydrate	BDH, England
Folin-phenol reagent	Fluka, Switzerland
sodium potassium tartrate	APS Ajax Finechem, Australia
Anson reagent	
Folin-phenol reagent	Fluka, Switzerland
anhydrous sodium carbonate and trichloroacetic acid	Sigma-Aldrich, Germany
Winker and Stuckmann reagent	
2-propanol	BDH, England
gum arabic	Fluka, Switzerland
potassium hydrogen phosphate	APS Ajax Finechem, Australia
Womine TE (wetting agent)	Tokai Seiyu, Japan
Kjeldahl reagent	
concentrated sulphuric acid	Merck, Germany
anhydrous sodium sulphate, boric acid, bromocresol green indicator, methyl red indicator, and selenium oxide	APS Ajax Finechem, Australia
cupric sulphate pentahydrate and hydrochloric acid	BDH, England
sodium hydroxide	Lab-scan, Thailand

Table 3.2 (continued)

Chemical	Manufacturer
Amino acid reagent	
6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ•flour reagent)	Fluka, Switzerland
Fatty acid reagent	
sodium chloride, boron trifluoride, sodium hydroxide, methanol, isooctane	Merck, Germany
Others	
1-phenyl-3-methyl-5-pyrazolone (PMP)	Fluka, Switzerland
Ecowet DA (wetting agent)	East Asiatic, Thailand
glacial acetic acid	Merck, Germany
methylene blue and trichloroethylene	Lab-scan, Thailand
potassium hydrogen phosphate, concentrate hydrochloric acid, sodium acetate, and disodium hydrogen phosphate	APS Ajax Finechem, Australia

3.2 Equipment

1. pH meter, Model 511, Denver Instrument
2. Shaker bath, Model L-24p-1, Labtec
3. UV-Vis spectrophotometer, Model Specord S100, Analytik Jena
4. High-performance liquid chromatographs, Model Waters Alliance 2695, Waters
5. Gas chromatograph, Model Shimadzu GC-14A , Shimadzu Corporation
6. Sample cutter, diameter 11.3 cm, Jen-Haur Co.,Ltd.
7. Digestion unit, Model K-424, Bü chi
8. Distillation unit, Model B-324, Bü chi
9. Soxhlet extraction assembly
10. X-ray diffractometer, Model JDX-3530 , JEOL
11. Infrared Moisture Balance, Model AD-4715, A&D Company
12. Scanning Electron Microscope, Model JSM-5410LV, JEOL
13. Bursting strength tester, Mullen type, Yasuda
14. MacBeth reflectance spectrophotometer, COLOR-EYE 7000
15. Ultrasonic cleaner, Model 575HTAE, Crest

3.3 Enzymatic scouring process

Prior to scouring, greige cotton fabric was prewashed in boiling water for 10 minutes then water rinsed and dried in order to remove the water soluble materials depositing on the fabric surface. The prewashed fabric was tested for water absorbency according to the test procedure outlined in section 3.6.6 in order to determine whether a scouring was needed after prewashing the greige fabric, and found that the prewashed fabric did not absorb water sufficiently and thus it required a further scouring. Various enzymatic scouring treatments were performed on the prewashed fabric according to the methods introduced by Sangwatanaroj *et al.* [2]. All scouring treatments are summarized in Table 3.3 and Diagram 3.1.

Table 3.3 Formulations for various enzymatic scouring methods.

| Method | Symbol | Enzyme | | | Wetting agent (g/l) | Scouring condition | | | Fabric absorbency |
		Step	Type	g/l		Temp (°C)	pH	Time (min)	
1	PEC	1	Pectinase	5.00	0.5	37	4.0	120	adequate
2	L	1	Lipase	0.50	1.0	37	8.0	30	inadequate
	CL	2	Cellulase	0.50	1.0	40	4.5	30	adequate
3	P	1	Protease	0.50	1.0	37	7.0	30	inadequate
	CP	2	Cellulase	0.50	1.0	40	4.5	30	adequate
4	LP	1	Lipase	0.25	1.0	37	7.5	30	inadequate
		1	Protease	0.25					
	CLP	2	Cellulase	0.50	1.0	40	4.5	30	adequate
5	WA	1	-	-	0.5, 1.0	37, 40	4.0, 4.5, 7.0, 7.5, 8.0	30,120	inadequate

3.3.1 One-step pectinase process

Prewashed fabric was scoured with pectinase and a nonionic wetting agent "Ecowet DA" using a shaker bath at 37°C, liquor to fabric ratio of 50:1, and an optimal condition of pH 4 for 2 hours (see method 1 in Table 3.3). It was then boiled in distilled water for 10 minutes to terminate the enzyme reaction, water rinsed and dried.

3.3.2 Two-step lipase/protease/cellulase process

Data in a previous work by Sangwatanaroj *et al.* [2] indicate that cellulase will not act on the cellulose of cotton unless protease and/or lipase were applied first on the cotton. It appears to be necessary to firstly remove waxes, fats, and proteins before cellulase can reach the cellulose layers.

In the first scouring step prewashed fabric was scoured with Ecowet DA and either lipase or protease or lipase and protease using a shaker bath at 37°C, liquor to fabric ratio of 50:1, and an optimal condition of pH 8, 7, or 7.5, respectively, for 30 minutes. Then the fabric was boiled in distilled water for 10 minutes, water rinsed and dried. In the second scouring step the fabric was scoured with cellulase and Ecowet DA at 40°C, liquor to fabric ratio of 50:1, and an optimal condition of pH 4.5 for 30 minutes (see methods 2, 3, and 4 in Table 3.3). It was then boiled in distilled water for 10 minutes, water rinsed and dried.

3.3.3 Blank Treatment

To determine whether the nonionic wetting agent had a direct scouring effect on the fabric, the prewashed fabric was treated with 0.5 and 1 g/l Ecowet DA at 37°C and 40°C; liquor to fabric ratio of 50:1, pH 4, 4.5, 7, 7.5, and 8; for 30 and 120 minutes (all scouring conditions used in this study). It was then rinsed with water, dried, and tested for water absorbency according to the method described in section 3.6.6.

An evaluation of the scouring effect of the nonionic wetting agent "Ecowet DA" without enzymes shown in method 5 of Table 3.3 indicated that all treated fabrics did not absorb water adequately and therefore this nonionic wetting agent did not play a major role in the enzymatic scouring. Scouring without the presence of an enzyme could not effectively treat the fabric to increase water absorbency.

<u>Pectinase Process</u>

100°C

37°C
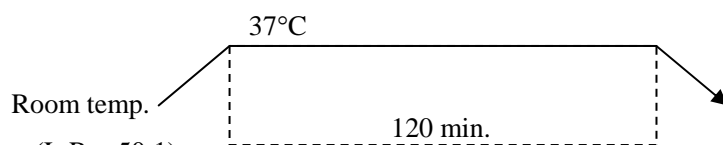
Room temp.
- (L:R = 50:1)
- cotton fabric
- pectinase 5 g/l
- pH 4
- Ecowet DA 0.5 g/l

120 min.

10 min.
- Distilled water
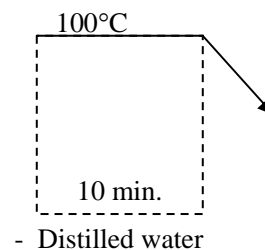
<u>Lipase/protease process</u>

100°C

37°C
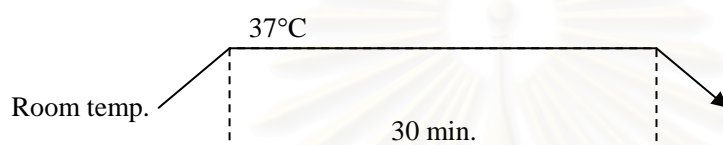
Room temp.
- (L:R = 50:1)
- cotton fabric
- lipase, protease, or lipase-protease 0.5 g/l
- pH 8.0, 7.0, or 7.5
- Ecowet DA 1 g/l

30 min.

10 min.
- Distilled water

<u>Cellulase process</u>

100°C

40°C
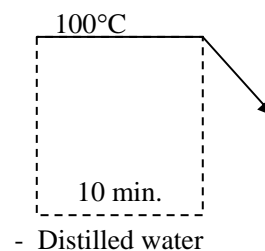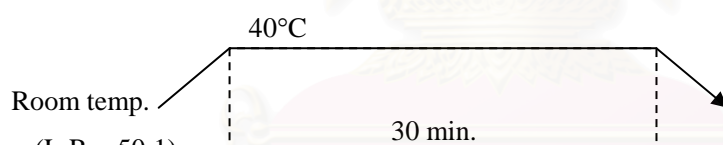
Room temp.
- (L:R = 50:1)
- cotton fabric
- cellulase 0.5 g/l
- pH 4
- Ecowet DA 1 g/l

30 min.

10 min.
- Distilled water

<u>Blank treatment</u>

100°C

37, 40°C
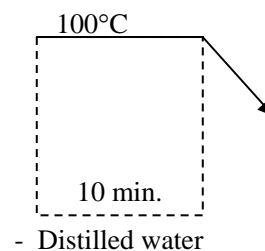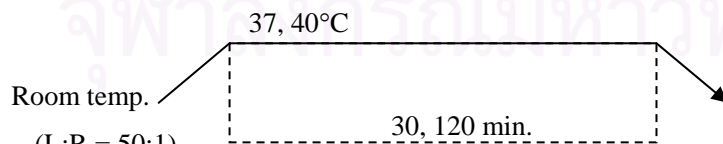
Room temp.
- (L:R = 50:1)
- cotton fabric
- pH 4.0, 4.5, 7.0, and 8.0
- Ecowet DA 0.5 and 1.0 g/l

30, 120 min.

10 min.
- Distilled water
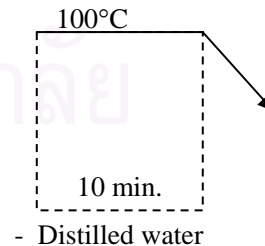
Diagram 3.1 Enzymatic scouring procedures.

3.4 Determination of enzyme activity

Enzyme activity is expressed in units where one unit is defined as the amount of enzyme used for transforming one mole of substrate in one minute into micromoles of product per milligram of protein or product (volume) under standard conditions of temperature, optimal pH and optimal substrate concentration. In this work, four enzymes consisting of pectinase, lipase, protease, and cellulase were determined for enzyme activities on specific and unspecific substrates. In addition, scouring solutions containing these enzymes were assayed for enzyme activities both before and after scouring as follows.

3.4.1 Effect of wetting agent on enzyme action

In this work a nonionic wetting agent "Ecowet DA" was added into all enzymatic scouring solutions and thus its effect on enzyme activities was studied. Various enzyme solutions in the presence and in the absence of Ecowet DA were analyzed for enzyme activity according to the methods shown in section 3.4.2.

3.4.2 Enzyme activity on specific substrate

3.4.2.1 Cellulase activity

Cellulase activity was determined using the Mandels method [56] with D-glucose as a standard and filter paper as a substrate. This method concerns the hydrolysis of filter paper into glucose using cellulase in the scouring solutions (with and without Ecowet DA). The absorption of the solutions (for the presence of reducing sugars including glucose) was measured using UV-Vis spectrophotometry after incubation at 520 nm by the Nelson-Somogyi method [79]. One unit of cellulase activity was defined as the amount of cellulase which produced 1 μmol of reducing sugars including D-glucose per minute at pH 4.5 and 40°C (cellulase scouring condition). In this test, copperarsenomolybdate solution was prepared in order to detect for the existence of reducing sugars including D-glucose in the test solution. The D-glucose standard curve relating between its concentration and light absorbance at 520 nm was also prepared for the test. Details of the test method are described as follows.

Copperarsenomolybdate solution was made from two solutions consisting of copper and arsenomolybdate solutions.

Copper solution was prepared from a mixture of solutions A and B (4:1 by volume). To prepare solution A, two solutions were made. The first one was made by dissolving 15 g of sodium potassium tartrate, 30 g of anhydrous sodium carbonate and 20 g of sodium hydrogen carbonate in 300 ml of water. The second solution was prepared by dissolving 180 g of anhydrous sodium sulphate in 500 ml of boiling water and then the solution was cool to room temperature. Finally, both solutions were mixed and the total volume was adjusted to 1 lit with water. Solution B was prepared by dissolving 5 g of cupric sulphate pentahydrate and 45 g of anhydrous sodium sulphate in 250 ml of water.

To prepare arsenomolybdate solution, two solutions were made. The first one was made by dissolving 25 g of ammonium molybdate in 450 ml of water and then 21 ml of concentrated sulphuric acid was added into the solution. The second solution was prepared by dissolving 3 g of disodium hydrogen arsenate heptahydrate in 25 ml of water. Both solutions were mixed, incubated at 37°C for 24 hr and stored in a brown bottle. Just before using, 2 ml of 0.75 M sulphuric acid was added.

Cellulase assay was initiated by first mixing 0.5 ml of the cellulase scouring solution (with and without Ecowet DA) with 50 mg of filter paper as a substrate (Whatman no.1) and 0.5 ml of 0.04 M acetate buffer to obtain the pH of 4.5. The mixture was then incubated at 40°C for 20 min. After 20 min, 0.5 ml of the liquid was drawn from the mixture. It was immediately mixed with 0.5 ml of the copper solution in order to terminate the cellulase action and then incubated in a boiling water bath for 15 min. After the sample was rapidly cooled to room temperature, 0.5 ml of the arsenomolybdate solution was added, next 1.5 ml of water was added and mixed well. After 15 min incubation at room temperature, the assay mixture was filtered by a filter paper (Whatman no.40) in order to remove precipitated residual substrates. Finally, the solution was tested for its absorption at 520 nm using UV-Vis spectrophotometry. The absorbance was then converted into the concentration of reducing sugars including glucose presented in the solution according to the concentration and absorbance calibration curve of the standard D-glucose solution shown in Figure 3.1. The cellulase assay was conducted three times and the test results were averaged.

The D-glucose calibration curve relating between the concentration of glucose solution and it light absorbance was constructed by preparing various concentrations of glucose solutions (20, 40, 60, 80 and 100 μg/ml) and testing for their light absorbance in the range of 0 to 1 at wavelength 520 nm using UV-Vis spectrophotometry.
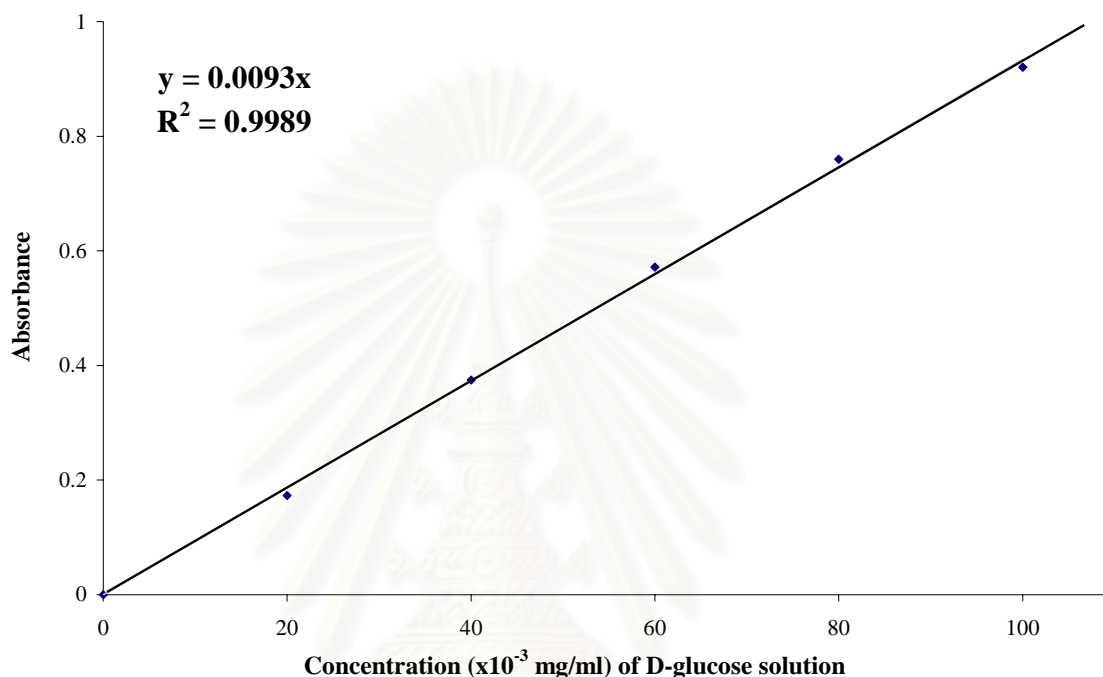
Figure 3.1 Concentration and absorbance calibration curve of standard D-glucose solution using UV-Vis spectrophotometry.

3.4.2.2 Pectinase activity

Pectinase activity was assayed by measuring the release of galacturonic acid using the Nelson-Somogyi method [79, 80] with D-galacturonic acid as a standard and polygalacturonic acid as a substrate. This method concerns the hydrolysis of polygalacturonic acid into galacturonic acid using pectinase in the scouring solutions (with and without Ecowet DA) and measuring the absorption of the solutions (for the presence of galacturonic acid) at 500 nm using UV-Vis spectrophotometry after incubation. One unit of pectinase activity was defined as the amount of pectinase which produced 1 μmol of galacturonic acid per minute at pH 4 and 37°C (pectinase scouring condition). In this test, copperarsenomolybdate solution was also used and thus it was prepared following the same procedure as shown in section 3.4.2.1. The

concentration and absorbance calibration curve of D-galacturonic acid solution was prepared as well. Details of the test method are described as follows.

Prior to pectinase assay, polygalacturonic acid solution as a substrate was prepared by mixing 20 ml of 0.6 M sodium chloride with 80 ml of a solution containing 75 mM sodium acetate, 7.5 mM EDTA, and 0.3% (w/v) of polygalacturonic acid. Then, the final pH was adjusted to 4.0 with 1 N sodium hydroxide. Pectinase assay was initiated by mixing 2.5 ml of the prepared polygalacturonic acid solution with 0.5 ml of the pectinase scouring solution (with and without Ecowet DA). The solution was then incubated at 37°C for 20 min. After 20 min, 1 ml of the solution was drawn and immediately mixed with 1 ml of the copper solution. The sample was subsequently incubated in a boiling water bath for 15 min. After it was rapidly cooled to room temperature, 1 ml of the arsenomolybdate solution was added, next 3 ml of water was added and mixed well. After 15 min incubation at room temperature, the assay mixture was filtered by a filter paper (Whatman no.40) in order to remove precipitated residual substrates. Finally, the solution was tested for its absorption at 500 nm using UV-Vis spectrophotometry. The absorbance was then converted into the concentration of galacturonic acid presented in the solution using information shown in the calibration curve of Figure 3.2. The pectinase assay was conducted three times and the test results were averaged.

The D-galacturonic acid calibration curve relating between the concentration of galacturonic acid solution and its light absorbance was constructed by preparing various concentrations of galacturonic acid solutions (20, 60, 100, 140, 180 and 200 µg/ml) and testing for their light absorbance in the range of 0 to 1 at wavelength 500 nm using UV-Vis spectrophotometry.

Figure 3.2 Concentration and absorbance calibration curve of standard D-galacturonic acid solution using UV-Vis spectrophotometry.

3.4.2.3 Protease activity

Protease activity was determined using the Anson method [81] with tyrosine as a standard and casein as a substrate. This method concerns the hydrolysis of casein into amino acids including tyrosine using protease in the scouring solutions (with and without Ecowet DA) and measuring the absorption of the solutions (for the presence of amino acids including tyrosine) at 660 nm using UV-Vis spectrophotometry after incubation. One unit of protease activity was defined as the amount of protease which produced 1 µmol of amino acids including tyrosine per minute at pH 7 and 37°C (protease scouring condition). The tyrosine standard curve relating between its concentration and light absorbance at 660 nm was also prepared for the test. Details of the test method are described as follows.

Prior to protease assay, 1.5% (w/v) of the fresh casein solution as a substrate was prepared at pH 7. Protease assay was initiated by mixing 1 ml of the prepared casein solution with 1 ml of the protease scouring solution (with and without Ecowet DA). The solution was then incubated at 37°C for 20 min. After 20 min, 2 ml of 0.4 M trichloroacetic acid was added in order to terminate the protease action and then the sample was filtered by a filter paper (Whatman no.40) in order to remove

precipitated residual substrates. The clear solution was drawn and mixed with 2.5 ml of 0.4 M anhydrous sodium carbonate and 0.5 ml of 2N Folin-phenol solution. After 10 min incubation at room temperature, the solution was tested for its absorption at 660 nm using UV-Vis spectrophotometry. The absorbance was then converted into the concentration of tyrosine presented in the solution using information shown in the calibration curve of Figure 3.3. The protease assay was conducted three times and the test results were averaged.

The tyrosine calibration curve relating between the concentration of tyrosine solution and its light absorbance was constructed by preparing various concentrations of tyrosine solutions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mg/ml) and testing for their light absorbance in the range of 0 to 1 at wavelength 660 nm using UV-Vis spectrophotometry.
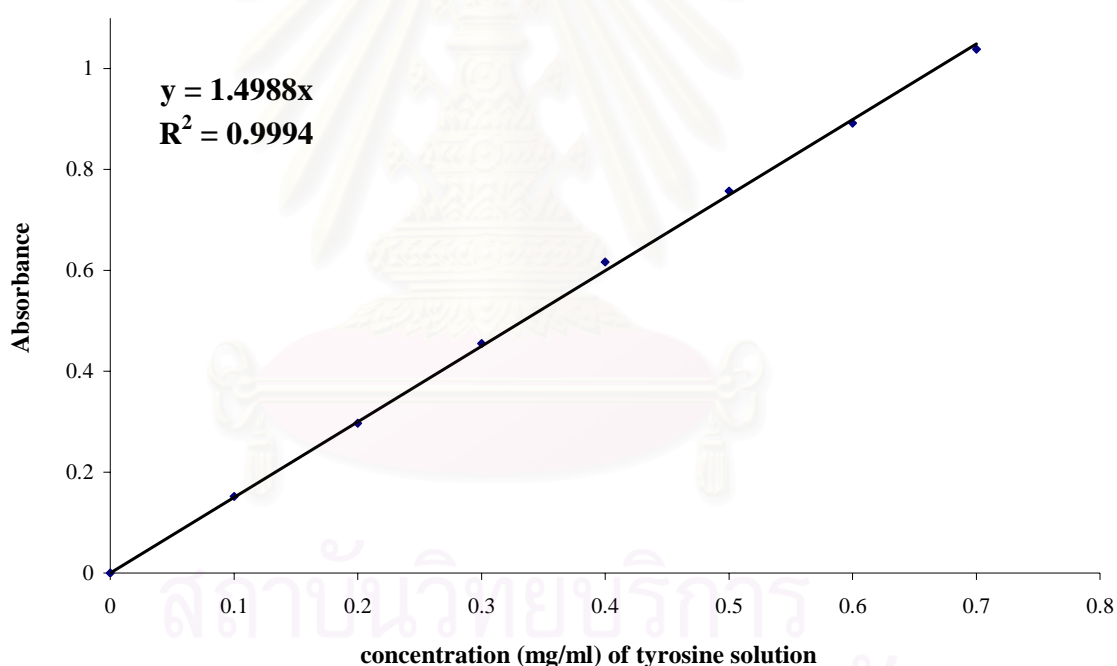
Figure 3.3   Concentration and absorbance calibration curve of standard tyrosine solution using UV-Vis spectrophotometry.

3.4.2.4 Lipase activity

Lipase activity was determined using a modified method of Winkler and Stuckmann [82] with p-nitrophenol as a standard and p-nitrophenyl palmitate (pNPP) as a substrate. This method concerns the hydrolysis of pNPP into p-nitrophenol using lipase in the scouring solutions (with and without Ecowet DA) and measuring the absorption of the solutions (for the presence of p-nitrophenol) at 410 nm using UV-Vis spectrophotometry after incubation. One unit of lipase activity was defined as the amount of lipase which produced 1 μmol of p-nitrophenol per minute at pH 8 and 37°C (lipase scouring condition). In this test, reagents A and B were prepared in order to detect for the existence of p-nitrophenol in the test solution. The concentration and absorbance calibration curve of p-nitrophenol solution was prepared for the test. Details of the test method are described as follows.

Reagent A was prepared by dissolving pNPP as a substrate in 10 ml of 2-propanol until reaching the concentration of 16.5 mM, then sonicating the mixture by an Ultrasonic instrument for 6 min at room temperature.

Reagent B was prepared by mixing 100 ml of 50 mM potassium hydrogen phosphate, 4 g of Womine TE and 1 g of gum Arabic. Then the pH of the mixture was adjusted to 8.

Prior to lipase assay, a solution mixture was prepared by mixing 1 part of reagent A and 9 parts of reagent B. Lipase assay was initiated by mixing 1 ml of the lipase scouring solution (with and without Ecowet DA) and 9 ml of the prepared solution mixture. The sample was then incubated in a water bath at 37°C for 20 min. Finally, the sample was tested for its absorption at 410 nm using UV-Vis spectrophotometry. The absorbance was then converted into the concentration of p-nitrophenol presented in the solution using information shown in the calibration curve of Figure 3.4. The lipase assay was conducted three times and the test results were averaged.
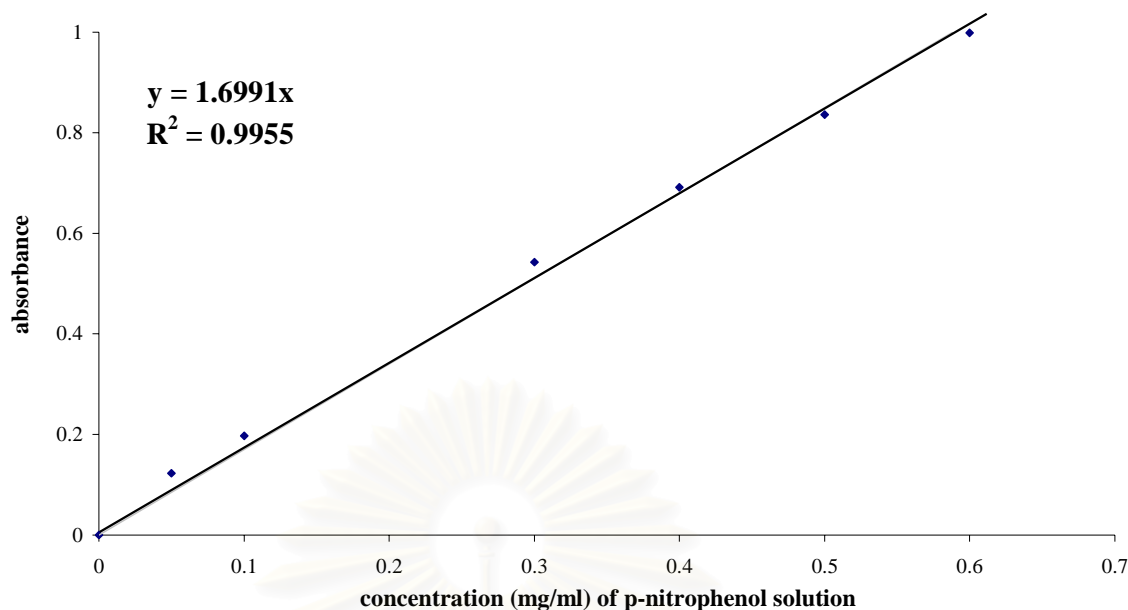
Figure 3.4 Concentration and absorbance calibration curve of standard p-nitrophenol
           solution using UV-Vis spectrophotometry.

The p-nitrophenol calibration curve relating between the concentration of p-nitrophenol solution and its light absorbance was constructed by preparing various concentrations of p-nitrophenol solutions (0.05, 0.1, 0.3, 0.4, 0.5 and 0.6 mg/ml) and testing for their light absorbance in the range of 0 to 1 at wavelength 410 nm using UV-Vis spectrophotometry.

3.4.3 Enzyme activity on unspecific substrate

Pectinase, cellulase, lipase, and protease were also tested for their activities on the unspecific substrates using the methods previously shown in sections 3.4.2.1 – 3.4.2.4. Pectinase activity was tested on 3 substrates consisting of filter paper (cellulose), pNPP (fats), and casein (protein). Cellulase activity was tested on polygalacturonic acid (pectins), pNPP, and casein. Lipase activity was tested on polygalacturonic acid, filter paper, and casein. Protease activity was tested on polygalacturonic acid, filter paper, and pNPP.

3.5 Analysis of scouring solution

During enzymatic scouring of cotton, it is speculated to observe the hydrolyses of cotton cellulose and its impurities in the scouring system. The possible hydrolyzed products consist of reducing sugars from cellulose hydrolysis, galacturonic acid from polygalacturonic acid hydrolysis, and amino acids from protein hydrolysis. To examine the hydrolyzed products from scouring of cotton, the scouring solution from each enzymatic scouring process was tested for reducing sugars and galacturonic acid by UV-Vis spectrophotometry after various scouring time periods. In addition, the scouring solution was also tested for amino acids by HPLC and proteins by UV-Vis spectrophotometry after each scouring step.

3.5.1 Reducing sugars content

The scouring solution from each enzymatic scouring process was tested for reducing sugars (generally consisting of glucose, cellobiose, arabinose, xylose, galactose, and other monosaccharides, confirmed by HPLC analysis), using UV-Vis spectrophotometry after various scouring time periods according to the Nelson-Somogyi method [79]. In this test, the copperarsenomolybdate solution was used in the analysis and thus was prepared following the same procedure shown in section 3.4.2.1.

Determination of reducing sugars was initiated by mixing 2 ml of the scouring solution with 2 ml of the copper solution. The sample was subsequently incubated in a boiling water bath for 15 min. After the sample was rapidly cooled to room temperature, 2 ml of the arsenomolybdate solution was added. Then 6 ml of water was added and the sample was mixed well. After 15 min incubation at room temperature, the sample was measured for its light absorption at 520 nm using UV-Vis spectrophotometry. The absorbance was then converted into the concentration of reducing sugars presented in the scouring solution using information shown in the calibration curve of Figure 3.1. The amount of reducing sugars in the scouring solution was determined every 5 min up to 30 min for the lipase/protease/cellulase scouring time and then at 30, 60, 80, 90, 100, 110, and 120 min of the pectinase scouring time. A solution of Ecowet DA with enzyme at 0 min of the scouring time was also tested for reducing sugars and was used as a blank sample. In addition, water

from the process of prewashing greige fabric was tested for reducing sugars as well. The whole test was conducted five times and the test results were averaged.

3.5.2 Galacturonic acid content

The scouring solution was tested for galacturonic acid using UV-Vis spectrophotometry, according to the Nelson-Somogyi method [79], every 5 min up to 30 min for the lipase/protease/cellulase scouring time and then at 30, 60, 80, 90, 100, 110, and 120 min of the pectinase scouring time.

In this test, the determination method of galacturonic acid mostly resembled that of reducing sugars previously shown in section 3.5.1. However, when measuring for sample's light absorption using UV-Vis spectrophotometry, it's needed to measure at wavelength of 500 nm instead. The absorbance was then converted into the concentration of galacturonic acid presented in the scouring solution using information shown in the galacturonic acid calibration curve of Figure 3.2. The test was conducted five times and the test results were averaged.

3.5.3 Amino acids content

The presence of 17 amino acids in the scouring solutions were determined using HPLC, according to the JAOAC (1995) [83], at 30 min of the lipase/protease/cellulase scouring time and at 120 min of the pectinase scouring time. A solution containing Ecowet DA and enzyme was also tested for the existence of amino acids and was used as a blank sample. 17 standard amino acids used in this test were alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionin, phenylalanine, proline, serine, threonine, tyrosine, and valine.

Prior to analysis, the enzymatic scouring solution was freeze-dried to concentrate the solution. Then 10 μl of the internal standard (α-amino butyric acid), 70 μl of AccQ•flour derivatization buffer and 20 μl of AccQ•flour reagent were added and heated at 55°C for 10 min in a heating block to derivatize. Finally, the solution sample was analyzed on the WATERS Alliance 2695 with heater system, and WATERS 2475 Multi λ fluorescence detector (EX:250, EM395 nm). The column was

AccQ•Tag column (dimension 3.9 x 150 mm, particle size 4 μm) and the temperature was controlled at 35±1°C. The sample volume for each test was 5 μl and the eluents consisted of AccQ•Tag Eluent A, acetonitrile, and deionized water.

3.5.4 Protein content

Protein content in the enzymatic scouring solution was determined according to the Lowry assay method [84] using bovine serum albumin (BSA) as a standard and measuring the absorption of the enzymatic scouring solutions (with Ecowet DA), after incubation, at 540 nm using UV-Vis spectrophotometry. In this test, Lowry solution was prepared from three solutions (A, B, and C) in order to detect for the existence of protein from enzyme and that released from cotton into the scouring solution. The concentration and absorbance calibration curve of BSA solution was prepared.

Solution A was prepared by dissolving 100 g of sodium carbonate in 1 lit of 0.5N sodium hydroxide. Solution B was prepared by dissolving 1 g of cupric sulphate pentahydrate in 100 ml of distilled water. Solution C was prepared by dissolving 2 g of potassium tartrate in 100 ml of distilled water. Just before the protein assay, 20 parts of solution A, 1 part of solution B, and 1 part of solution C were mixed to generate the Lowry solution.

Protein assay was initiated by mixing 1 ml of the enzymatic scouring solution (with Ecowet DA) with 1 ml of the Lowry solution. The sample was then incubated for 20 min at room temperature. During that time, 5 ml of 2N Folin-phenol solution was mixed with 50 ml distilled water. After 20 min of incubation, 3 ml of Folin-phenol solution was added. After another 45 min of incubation, the solution was tested for its absorption at 540 nm using UV-Vis spectrophotometry and the absorbance was converted into BSA concentration according to the information shown in Figure 3.5. The test was conducted five times and the test results were averaged.

The BSA calibration curve relating between the concentration of BSA solution and its light absorbance was constructed by preparing various concentrations of BSA solutions (24, 45, 90, 120, 180, 240, and 300 μg/ml) and testing for their light absorbance in the range of 0 to 1 at wavelength 540 nm using UV-Vis spectrophotometry.
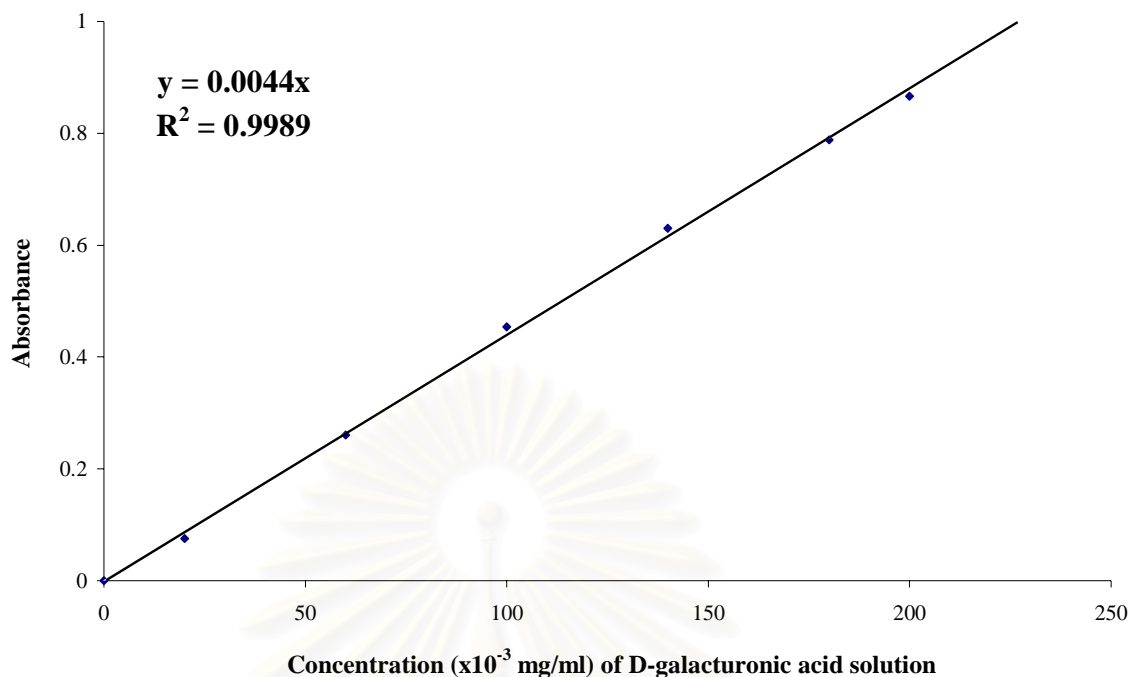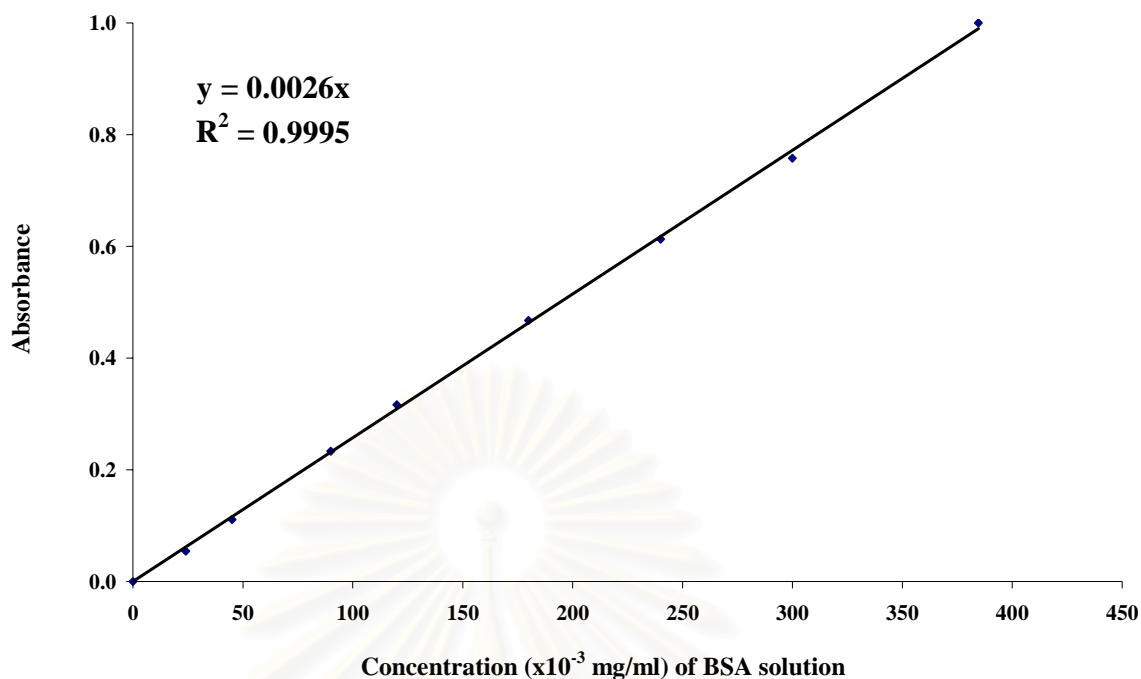
Figure 3.5 Concentration and absorbance calibration curve of BSA standard.

3.6 Analysis of fabric

Greige, prewashed and scoured fabrics were tested for impurities originally existing on the greige fabric and those remaining on the fabric after prewashing and scouring, respectively. Impurities being studies were proteins, anionic components including pectin, extractable substances and waxes, and fatty acids. In addition, these fabrics were also tested for various properties such as weight, water absorbency, weight loss after prewashing and scouring, whiteness and yellowness, bursting strength, crystallinity index, and appearance under SEM (Scanning Electron Microscope). Details of the test methods are described as follows.

3.6.1 Protein content

Protein content of the fabric was analyzed using the Kjehdahl method, according to the AOAC official method 3.5.09 (2000) [85], in order to determine the residual protein on the scoured fabric. This method contained 2 steps: the digestion step and the distillation and titration step. First, a concentrated sulphuric acid was used to digest and convert combined nitrogen in fabric-protein into ammonium sulphate. This acid solution was then adjusted to an alkaline condition and ammonia

was liberated. The nitrogen content was determined by titration with a standard acid solution. The protein content was then obtained by multiplying the percentage of nitrogen in an aliquot of fiber by an empirical factor of 6.25. Digestion and distillation/titration steps are described in detail as follows.

In the digestion step, 20 ml of concentrated sulphuric acid was added into a Kjehdahl tube containing 2 g of fabric sample and 5 g of a catalyst selenium mixture in order to completely digest proteins in fabric in about an hour as the mixture turned into a clear solution or showed a light green color. The solution was cooled to room temperature and transferred to a distillation unit in the distillation and titration step.

In the distillation and titration step, 50 ml of distilled water, 75 ml of 35% sodium hydroxide and 50 ml of 4% boric acid were added into the solution and then the solution was distilled for 5 min. After that the solution was cooled to room temperature and was removed from the distillation unit. Then 50 ml of 4% boric acid and 5 drops of a mixed indicator consisting of 0.125 g of methyl red and 0.0825 g of methylene blue in 100 ml of 90% ethanol were added into the solution and the solution was titrated with 0.1 N of hydrochloric acid. The nitrogen and the protein contents in the fabric sample were calculated according to the equations 3.1 and 3.2. The test was conducted two times for each sample and the test results were averaged.

$$\text{Nitrogen content (\%)} = \frac{\textit{HCl titration volume (ml) X HCl concentration (N) X 14 X 100}}{\textit{Fabric weight (g)}} \quad \text{(Equation 3.1)}$$

$$\text{Protein content (\%)} = \textit{6.25 X Nitrogen content (\%)} \quad \text{(Equation 3.2)}$$

3.6.2 Content of pectins and other anionic components

Fabric sample was tested for the presence of pectins and other anionic components by measuring the absorption of methylene blue on the fabric [86]. This method is based on the interaction between the cationic dye of methylene blue and the anionic components including pectins on the fabric surface. The amount of dye adsorbed by the sample is proportional to the number of anionic components present, including pectins. The methylene blue standard curve relating between its concentration and light absorbance at 662 nm was also prepared for the test.

The measurement of methylene blue content on the fabric was initiated by immersing the fabric sample in a solution containing 0.5 g/l methylene blue in a shaker bath at a liquor ratio of 30:1 at 70°C for 8 hr. After dyeing, the remaining dye solution was diluted 40 times with distilled water and the diluted solution was tested for its absorption at 662 nm using UV-Vis spectrophotometry. The absorbance was then converted into the concentration of methylene blue presented in the solution according to the concentration and absorbance calibration curve of standard methylene blue solution shown in Figure 3.6. The methylene blue concentration found in the remaining dye solution was used to calculate the dye concentration (content) on the fabric by subtracting it from the dye concentration before dyeing. The test was conducted three times for each sample and the test results were averaged.
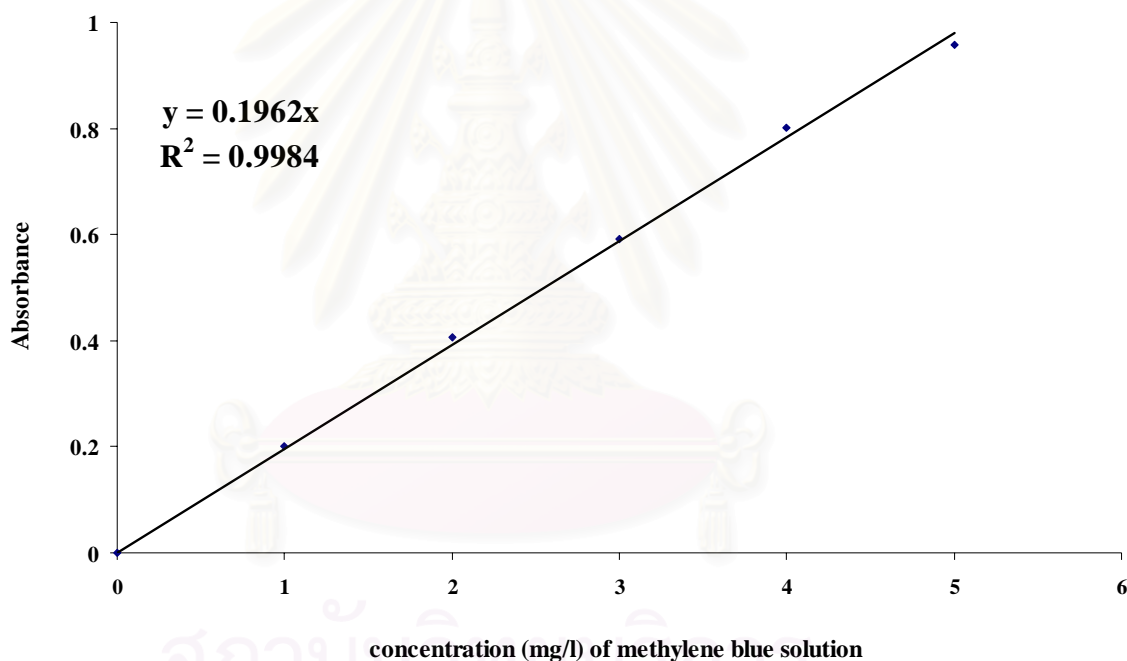
Figure 3.6   Concentration and absorbance calibration curve of methylene blue standard.

The methylene blue calibration curve relating between the concentration of methylene blue solution and it light absorbance was constructed by preparing various concentrations of methylene blue solutions (1, 2, 3, 4, and 5 mg/l) and testing for their light absorbance in the range of 0 to 1 at wavelength 662 nm using UV-Vis spectrophotometry.

3.6.3 Contents of extractable substances and waxes

Extractable content and wax content on the fabric were determined according to the AATCC test method 97 (1999) [87] in order to determine the residual impurities consisting of water soluble substances, starch, and waxes on the scoured fabric. Extractable content on the fabric was measured from the fabric weight loss after a 3-step extraction with water, α-amylase enzyme, and trichloroethylene, respectively. Wax content on the fabric was measured from the fabric weight loss after extraction with trichloroethylene. Details of the test are described as three extracting steps as follows.

In the first step of water extraction, the fabric was heated and weighed at 105°C using an Infrared moisture balance. Then it was immersed in 200 ml of distilled water at 82 ± 3°C for 2 hr. After that, it was rinsed twice with 25 ml of distilled water in a Buchner funnel secured in a filtration flask, air dried, and weighed again.

In the second step of enzymatic extraction, the fabric was heated and weighed using an Infrared moisture balance and then immersed in 200 ml of 4 g/l amylase at pH 6.5, 75°C for 1 hr. It was rinsed with 100 ml of distilled water for 10 successive times at 82 ± 3°C in a Buchner funnel secured in a filtration flask, air dried, and weighed again.

In the last step of extraction, the fabric was heated and weighed using an Infrared moisture balance and was then extracted with 200 ml of trichloroethylene for 16 times in a Soxhlet extractor. The percentage of the extractable substances was calculated from the difference of the fabric weights at 105°C before and after each extracting step as shown in equation 3.3.

$$E = \frac{[B - A]}{B} \times 100 \qquad \text{(Equation 3.3)}$$

where

E is extractable substances on the fabric, %

B is fabric weight at 105°C before a particular extraction, g

A is fabric weight at 105°C after a particular extraction, g

This experiment was conducted three times and the test results were averaged.

3.6.4 Fatty acids content

For this work, the existence of 18 fatty acids (C_8-C_{24}) on the fabric was determined by GC (gas chromatography) in order to measure the residual waxes (in terms of fatty acids) on the scoured fabric. The test was conducted by extracting fatty acids from the fabric with trichloroethylene then drying and measuring for the contents of methyl ester of fatty acids according to the AOAC official method 47.3.43 (2000) [88]. Details of the test method are described as follows.

First, the fabric was extracted with trichloroethylene following the trichloroethylene procedure shown in section 3.6.3. Then 2.5 ml of the extracted compound (consisting of various fatty acids) and 1.5 ml of 0.5 N methanolic sodium hydroxide solution was added into a glass tube containing an internal standard (methyl ester standard) in order to generate methyl ester of fatty acids when heated at 100°C for 5 min. It was then cooled to room temperature. After that 2 ml of boron trifluoride in methanol was added in an atmosphere of nitrogen gas and the sample was heated at 100°C for 30 min, then cooled to 30 - 40°C. 1 ml of isooctane was added in an atmosphere of nitrogen gas and the sample was shaken for 30 second at warm. Immediately 5 ml of saturated sodium chloride was added and mixed thoroughly in an atmosphere of nitrogen gas. The solution was cooled to room temperature in order to separate the isooctane layer from the aqueous lower phase. Finally, 1 μl of the extracted isooctane was analyzed in a GC-14A Shimadzu, C-R4A CHROMATOPAC Shimadzu integrator. The column was a capillary column, CBP20M (dimension 25 m x 0.25 mm). The initial temperature was set at 170°C with nitrogen gas as a carrier gas at 1 kg/cm^2, hydrogen flow at 0.6 kg/cm^2, and air flow at 0.5 kg/cm^2. The column was ramped at 1°C/min from 170°C to 225°C. 18 standard fatty acids used in this study were arachidic acid, behenic acid, caprylic acid, capric acid, cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA), heptadecanoic acid, heneicosanoic acid, lauric acid, lignoceric acid, linoleic acid, myristic acid, oleic acid, palmitic acid, pentadecanoic acid, stearic acid, tricosanoic acid, tridecanoic acid, and undecanoic acid

3.6.5 Fabric weight

Greige fabric weight was determined by cutting the fabric with an 11.3 cm diameter standard circular cutter and weighing the cut sample. Three pieces were cut and weighed, and the average fabric weight in gram per 100 cm^2 area was calculated.

3.6.6 Water absorbency of fabric

After a complete scouring, the fabric is required to absorb water immediately or within 3 seconds and to absorb evenly all over the fabric. The absorbency test was conducted according to the AATCC Test Method 79-2000 "Absorbency of Bleached Textiles [89]". A drop of water is allowed to fall onto the surface of the test specimen. The time required for the specular reflection of water drop to disappear is measured and recorded as wetting time. The fabric that absorbs water within three seconds or less and absorbs evenly all over the fabric is generally considered to have an adequate absorbency. The test was conducted five times for each sample.

3.6.7 Fabric weight loss

Fabric weight loss was measured in order to determine for the amount of materials being removed from the fabric by means of scouring. It was determined by weighing and drying the fabric before and after treatment using an Infrared moisture balance. The test was conducted three times for each sample and the average was calculated.

3.6.8 Fabric whiteness and yellowness

Whiteness and yellowness of the fabric was measured based on the ASTM D1925 [90] using the MacBeth Color-Eye 7000 reflectance spectrophotometer. Each sample was measured five times and data were averaged.

3.6.9 Fabric strength

Fabric was tested for bursting strength according to the JIS L1018 (1999) [91] using a Mullen type bursting strength tester in order to determine the strength of the scoured fabric. Bursting strength is the maximum pressure (kg/cm^2) of the fluid that pushed the test fabric until break down. Details of the method are described as

follows. Ten test pieces of approximately 15 cm x 15 cm were prepared for each sample and each piece was clamped on the testing grip with its surface upward. A uniform force was applied upward to a rubber diagram located under the sample in order to push the sample to burst. The bursting strength of the fabric was calculated from subtracting the burst force with the correction factor of the rubber diaphragm while this factor was defined as the residual force on the clamp when the fabric was removed after breakage (see equation 3.4).

$$\text{Bursting strength (kg/cm}^2) \ = \ A - B \qquad \text{(Equation 3.4)}$$

where

A is fabric burst force (kg/cm^2)

B is residual force on the clamp when the fabric was removed (kg/cm^2)

3.6.10 Crystallinity index

A JEOL diffractometer with Cu radiation was used to investigate the crystallinity index of the fabric at a scanning condition of 30 Kv and 40 mA, at a speed of 0.02°, 2θ/sec, from 2θ = 8 to 2θ = 30 [92]. Intensities of amorphous and crystalline peaks at diffraction angles 2θ equal to 18 and 22.8 were measured respectively. Crystallinity index was finally calculated from the following equation 3.7.

$$CrI = \frac{(I_{002} - I_{AM})}{I_{002}} \times 100 \qquad \text{(Equation 3.5)}$$

Where

CrI   is crystallinity index

I_{002} is maximum intensity of crystalline peak

I_{AM} is base line of amorphous peak.

3.6.11 Surface appearance

Fabric was examined for the appearance of the fiber surface using the Scanning Electron Microscope (SEM), Model JSM-5410LV, JEOL. The sample was prepared by mounting on SEM stubs, and coating with gold in the nanometer level by a sputter-coater. The gold was inonized during scanning using the Argon gas in the vacuum condition. Finally, the sample was observed under the 90, 2,000 and 3,500 X magnifications.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Properties of greige and prewashed cotton fabrics

Before scouring, greige cotton fabric was prewashed in boiling water in order to remove water soluble substances located on the cotton surface.

Greige and prewashed cotton fabrics were tested for various properties according to the test procedures outlined in section 3.6. Their properties are shown in Table 4.1.

Table 4.1 Properties of greige and prewashed cotton fabrics.

Property	Reference	Greige fabric	Prewashed fabric
Protein content (%)	1.3	1.1226	0.8423
Methylene blue content (g/kg fabric)		8.72	6.83
Anionic component including pectin (%)	0.9	0.87	0.68
Extractable content (%)	2.3	2.3897	0.7182
Wax content (%)	0.6	0.3095	0.3086
Weight loss (%)		-	2.69
Crystallinity index	70	75.4	75.4
Bursting strength (kg/cm^2)		6.2	6.1
Whiteness index		undetectable	14.0
Yellowness index		31.9	23.2
Water absorbency		none	none

Previously, a reference [9] has shown that the content of cotton impurities presented in the cuticle of raw cotton, typically consists of 1.3% proteins, 0.9% pectins, 0.6% waxes, and 2.3% other substances with an overall content of 5.1%. Crystallinity index of cotton is generally found at 70%.

In this work, our greige cotton fabric was tested and found that its impurities consisted of 1.1% proteins, 0.9% anionic components including pectins (methylene

blue content), 0.3% waxes and 2.4% total extractable substances (see Table 4.1). These amounts of pectins and waxes have made greige cotton hydrophobic and thus a scouring process was required for greige cotton in order to remove these impurities and to provide a sufficient absorbency to cotton. Crystallinity index of the greige fabric was found at 75.4 and its bursting strength was at 6.2 kg/cm^2. Greige fabric was in light yellow with a yellowness index of 32.

Prewashed fabric was also tested for various properties and found that its impurities consisted of 0.8% proteins, 0.7% anionic components including pectins (methylene blue content), 0.3% waxes and 0.7% total extractable substances (see Table 4.1). Compared the content of impurities on prewashed fabric with that on greige fabric, it shows that the prewashed fabric contained lower contents of proteins, anionic components, and extractable substances. Prewashing the greige fabric with boiling water could partially help removing these impurities from the fabric. On the contrary, waxes remained intact with the fabric after prewashing. Prewashed fabric lost 2.7% of its weight after prewashing and this might be due to the loss of water soluble substances and other hydrolyzed components including natural coloring matters. It still maintained its hydrophobic property and needed to be scoured to improve its absorbency.

Both greige and prewashed fabrics had nearly the same crystallinity index and bursting strength while their whiteness and yellowness indices were different. Prewashing the fabric could increase its whiteness and decrease yellowness due to the loss of natural yellowish colorants. On the other hand, the boiling water in prewashing process did not affect the bulk property of the fabric such as the physical strength and the crystalline.

4.2 Enzyme activity

4.2.1 Effect of wetting agent on enzyme

Prior to determining the enzyme action, effect of the nonionic wetting agent on enzyme was studied by measuring the enzyme activity (on the specific and unspecific substrates) in the presence and in the absence of the nonionic wetting agent according to the test procedures outlined in section 3.3. Table 4.2 showed insignificant different of enzyme activity in both systems. Results from this study

have led to a conclusion saying that the nonionic wetting agent used in this work had no action on enzyme.

Table 4.2 Enzyme activity in the presence and in the absence of the nonionic wetting agent.

Enzyme	Activity on Substrate (with wetting agent) (U/ml)				Activity on Substrate (without wetting agent) (U/ml)			
	Polygalacturonic acid	Filter paper	pNPP*	Casein	Polygalacturonic acid	Filter paper	pNPP*	Casein
Pectinase	480	10	0	0	486	11	0	0
Lipase	0	0	37	19	0	0	39	18
Protease	0	0	0	252	0	0	0	252
Cellulase	365	17	0	0	363	15	0	0

*pNPP = p-nitrophenylpalmitate

4.2.2 Enzyme activity on specific and unspecific substrates

All four enzymes including pectinase, lipase, protease, and cellulase were tested for their activities on specific and unspecific substrates in the presence of the nonionic wetting agent and results are shown in Table 4.2. Pectinase showed an activity of 480 U/ml towards the polygalacturonic acid (pectins), an activity of 10 U/ml towards the filter paper (cellulose), and no activity on either pNPP (fat) or casein (protein). Cellulase showed an activity of 365 U/ml towards the polygalacturonic acid, an activity of 17 U/ml towards the filter paper, and no activity on pNPP and casein. These results indicate that both enzymes (pectinase and cellulase) can act on cellulose and a composition of pectin or polygalacturonic acid. This may be because both cellulose and pectins have a very similar chemical structure with glycosidic linkage oxygen between glucose rings and pyranose rings respectively (see Figures 2.2 and 2.4). Pectinase and cellulase may be capable of randomly catalyzing the hydrolysis of pectins and cellulose at the α-1,4 and β-1,4 glycosidic linkage oxygen between the pyranose rings in polygalacturonic acid and the glucose rings in cellulose resulting in the production of galacturonic acid and reducing sugars. Pectinase showed a higher activity towards polygalacturonic acid than cellulase while cellulase showed a higher activity towards filter paper than pectinase. Protease only

showed a specific activity of 252 U/ml on casein and played no action to other unspecific substrates. Lipase used in this experiment contained amylase and protease and thus it showed higher activity on pNPP and lower activity on casein.

Results from Table 4.2 have indicated that pectinase and cellulase not only showed activities on their specific substrates, they also showed activities on unspecific substrates which having similar chemical structure such as pectins and cellulose. On the contrary, lipase and protease showed activities only on specific substrates.

4.2.3 Enzyme activity before and after scouring

All enzymes in scouring solutions were determined for their activities both before and after scouring and results are shown in Table 4.3.

Table 4.3   Enzyme activity in scouring solution before and after scouring.

	Enzyme Activity (U/ml)					
	PEC	L	CL	P	CP	CLP
Enzyme solution (before)	480	37	17	252	17	17
Enzyme solution (after)	311	35	16	247	16	16
Enzyme solution + fabric (after)	210	30	14	243	13	12

PEC = pectinase	P	= protease
L = lipase	CP	= cellulase after protease
CL = cellulase after lipase	CLP	= cellulase after lipase/protease

First of all, a comparison of enzyme activity between the samples of enzyme solutions in the absence of cotton fabric before and after passing the scouring conditions was determined. It was found that pectinase lost 35% of its activity when the pectinase solution was left in the scouring condition (pH 4, 37°C, 2 hr), lipase lost 5% (pH 8, 37°C, 30 min), protease lost 2% (pH 7, 37°C, 30 min), and cellulase lost 6% (pH 4.5, 40°C, 30 min). This loss of enzyme activity was purely due to a long period of heating in each enzymatic scouring (120 min for pectinase scouring and 30 min for lipase/protease/cellulase scouring).

After scouring of cotton fabric, the fabric was removed from the scouring solution and the solution was analyzed for enzyme activity comparing with its activity before scouring. Results are also shown in Table 4.3. An activity loss of 56% was found in the two-hour pectinase scouring, 19% in the half-an-hour lipase scouring, 4% in the half-an-hour protease scouring, and 24% in the half-an-hour cellulase scouring. The enzyme activity tended to decrease as the scouring time increased. The decrease of enzyme activity could involve the loss of the enzyme through adsorbing onto the fabric surface as well as the presence of an interference by impurities in the scouring solution. Impurities found in the scouring solutions after scouring could have come from the hydrolyses of pectins, fats, proteins, and cellulose into galacturonic acid, fatty acids, amino acids, and reducing sugars respectively, as well as could be some other substances such as cotton's natural coloring matters and dirt. These impurities could involve in destroying the enzyme structure and decreasing the enzyme activity.

4.3 Analysis of scouring solution

To study the hydrolyzed products from scouring of cotton, the scouring solution from each scouring process was analyzed for reducing sugars, galacturonic acid, amino acids, and proteins after various scouring time periods. Results are shown as follows.

4.3.1 Reducing sugars content

Prior to enzymatic scouring, greige cotton fabric was prewashed in boiling water for 10 min to remove dirt. This prewashed water was then tested for reducing sugars and found only a small amount of 0.8 μg/ml$\cdot$g cotton. Boiling water may initially hydrolyze cellulose into reducing sugars dissolving in water.

Prewashed fabric was then scoured with pectinase or lipase/protease/cellulase and the scouring solution was analyzed for the presence of reducing sugars at various scouring times. The results are shown in Table 4.4 and Figures 4.1 and 4.2.

Table 4.4   Presence of reducing sugars in scouring solutions at various scouring times.

Scouring Time (min)	Reducing sugars (μg/ml.g cotton)					
	PEC	CL	P	CP	LP	CLP
0	0	0	0	0	0	0
5		11.96	13.19	17.01	2.84	15.46
10		19.35	20.21	22.88	4.52	22.47
15		25.62	22.52	27.32	5.79	25.23
20		30.53	25.34	32.07	6.36	30.72
25		36.14	26.73	39.52	10.06	31.87
30	647.37	39.57	27.37	44.86	10.98	42.35
60	754.48					
80	852.97					
90	900.89					
100	919.23					
110	937.10					
120	985.60					

Note: Lipase scouring did not produce reducing sugars.

Table 4.4 and Figure 4.1 indicate that the one-step two-hour pectinase scouring process produced the highest amount of reducing sugars at 985.60 μg/ml.g cotton, compared to other two-step scouring processes.

The pectinase scouring process in this work generated a high amount of reducing sugars probably from the hydrolysis of cellulose. According to the results of the enzyme activity shown in the section 4.2.2, pectinase acted on both pectins and cellulose to different degrees and therefore cotton scouring with pectinase can produce reducing sugars (from the hydrolysis of cellulose) as found in this work. In addition, pectins and cellulose may connect to each other in cotton fiber. Removing the pectin part via the pectinase scouring process may cause the removal of the cellulose part (as reducing sugars) attached to the pectin and vice versa.

Figure 4.1 shows that there was a rapid increase of reducing sugars content during the first 40 min of the process and a slow increase took place later till the end possibly due to a suppression of the pectinase activity from an increase of impurities in the scouring solution.
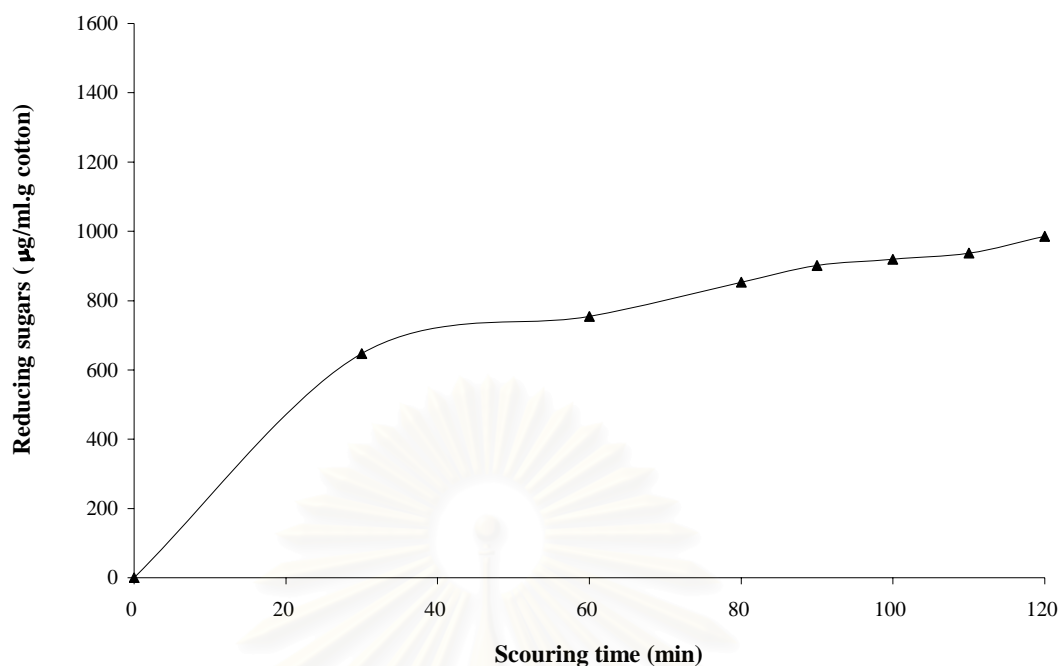
Figure 4.1 Presence of reducing sugars in pectinase scouring solution at various scouring times.

Table 4.4 also illustrates the presence of reducing sugars in the lipase/protease/cellulase scouring solutions at various scouring times. Table 4.4 and Figure 4.2 indicate that the two-step scouring with lipase/protease/cellulase produced lower amount of reducing sugars than the one-step scouring with pectinase, and the two-step protease then cellulase scouring processes produced the maximum reducing sugars at 72.23 μg/ml•g cotton, compared to other two-step scouring processes. The first step of protease scouring produced 27.37 μg/ml•g cotton and the second step of cellulase scouring produced 44.86 μg/ml•g cotton. The test for the protease activity on substrates has previously shown that this enzyme had an effect only on a specific substrate like protein casein. Its action on cellulose (producing reducing sugars) may be because the protein is attached to cellulose in cotton fiber. Removing the protein as amino acids component may cause the removal of cellulose as reducing sugars. In the case of cellulase scouring, cellulose can easily be hydrolyzed into reducing sugars by the cellulase catalysis as can be seen in Table 4.4 and Figure 4.2 that the production of reducing sugars by cellulase scouring was always higher than that by lipase and/or protease scouring.

Results from Table 4.4 and Figure 4.2 also show that the lipase scouring did not produce reducing sugars which means that lipase had no action on unspecific substrates such as cellulose. The test for the lipase activity on substrates has previously indicated that this enzyme contained residual protease and showed high activity on fat, low activity on protein, and no activity on cellulose. Cellulose and fat may not connect to each other as complex structures in cotton fiber as removing the fat component by lipase scouring did not cause the removal of cellulose as reducing sugars. Instead, the lipase then cellulase scouring produced reducing sugars at 39.57 µg/ml·g cotton by the cellulase action.
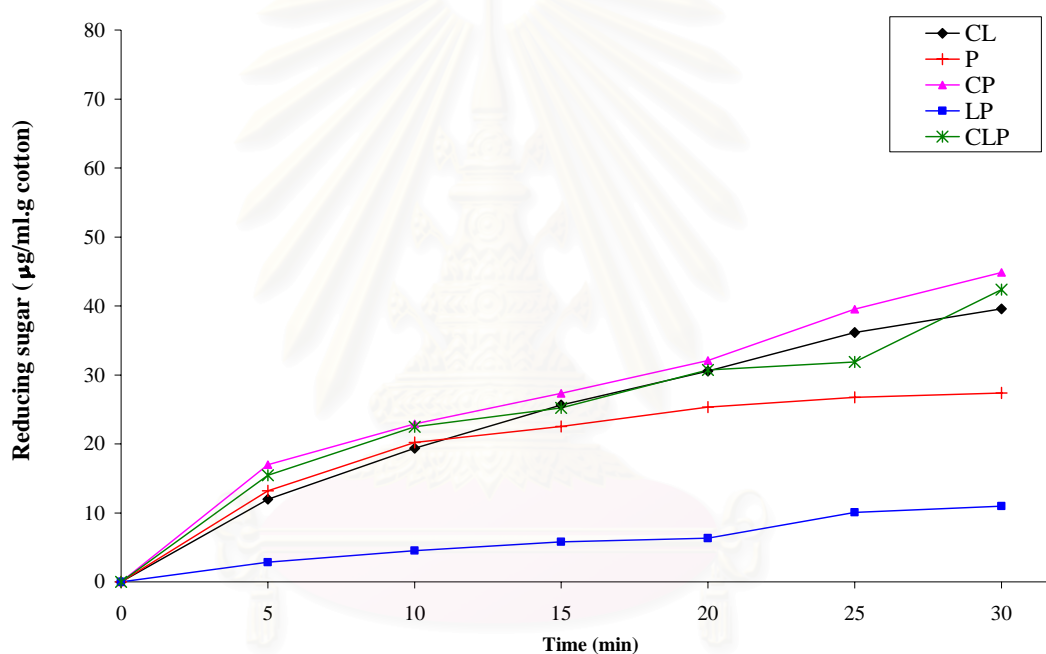
Figure 4.2   Presence of reducing sugars in lipase/protease/cellulase scouring solutions at various scouring times.

The two-step scouring process with a mixture of lipase and protease then cellulase totally produced reducing sugars at 53.33 µg/ml·g cotton. The first step of lipase/protease scouring produced 10.98 µg/ml·g cotton and the second step of cellulase scouring produced 42.35 µg/ml·g cotton. In this two-step scouring process, only protease and cellulase acted on cellulose while lipase took no part of the action on cellulose. The indirect action of protease on cellulose has been clarified previously as well as the direct action of cellulase on cellulose.

In the case of the two-step scouring, it has been shown that scouring with lipase and/or protease was required in the first step before scouring with cellulase in the second step. In the other words, cellulase would not show its influence on cotton unless protease and/or lipase were applied first on cotton. It would be necessary to firstly removed waxes, fats, proteins, and pectins before cellulase could reach the cellulose layers.

Table 4.4 and Figures 4.1 and 4.2 show that the one-step scouring with pectinase produced several times higher content of reducing sugars than the two-step scouring with lipase/protease/cellulase. Different amounts of reducing sugars were produced in the various enzymatic scouring processes. However, all of the scouring processes proved effective because all scoured fabrics showed an immediate improvement in wetting with water drops while the untreated greige fabric and the prewashed fabric showed a hydrophobic property.

4.3.1.1 Kinetic empirical equation for the presence of reducing sugars

The enzymatic scouring of cotton fabric is classified as a heterogeneous reaction system with cotton fabric being a solid substrate and the enzymatic scouring solution being an aqueous phase. The classic enzymatic reaction kinetic equation-Michaelis-Menten model cannot be used in this case because it is only suitable for a homogeneous reaction system. To adjust our experimental data, the Ghose-Walseth kinetic empirical equation was used in this study [26], expressed in equation 4.1. The k and m parameters were calculated using non-linear regression (NLR) in SPSS 12.0 for windows. The experimental profiles in Figures 4.1 and 4.2 showing the presence of reducing sugars in the scouring solution at various scouring times were adjusted based on equation 4.1. Their new profiles are shown in Figures 4.3 - 4.5 respectively.

$$P = kt^{m}$$

(Equation 4.1)

where

P is product content at time t (µg/ml·g cotton)

t is scouring time (min)

k is kinetic constant

m is characteristic parameter of the substrate-enzyme system

Figure 4.3 Ghose-Walseth kinetic model of reducing sugars presented in pectinase scouring solution at various scouring times.

Figure 4.4 Ghose-Walseth kinetic model of reducing sugars presented in lipase/protease/cellulase scouring solutions at various scouring times.

Figure 4.5   Ghose-Walseth kinetic model of reducing sugars presented in cellulase
               scouring solution (C*) at various scouring times.

Based on the *k* and *m* parameters calculated by the NLR, six kinetic empirical equations were established as kinetic models of reducing sugars content in the enzymatic scouring solutions at various scouring times shown in Table 4.5 along with the parameter r^2 (correlation coefficient) closed to 1. These r^2 parameters showed the reliability and the applicability of the kinetic models to the experimental data, and also indicated their validity to make quantitative prediction of the product during the enzymatic scouring process.

Seven equations proposed in Table 4.5 were used to calculate for the contents of reducing sugars in the scouring solutions at various scouring times shown in Table 4.6. Some insignificant differences between the data shown in Table 4.6 (kinetic model) and in Table 4.4 (experimental result) are seen as well as those differences between Figures 4.3 and 4.1, and between Figures 4.4, 4.5 and 4.2.

Table 4.5 Ghose-Walseth kinetic model of the production of reducing sugars from pectinase and lipase/protease/cellulase scouring processes.

Enzyme	Production of reducing sugars	
	Equation	r^2
PEC	$P = 218.1217t^{0.3122}$	0.9893
CL	$P = 4.2276\ t^{0.6613}$	0.9549
P	$P = 8.1310\ t^{0.3688}$	0.9371
CP	$P = 5.8438\ t^{0.5896}$	0.9467
LP	$P = 0.6569\ t^{0.8364}$	0.9205
CLP	$P = 5.9671\ t^{0.5519}$	0.9652
C*	$P = 5.2896t^{0.6010}$	0.9771

Note: kinetic equations from CL, CP, and CLP were combined into one equation C*.

Table 4.6 Presence of reducing sugars in scouring solutions at various scouring times according to the Ghose-Walseth kinetic model.

Scouring Time (min)	Reducing sugars (µg/ml•g cotton)						
	PEC	CL	P	CP	LP	CLP	C*
0	0	0	0	0	0	0	0
5		12.26	14.72	15.09	2.52	14.51	13.92
10		19.38	19.01	22.71	4.51	21.26	21.11
15		25.34	22.07	28.85	6.33	26.60	26.93
20		30.65	24.55	34.18	8.05	31.17	32.01
25		35.53	26.65	38.99	9.70	35.26	36.61
30	630.75	40.08	28.50	43.41	11.30	38.99	40.85
60	783.14						
80	856.73						
90	888.82						
100	918.54						
110	946.28						
120	972.34						

Note: kinetic equations from CL, CP, and CLP were combined into one equation C*.

4.3.1.2 Production rate of reducing sugars

      Previously, the presence of reducing sugars in the scouring solutions at various scouring times was shown in experimental data and in kinetic equations. In this section, those experimental data were used to calculate for the production rates of reducing sugars from the scouring processes.

      The rate of reaction, the reaction velocity (υ) or in this case "the production rate" is a quantitative expression of the change in concentration of substrate (s) or product (p) with time (t). In this work, the production rates of reducing sugars were calculated using equation 4.2. Results are shown in Table 4.7 and Figures 4.6 and 4.7.

$$\upsilon = -\frac{dS}{dt} = \frac{dP}{dt}$$
        (Equation 4.2)

Table 4.7 Production rates of reducing sugars presented in scouring solutions at various scouring times.

Scouring Time (min)	Reducing sugars (μg/ml$\cdot$g cotton$\cdot$min)					
	PEC	CL	P	CP	LP	CLP
5		2.39	2.64	3.40	0.57	3.09
10		1.93	2.02	2.29	0.45	2.25
15		1.71	1.50	1.82	0.39	1.68
20		1.53	1.27	1.60	0.32	1.54
25		1.45	1.07	1.58	0.40	1.27
30	21.58	1.32	0.91	1.50	0.37	1.41
60	12.57					
80	10.66					
90	10.01					
100	9.19					
110	8.52					
120	8.21					
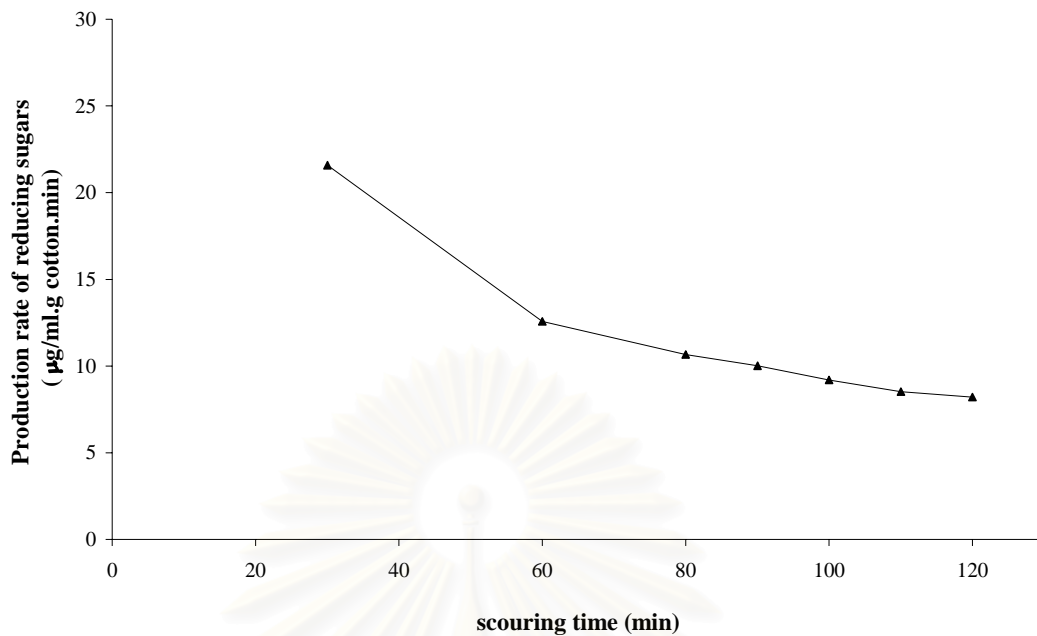
Figure 4.6   Production rates of reducing sugars presented in scouring solutions at various scouring times from the pectinase scouring process.
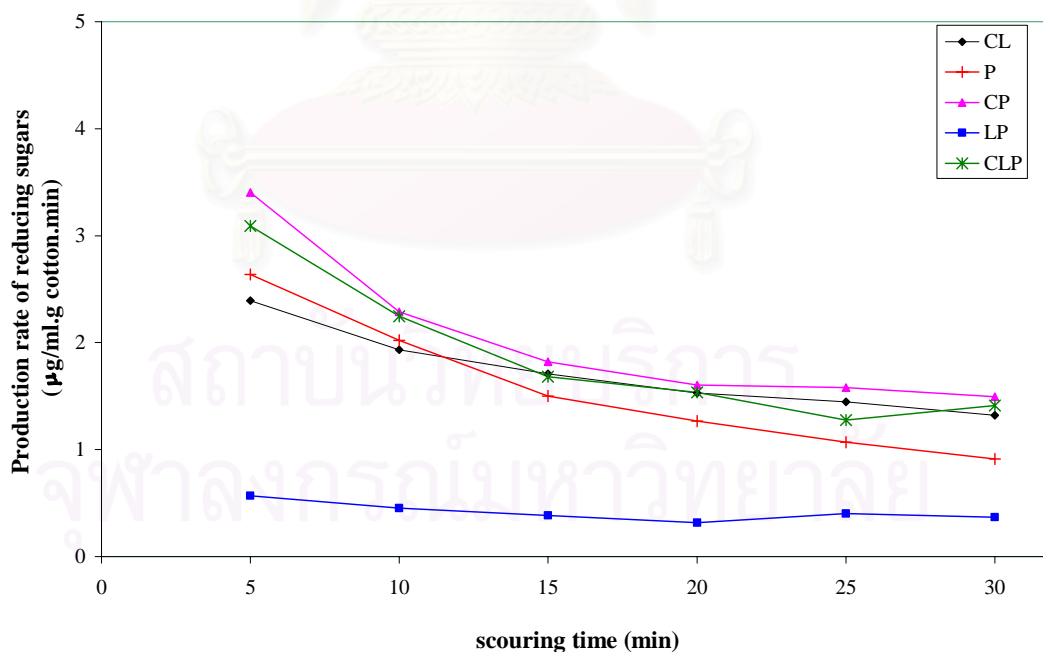
Figure 4.7   Production rates of reducing sugars presented in scouring solutions at various scouring times from the lipase/protease/cellulase scouring processes.

Table 4.7 and Figures 4.6 and 4.7 illustrate the production rates of reducing sugars presented in the scouring solutions at various scouring times, based on experimental data shown in Table 4.4 and Figures 4.1 and 4.2. Data indicated that production rate from the pectinase scouring process was several times higher than those from the lipase/protease/cellulase scouring processes since the beginning to the end of the process. In this work, the amount of pectinase required for complete scouring was 10 times higher than that of other enzymes. This increase could help generate reducing sugars at a much higher level than from the lipase/protease/cellulase scouring processes. Both Figures 4.6 and 4.7 show a decrease of the production rate with scouring time in a quadratic relationship. A rapid drop of the production rate is found after the first half of the total scouring time (15 min for the lipase/protease/cellulase scouring processes and 60-80 min for the pectinase scouring process). This drop may be due to a suppression of the enzyme activity from an increase of impurities in the scouring solutions. The production rate of the second half of the scouring time shows a slow decrease to plateau. Figure 4.7 shows a constant production rate over time for scouring with lipase and protease mixed enzymes, while other scouring processes show the original decreasing trend. It has been shown previously that lipase scouring did not produce reducing sugars and therefore only protease would influence the production rate of reducing sugars from the lipase/protease scouring process. The amount of protease used in the lipase/protease scouring process was half of other enzymes used in the lipase and the protease scouring processes and this could affect the production rate of reducing sugars from the lipase/protease scouring process, showing the lowest constant production rate. Figure 4.7 also shows that the cellulase scouring process generated reducing sugars at the highest rate during the process and this is because reducing sugars are direct products from the cellulose hydrolysis using cellulase.

4.3.2 Galacturonic acid content

Before scouring, greige cotton fabric was prewashed in boiling water for 10 min. This prewashed water was then tested for galacturonic acid and found a small amount of 1.5 μg/ml•g cotton. Boiling water may initially hydrolyze polygalacturonic acid in pectins into galacturonic acid dissolving in water.

Prewashed fabric was then scoured with pectinase or lipase/protease/cellulase and the scouring solution was analyzed for the presence of galacturonic acid at various scouring times. The results are shown in Table 4.8 and Figures 4.8 and 4.9.

Table 4.8　Presence of galacturonic acid in scouring solutions at various scouring times.

Scouring Time (min)	Galacturonic acid (μg/ml•g cotton)					
	PEC	CL	P	CP	LP	CLP
0	0	0	0	0	0	0
5		19.28	22.71	24.46	4.35	30.31
10		35.12	34.48	38.73	6.71	44.59
15		45.38	39.89	48.46	10.72	51.39
20		54.31	44.63	54.79	11.80	56.41
25		61.81	46.61	66.76	14.62	58.38
30	1156.19	66.38	47.07	70.49	15.83	75.45
60	1468.31					
80	1506.08					
90	1531.90					
100	1532.03					
110	1556.81					
120	1597.81					

Note: Lipase scouring did not produce galacturonic acid.
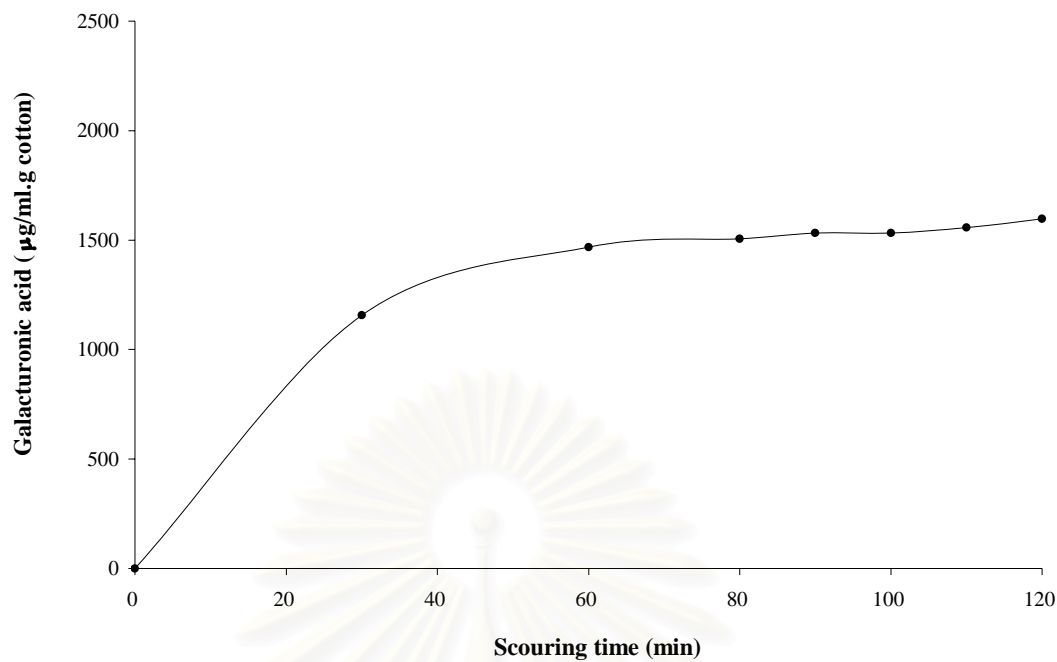
Figure 4.8 Presence of galacturonic acid presented in pectinase scouring solutions at various scouring times.
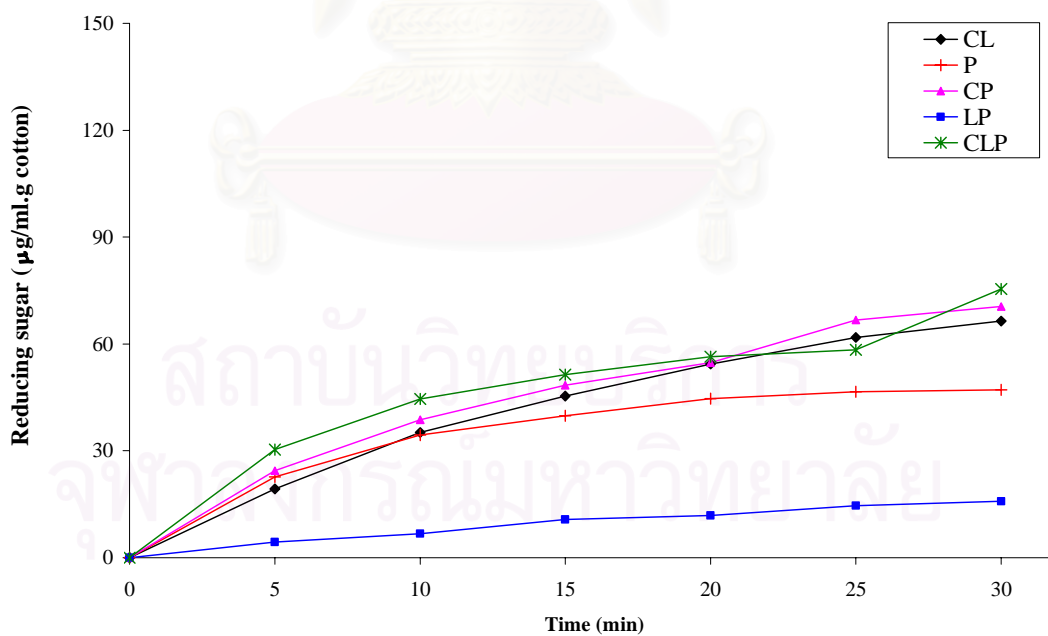
Figure 4.9 Presence of galacturonic acid in lipase/protease/cellulase scouring solutions at various scouring times.

Table 4.8 and Figure 4.8 indicate that the one-step two-hour pectinase scouring process produced the highest amount of galacturonic acid at 1,597.8 µg/ml•g cotton, compared to other two-step scouring processes. Generally, pectinase specifically catalyzes the hydrolysis of pectins found on cotton by transforming the polygalacturonic acid in pectins into galacturonic acid. Therefore, it is normal to find a high content of galacturonic acid in the pectinase scouring solution. Furthermore, the amount of pectinase required for complete scouring in this work was 10 times higher than that of other enzymes. This increase could help produce galacturonic acid at a much higher level than from the lipase/protease/cellulase scouring processes.

Figure 4.8 shows that there was a rapid increase of galacturonic acid content during the first 40 min of the process and a slow increase to plateau at the end took place with the same reason to Figure 4.1, the production of reducing sugars. When comparing the production of reducing sugars (Table 4.4 and Figure 4.1) to the production of galacturonic acid (Table 4.8 and Figure 4.8) from the pectinase scouring process, it can be seen that the production of galacturonic acid was approximately twice as high as that of the reducing sugars.

Table 4.8 and Figure 4.9 illustrate the presence of galacturonic acid in the lipase/protease/cellulase scouring solutions at various scouring times which is several times lower than that in the pectinase scouring solution. The two-step protease then cellulase scouring process produced the maximum galacturonic acid at 117.56 µg/ml•g cotton, compared to other two-step scouring processes. The test for the protease activity on substrates has previously shown that this enzyme had an effect only on a specific substrate like protein casein. Its action on pectins may be because the protein and pectin components are built-in complex structures and when removing the protein component as amino acids may cause the removal of pectins as galacturonic acid.

In the case of cellulase scouring, it has been shown previously in the results of the enzyme activity that cellulase could act on pectins directly via the hydrolysis to produce galacturonic acid. In addition, pectins and cellulose may connect to each other in cotton fiber. Hydrolysis of cellulose with cellulase could remove the pectins attached to the cellulose. Table 4.8 and Figure 4.9 also show that all cellulase scouring processes either after lipase scouring, protease scouring, or lipase/protease

scouring, produced the highest amount of galacturonic acid, compared to lipase and/or protease scouring.

Lipase scouring did not produce galacturonic acid because lipase had no action on unspecific substrate such as pectins and probably because fat (lipase's substrate) and pectins may not connect to each other as complex structures in cotton fiber as removing the fat component by lipase scouring did not cause the removal of pectins as galacturonic acid. The lipase/protease scouring process produced the lowest amount of galacturonic acid of all scouring processes and thus the only enzyme to act indirectly with pectins was protease with the same reason previously shown.

Table 4.8 and Figures 4.8 and 4.9 have shown that various amounts of galacturonic acid were produced from various scouring processes. However, all of the scouring processes proved effective as all scoured fabrics showed an immediate improvement in wetting with water drops while the untreated greige fabric and the prewashed fabric showed a hydrophobic property.

4.3.2.1 Kinetic empirical equation for the presence of galacturonic acid

The enzymatic scouring of cotton fabric has been classified previously as a heterogeneous reaction system with cotton fabric being a solid substrate and the enzymatic scouring solution being an aqueous phase. The Ghose-Walseth kinetic empirical equation (equation 4.1) was also used in this study in order to generate new profiles shown in Figures 4.10 - 4.12 from the experimental profiles in Figures 4.8 and 4.9.

Based on the k and m parameters calculated by the NLR, seven kinetic empirical equations were established as kinetic models of galacturonic acid content in the enzymatic scouring solutions at various scouring times shown in Table 4.10 along with the parameter r^2 (correlation coefficient) closed to 1. There r^2 parameters showed the reliability and the applicability of the kinetic models to the experimental data, and also indicated their validity to make quantitative prediction of the product during the enzymatic scouring process.
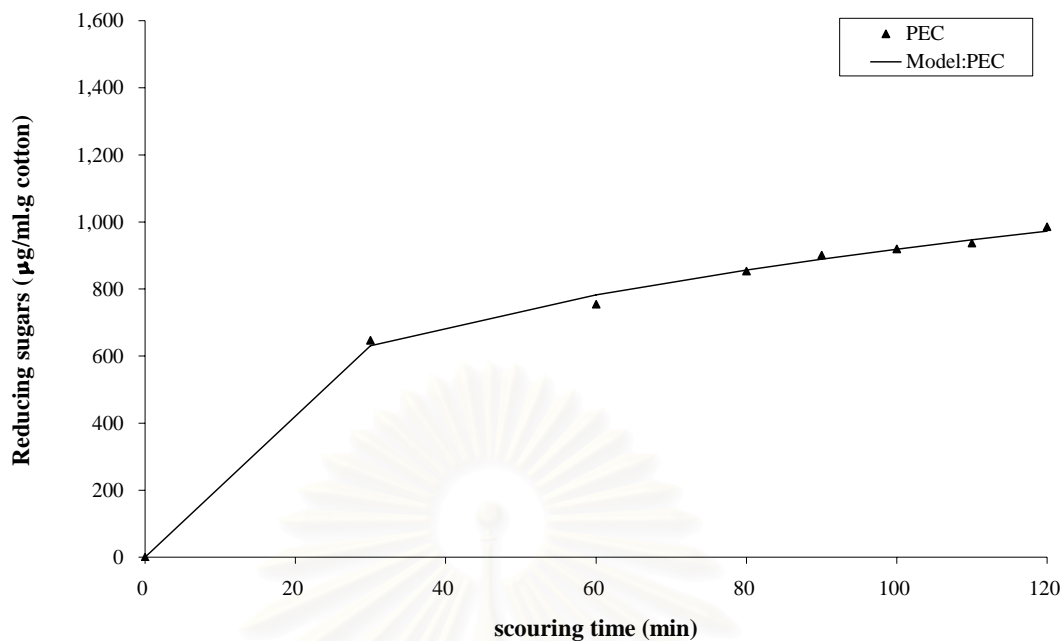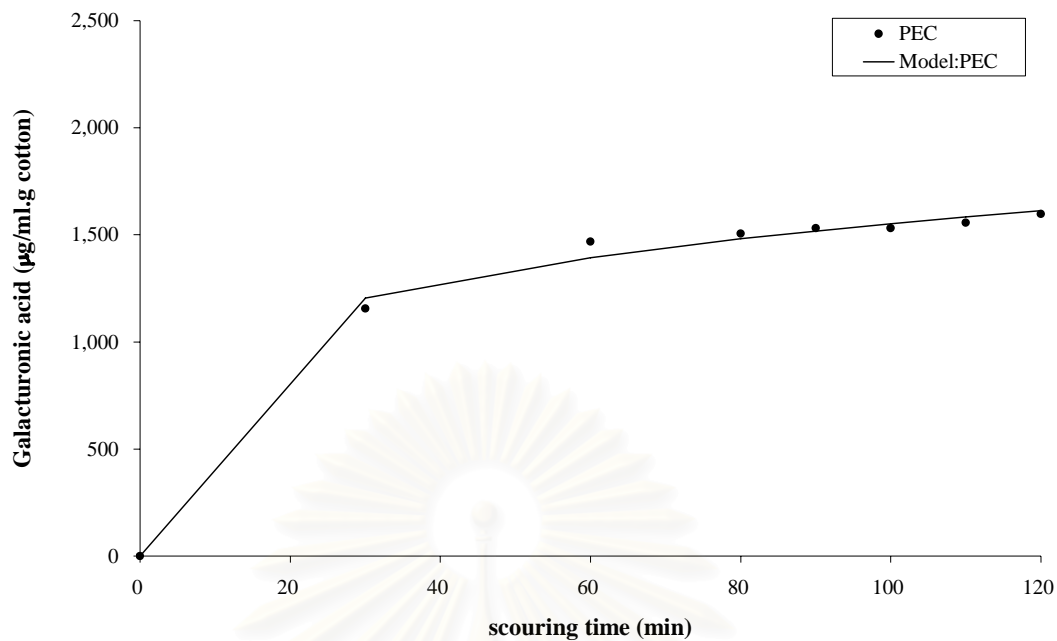
Figure 4.10 Ghose-Walseth kinetic model of galacturonic acid presented in pectinase scouring solution at various scouring times.
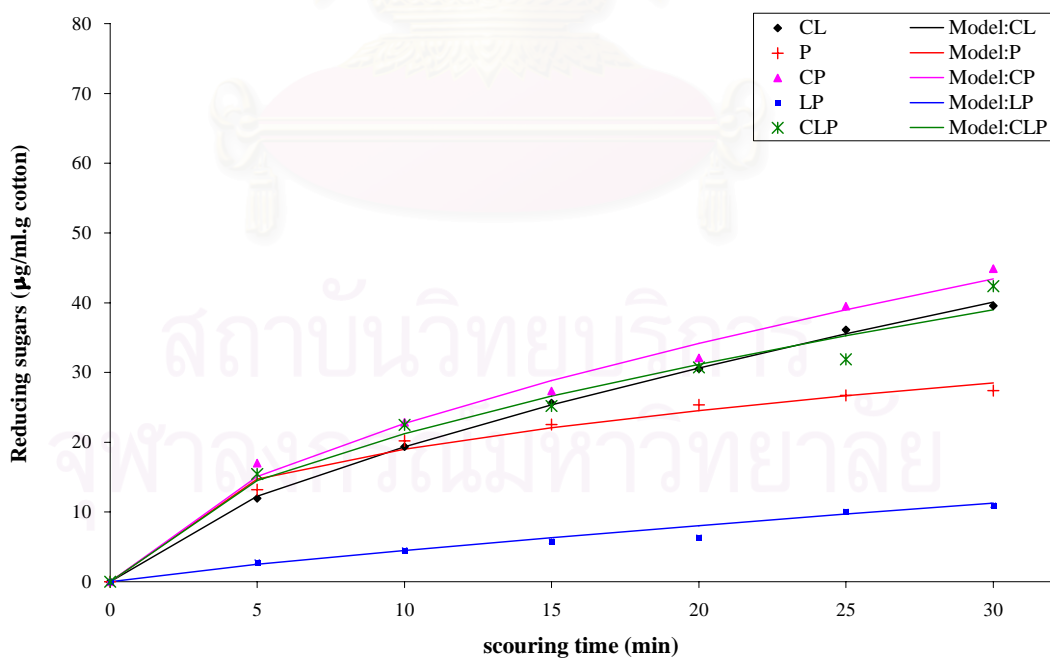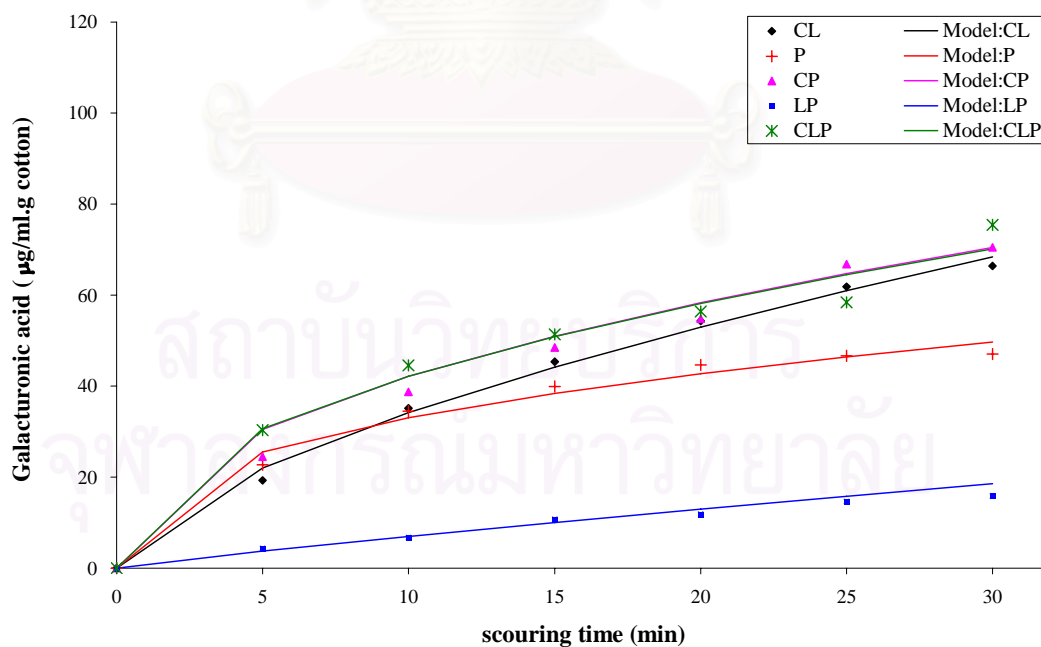

Figure 4.11 Ghose-Walseth kinetic model of galacturonic acid presented in lipase/protease/cellulase scouring solutions at various scouring times.
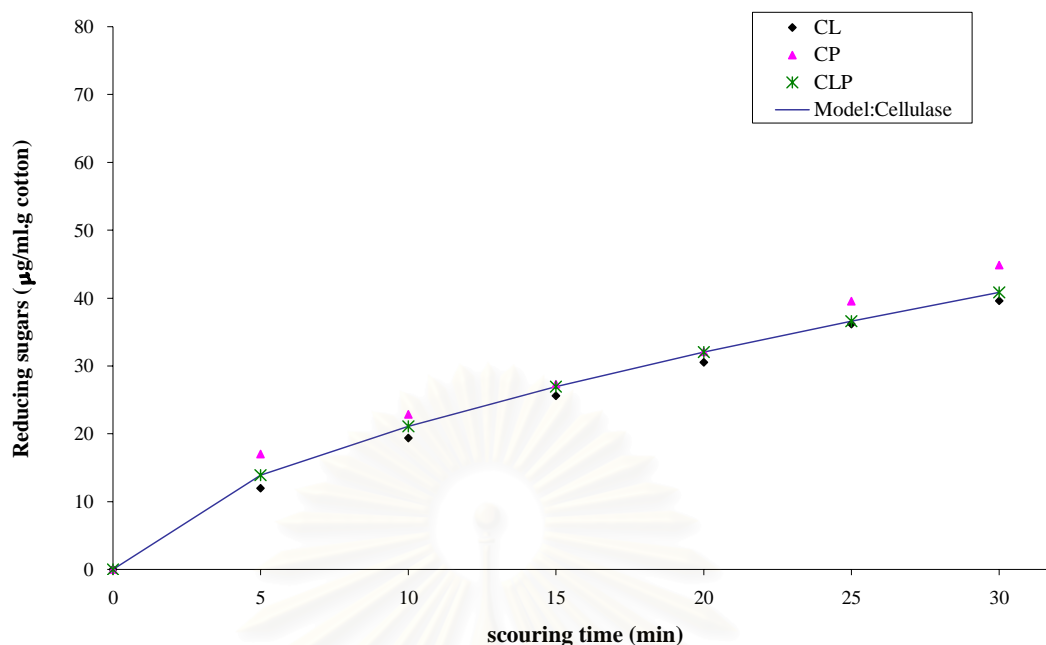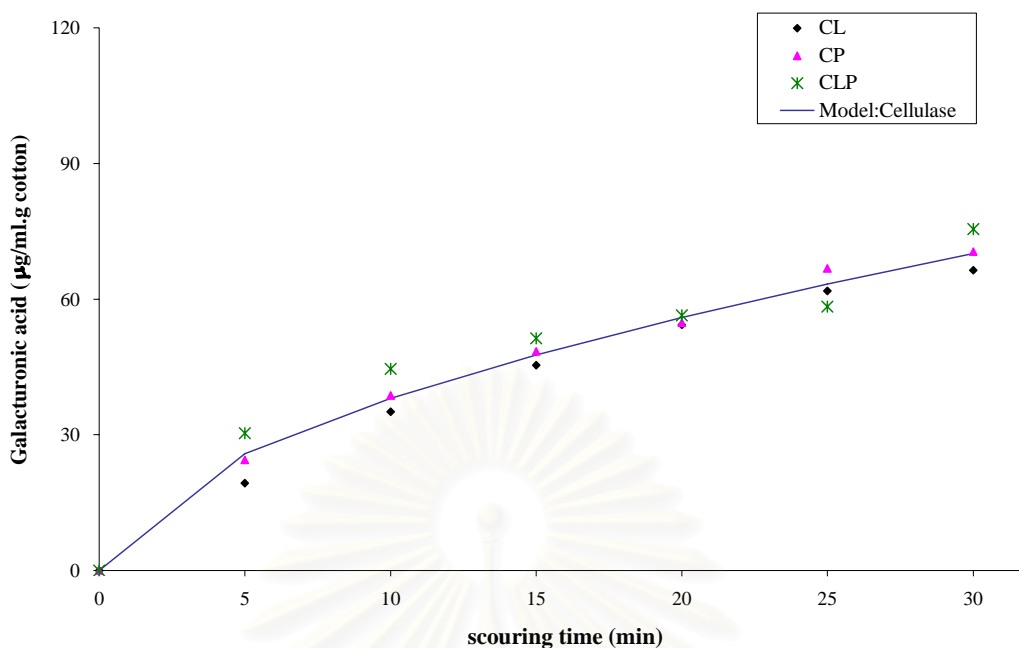
Figure 4.12 Ghose-Walseth kinetic model of galacturonic acid presented in cellulase scouring (C*) solutions at various scouring times.

Table 4.9 Ghose-Walseth kinetic model of the production of galacturonic acid from pectinase and lipase/protease/cellulase scouring processes.

Enzyme	Production of galacturonic acid	
	Equation	r^2
PEC	$P = 589.6783\,t^{0.2102}$	0.9924
CL	$P = 7.9694\,t^{0.6324}$	0.9601
P	$P = 14.0408\,t^{0.3713}$	0.9293
CP	$P = 14.3688\,t^{0.4674}$	0.9323
LP	$P = 0.8393\,t^{0.8919}$	0.9426
CLP	$P = 14.5499\,t^{0.4624}$	0.9752
C*	$P = 10.5716\,t^{0.5561}$	0.9794

Note: kinetic equations from CL, CP, and CLP were combined into one equation C*.

Six equations proposed in Table 4.9 were used to calculate for the contents of galacturonic acid in scouring solutions at various scouring times shown in Table 4.10. Some insignificant differences between the data shown in Table 4.10 (kinetic model) and in Table 4.8 (experimental result) are seen as well as those differences between Figures 4.10 and 4.8, and between Figures 4.11, 4.12 and 4.9.

Table 4.10   Presence of galacturonic acid in scouring solutions at various scouring times according to the Ghose-Walseth kinetic model.

Scouring Time (min)	Galacturonic acid (μg/ml$\cdot$g cotton)						
	PEC	CL	P	CP	LP	CLP	C*
0	0	0	0	0	0	0	0
5		22.02	25.52	30.49	3.76	30.62	25.87
10		34.14	33.01	42.15	6.97	42.19	38.04
15		44.12	38.38	50.95	10.01	50.90	47.66
20		52.92	42.70	58.28	12.93	58.14	55.93
25		60.94	46.39	64.69	15.78	64.46	63.32
30	1205.33	68.39	49.64	70.44	18.57	70.13	70.08
60	1394.38						
80	1481.31						
90	1518.44						
100	1552.44						
110	1583.86						
120	1613.09						

Note: kinetic equations from CL, CP, and CLP were combined into one equation C*.

4.3.2.2 Production rate of galacturonic acid

Previously, the presence of galacturonic acid in the scouring solutions at various scouring times was shown in experimental data and in kinetic equations. In this section, those experimental data were used to calculate for the production rates of galacturonic acid from the scouring processes. In this case, the production rates of galacturonic acid were also calculated using equation 4.2. Results are shown in Table 4.11 and Figures 4.13 and 4.14.

Table 4.11   Production rates of galacturonic acid presented in scouring solutions at various scouring times.

Scouring Time (min)	Galacturonic acid (μg/ml$\cdot$g cotton$\cdot$min)					
	PEC	CL	P	CP	LP	CLP
5		3.86	4.54	4.89	0.87	6.06
10		3.51	3.45	3.87	0.67	4.46
15		3.03	2.66	3.23	0.71	3.43
20		2.72	2.23	2.74	0.59	2.82
25		2.47	1.86	2.67	0.58	2.34
30		2.21	1.57	2.35	0.53	2.52
60	38.54					
80	24.47					
90	18.83					
100	17.02					
110	15.32					
120	14.15					
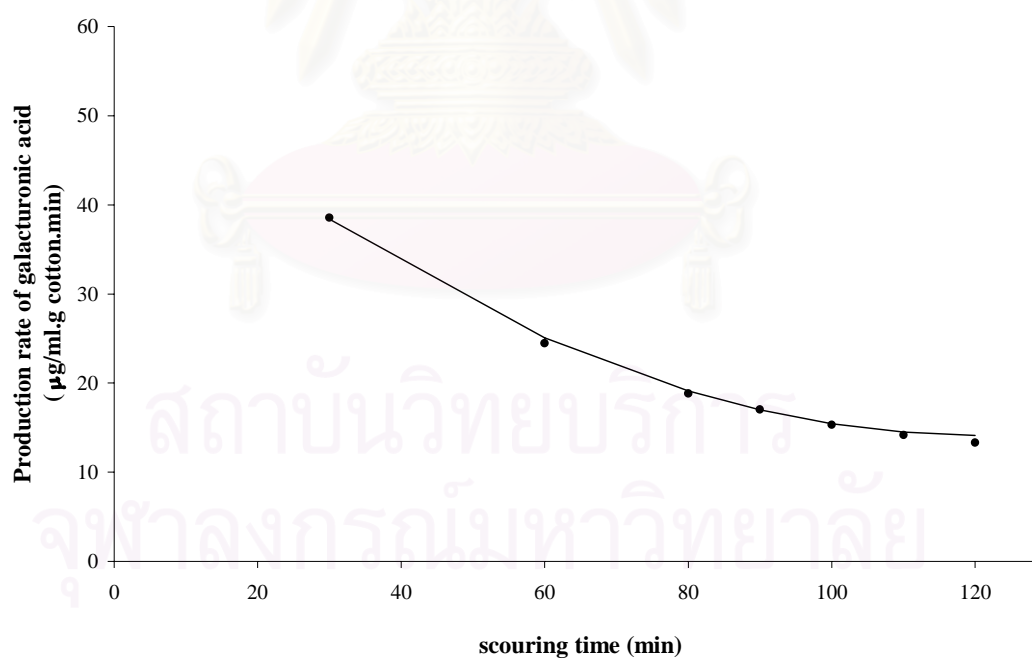
Figure 4.13   Production rates of galacturonic acid presented in scouring solutions at various scouring times from the pectinase scouring process.
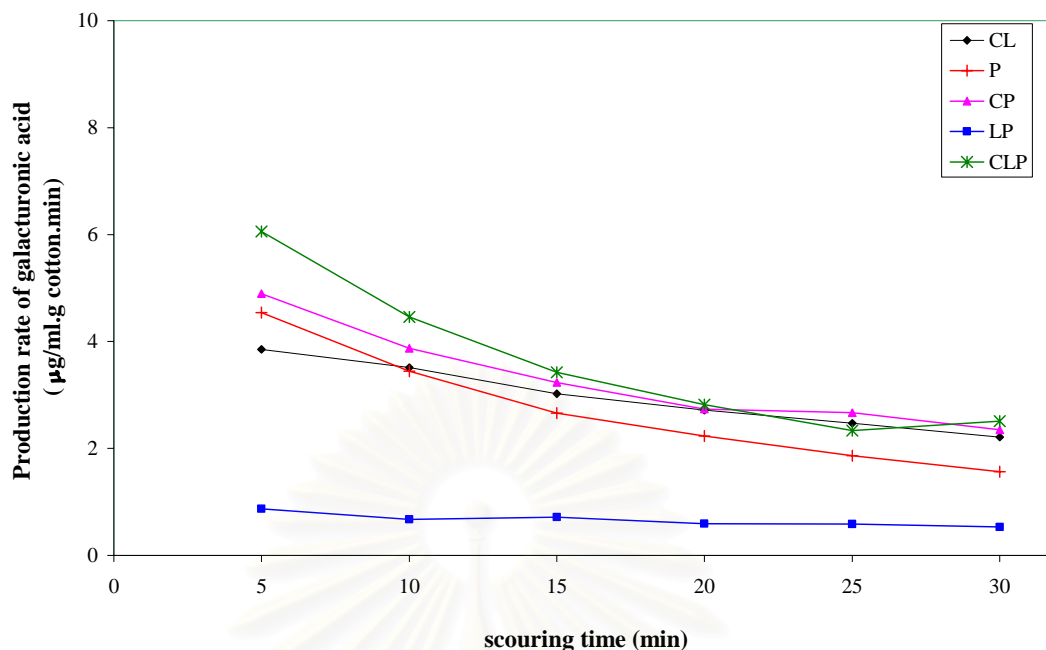
Figure 4.14   Production rates of galacturonic acid presented in scouring solutions at various scouring times from the lipase/protease/cellulase scouring processes.

Table 4.11 and Figures 4.13 and 4.14 illustrate the production rates of galacturonic acid presented in the scouring solutions at various scouring times, based on experimental data shown in Table 4.8 and Figures 4.8 and 4.9. Data indicated that production rate from the pectinase scouring process was several times higher than those from the lipase/protease/cellulase scouring processes since the beginning to the end of the process. In this work, the amount of pectinase required for complete scouring was 10 times higher than that of other enzymes. This increase could help generate galacturonic acid at a much higher level than from the lipase/protease/cellulase scouring processes. Both Figures 4.13 and 4.14 show a decrease of the production rate with scouring time in a quadratic relationship. A rapid drop of the production rate is found after the first half of the total scouring time (15 min for the lipase/protease/cellulase scouring processes and 60-80 min for the pectinase scouring process). This drop may be due to a suppression of the enzyme activity from an increase of impurities in the scouring solutions. The production rate of the second half of the scouring time shows a slow decrease to plateau. Figure 4.14 shows a constant production rate over time for scouring with lipase and protease mixed enzymes, while other scouring processes show the original decreasing trend. It

has been shown previously that lipase scouring did not produce galacturonic acid and therefore only protease would influence the production rate of galacturonic acid from the lipase/protease scouring process. The amount of protease used in the lipase/protease scouring process was half of other enzymes used in the lipase and the protease scouring processes and this could affect the production rate of galacturonic acid from the lipase/protease scouring process, showing the lowest constant production rate. Figure 4.14 also shows that the cellulase scouring process generated galacturonic acid at the highest rate during the process and this is because galacturonic acid is a direct product from the pectins hydrolysis using cellulase.

4.3.3 Amino acids content

The scouring solution was also analyzed for 17 amino acids from the hydrolysis of proteins in the cotton fiber using HPLC technique. Result are shown in Figure 4.15.
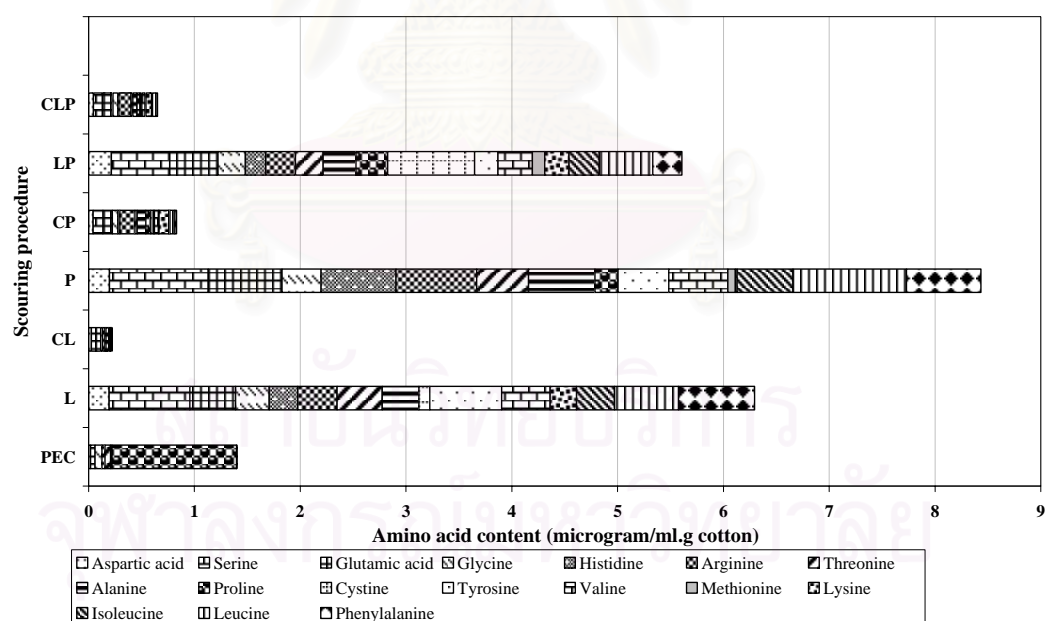
Figure 4.15 Presence of 17 amino acids in scouring solutions after various enzymatic scouring processes.

Figure 4.15 exhibits the existence of 17 amino acids in the scouring solution from various enzymatic scouring processes. It shows that the protease scouring process produced the highest amount of 17 amino acids at a total of 8.4 μg/ml•g cotton, the lipase scouring process was second at 6.3 μg/ml•g cotton, and the lipase/protease scouring process was third at 5.6 μg/ml•g cotton. The pectinase scouring process produced amino acids at 1.4 μg/ml•g cotton and the cellulase scouring processes produced less than 1 μg/ml•g cotton. Generally, protease is an enzyme to specifically catalyze the hydrolysis of protein. Therefore, it was expected to show the highest content of amino acids being produced from the protease scouring process. The lipase scouring process also generated a high content of amino acids and this is because the commercial lipase used in this work contained residual amylase and protease. Pectinase showed very little action on the proteins and cellulase showed no activity on proteins during the activity testing (see Table 4.2). Their little action on protein may be because the pectin and protein components are built-in complex structure attached to cellulose in cotton fiber. Removing the pectins by the pectinase action and hydrolyzing the cellulose by the cellulase action may also cause the removal of proteins as amino acids. Results from this section have shown that all enzymatic scouring processes used in this work could generate amino acids from the hydrolysis of proteins in cotton fiber either directly or indirectly, and thus it's possible to say that proteins could have existed everywhere in the fiber with a smaller amount near cellulose layers (cellulase scouring produced the lowest amount of amino acids). The 17 amino acids shown in Figure 4.15 were categorized based on the structural R group and the property of R group as illustrated in Tables 4.12 and 4.13.

Table 4.12 Classification of 17 amino acids (based on the structural R group) presented in the scouring solutions.

Amino acid (μg/ml•g cotton)	PEC	L	CL	P	CP	LP	CLP
1. Aliphatic amino acids	0.2259	4.6283	0.2150	6.3238	0.7374	4.6183	0.5918
2. Aromatic amino acids	0.0000	1.3976	0.0038	1.1825	0.0278	0.4975	0.0202
3. Heterocyclic aliphatic amino acids	1.1765	0.2686	0.0024	0.9265	0.0661	0.4922	0.0392
Total	1.4024	6.2944	0.2211	8.4328	0.8312	5.6080	0.6512

Table 4.12 reveals the levels of 17 amino acids found in the scouring solutions. They were classified into three categories based on the structural R group as aliphatic amino acids, aromatic amino acids, and heterocyclic aliphatic amino acids. Table 4.12 shows that the pectinase scouring process produced mainly heterocyclic aliphatic amino acids while the lipase/protease/cellulase scouring processes produced mainly aliphatic amino acids. Table 4.12 also shows that pectinase scouring process did not produce aromatic amino acids. Further work is needed in order to explain this phenomenon.

Table 4.13 Classification of 17 amino acids (based on the property of R group) presented in the scouring solutions.

Amino acid (μg/ml•g cotton)	PEC	L	CL	P	CP	LP	CLP
1. Non-polar hydrocarbon	0.0831	2.0884	0.0628	3.1563	0.2293	1.6855	0.1585
2. Mono functional side chain	0.0961	1.2899	0.051	1.4932	0.1431	1.7494	0.1207
3. Ion	0.0466	2.1983	0.1016	2.8622	0.3947	1.5965	0.3293
4. 2nd amino group + other	1.1765	0.7179	0.0057	0.9212	0.0641	0.5765	0.0427
Total	1.4023	6.2945	0.2211	8.4329	0.8312	5.6079	0.6512

Table 4.13 illustrates the classification of 17 amino acids found in the scouring solutions according to the property of R group. Table 4.13 shows that the lipase and/or protease scouring processes produced mostly amino acids with non-polar hydrocarbon and mono functional side chain, and ionic amino acids while the pectinase scouring process produced mostly amino acids with secondary amino group and other amino acids. Cellulase scouring produced the lowest amount of amino acids in which mainly were ionic amino acids. More research work must be conducted in order to explain all these results.

4.3.4 Protein content

Prior to enzymatic scouring, greige cotton fabric was prewashed in boiling water for 10 min. This prewashed water was tested for the existence of proteins and only 2 mg/ml•g cotton of proteins was found. Boiling water could partially remove proteins from the fiber surface and therefore it's possible to conclude that proteins exist at the outermost part of cotton fiber.

Table 4.14   Protein content in the scouring solutions.

	Protein Content (mg/ml)						
	PEC	L	CL	P	CP	LP	CLP
Enzyme solution (before)	136.49	521.74	161.82	339.74	158.91	423.36	175.01
Enzyme solution + fabric (after)	127.23	505.98	159.30	340.21	157.57	412.79	170.41

Concentration of proteins in the scouring solutions was determined both before and after scouring according to the test method shown in section 3.5.4. Theoretically, this protein content should mainly consist of the proteins removed from cotton via scouring and the proteins of various enzymes. Table 4.14 shows the content of proteins in the scouring solutions before and after scouring. The first group of data (Enzyme solution (before)) shows the proteins of enzymes and may be very small portions of proteins from water, buffer solution and nonionic wetting agent. On the other hand, the second group of data (Enzyme solution + fabric (after)) shows the proteins of enzymes and the proteins removed from cotton via scouring. The Table indicates that the protein content found in the scouring solutions after scouring were only a little lower than those found in the scouring solutions before scouring. Generally if proteins were removed from cotton fiber via scouring, the protein content in the scouring solution after scouring should be higher than that before scouring. But it did not take place that way in this work. Instead, the solution after scouring contained only a sight lower protein content than that before scouring. Therefore, it may be that the proteins were hydrolyzed or removed from cotton fiber mostly in the form of amino acids and only proteins were tested here. On the other hand, proteins of enzymes may partially be destroyed during scouring due to impurities in the system and thus protein content in the scouring solution after scouring slightly decreased lower than those before scouring as shown in Table 4.14

4.4 Analysis of fabric

Impurities on cotton fabric consisting of proteins, anionic components including pectins, extractable substances and waxes, and fatty acids were determined both before and after enzymatic scouring according to the test methods shown in sections 3.6.1-3.6.4. In addition, cotton fabric was also determined for various

properties such as water absorbency, weight loss, whiteness and yellowness, bursting strength, crystallinity index, and surface appearance under SEM (Scanning Electron Microscope) according to the test methods shown in sections 3.6.6-3.6.11. Finally, results from the analysis of the scouring solution (section 4.3) and the analysis of the fabric have been used to establish the structural profile of cotton impurities (see section 4.5). Data from the analysis of cotton fabric before and after scouring are shown as follows.

4.4.1 Protein content

Table 4.15 and Figure 4.16 show the protein content of the fabric before and after scouring. It was obtained by multiplying the percentage of nitrogen in an aliquot of fiber by an empirical factor of 6.25. Protein content of greige and prewashed fabrics have been previously shown in section 4.1 to be 1.12% and 0.84% respectively. Upon prewashing the fabric with boiling water, protein on the fiber surface was significantly removed. When comparing among scoured fabrics, it was found that the fabric scoured with protease then cellulase showed the lowest content of protein, followed by that scoured with lipase/protease then cellulase, with lipase then cellulase, and with pectinase respectively. Scouring cotton with protease generated the highest protein hydrolysis or removed the highest protein content at 26%, compared to scouring with other enzymes. This is due to the specific action of the protease on protein.

Table 4.15 Protein content (%) on unscoured and scoured fabrics.

Fabric	Scouring	% nitrogen	% protein
Greige fabric		0.1796	1.1226
Prewashed fabric		0.1348	0.8423
1-step scoured fabric	PEC	0.1183	0.7391
2-step scoured fabric	L	0.1074	0.6715
	CL	0.1066	0.6663
2-step scoured fabric	P	0.1004	0.6275
	CP	0.1001	0.6258
2-step scoured fabric	LP	0.1066	0.6660
	CLP	0.1037	0.6479

These results are consistent of the existence of 17 amino acids in the scouring solution shown in section 4.3.3 in which the protease scouring process produced the highest amount of amino acids. Scouring cotton with pectinase produced the lowest protein hydrolysis with approximately 12% decrease in protein content of the fabric, while scouring with lipase then cellulase decreased 21% of protein content and scouring with lipase/protease then cellulase decreased 23% of protein content in cotton. All enzymes could help removing protein from cotton, even with boiling water. This has pointed out that protein could exist anywhere on or in the fiber. It could be built-in complex structures with fats and pectins, could be attached to cellulose in cotton fiber, and could exist mainly on the outermost part of the fiber in which it was significantly removed by prewashing cotton with boiling water.
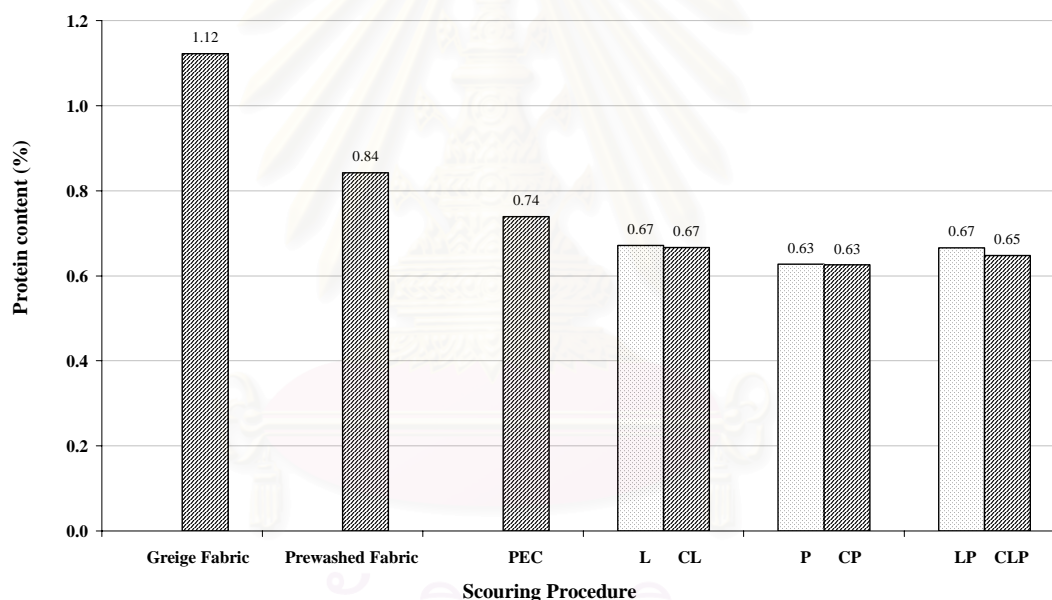
Figure 4.16 Protein content on unscoured and scoured fabrics.

4.4.2 Content of pectins and other anionic components

To determine the presence of pectins on cotton fabric, the fabric was dyed in a solution of methylene blue and the amount of methylene blue on the fabric was measured. Results are shown in Table 4.16 and Figure 4.17. The higher the amount of methylene blue absorbed by the fabric, the higher the content of anionic components, including pectin presented on the fabric. Table 4.16 and Figure 4.17 show that greige fabric absorbed the highest amount of methylene blue at 8.72 g/kg cotton. After

prewashing, the fabric absorbed approximately 22% lower content of methylene blue than the greige fabric. Prewashing the fabric with boiling water significantly removed the anionic components including pectins from the fabric as it has been shown previously in section 4.3.2 that the prewashed water also contained galacturonic acid (a product of pectins hydrolysis).

Table 4.16 Presence of methylene blue (pectins) on unscoured and scoured fabrics.

Fabric	Scouring	Methylene blue (g/kg cotton)	% Reduction of methylene blue
Greige fabric		8.72	-
Prewashed fabric		6.83	21.67
1-step scoured fabric	PEC	6.19	9.37
2-step scoured fabric	L	6.69	2.05
	CL	6.10	10.69
2-step scoured fabric	P	6.56	3.95
	CP	6.19	9.37
2-step scoured fabric	LP	6.44	5.71
	CLP	5.81	14.93
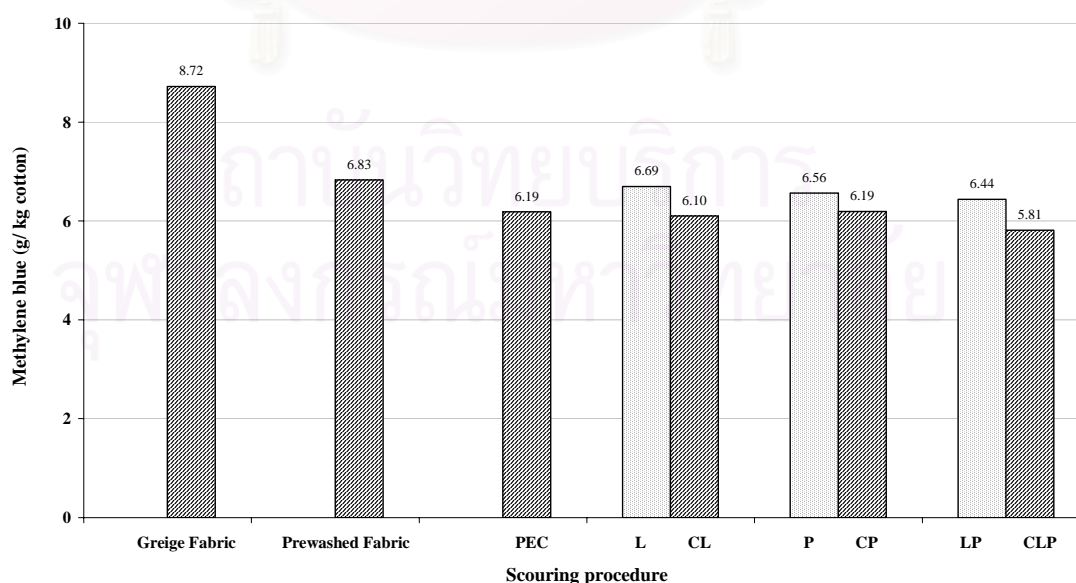
Figure 4.17 Presence of methylene blue (pectins) on unscoured and scoured fabrics.

After scouring, all scoured fabrics absorbed less methylene blue than the greige and the prewashed fabrics due to their lower content of anionic components including pectins. It was observed that the two-step scouring with lipase/protease/cellulase removed higher amounts of anionic components than the one-step pectinase scouring as the two-step scouring with lipase/protease then cellulase removed the highest amount of anionic components. All enzymes used in this work could help removing pectins directly and indirectly as it has been shown previously that pectinase and cellulase could act on pectins and cellulose directly while lipase and protease could remove pectins via removing fats and proteins which attached to pectins.

4.4.3   Extractable content

Extractable content on the fabric was determined according to the AATCC test method 97 (1999). Results are shown in Table 4.17 and Figure 4.18.

Greige fabric was found to have a total of 2.4% extractable substances, consisting of 2.08% water soluble substances and 0.31% wax content. Upon prewashing the fabric with boiling water, the fabric contained only 0.72% of extractable substances left on it and these substances consisted of 0.41% water soluble substances and 0.31% wax content. Approximately 1.67% of water soluble substances were removed from the fabric by boiling water while the wax substance remained intact on the fabric as it has been shown previously that both greige and prewashed fabrics did not absorb water adequately and they needed to be scoured in order to improve their wettability. Scouring can help removing the wax substance and increase the water absorbency of the fabric.

Previously, it has been revealed that all scoured fabrics showed an adequate water absorbency as their wax content was significantly lower than those found in greige and prewashed fabrics (see Table 4.17 and Figure 4.18). The total extractable content on scoured fabrics was between 0.4% to 0.5% while their wax content was between 0.001% to 0.005%. Results show that the two-step scouring with lipase then cellulase could remove the highest amount of wax from the fabric, followed by the two-step scouring with lipase/protease then cellulase, the two-step scouring with protease then cellulase, and the one-step scouring with pectinase, respectively.

Table 4.17 Extractable content on unscoured and scoured fabrics.

Fabric	Scouring	Waxes (%)	Total extractable substances (%)
Greige fabric		0.3095	2.3897
Prewashed fabric		0.3086	0.7182
1-step scoured fabric	PEC	0.0054	0.4150
2-step scoured fabric	L	0.0051	0.4147
	CL	0.0013	0.4109
2-step scoured fabric	P	0.1247	0.5343
	CP	0.0037	0.4133
2-step scoured fabric	LP	0.1512	0.5608
	CLP	0.0019	0.4115
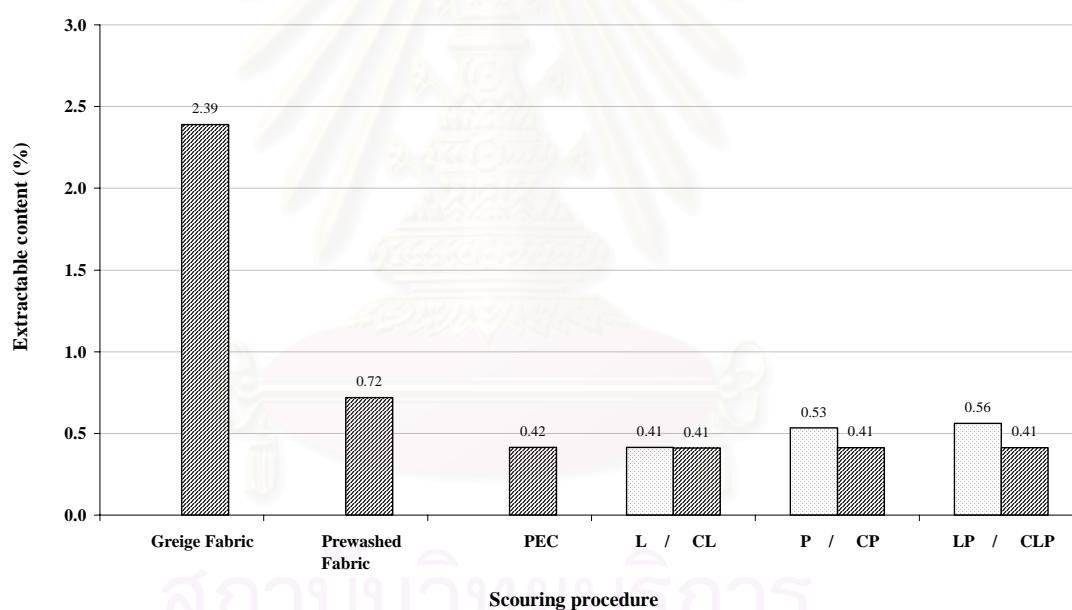
Figure 4.16 Extractable content on unscoured and scoured fabrics.

Although various scouring processes were used in this work, all processes could effectively remove waxes from the fabric to obtain adequate absorbency. Result from this section indicate that for cotton fabric to obtain adequate water absorbency, it was necessary to almost completely remove wax from the fabric (0.001 - 0.005% remained wax). Wax substances could exist in many places in cotton fiber because all enzymes used in this work could act either directly or indirectly on the wax removal.

They could be built-in complex structures or could attach to cellulose and while removing one substance, other substances next to it could be removed as well.

4.4.4 Fatty acids content

To determine residual wax (in terms of fatty acids) on the fabric after scouring, scoured fabric was tested instead of the scouring solution. This is because waxes or fatty acids can not be determined from the solution. First, scoured fabric was solvent extracted and the solvent was then analyzed for 18 fatty acids (C_8-C_{24}) using the GC analysis, and this was compared with extracts from the greige and prewashed fabrics.
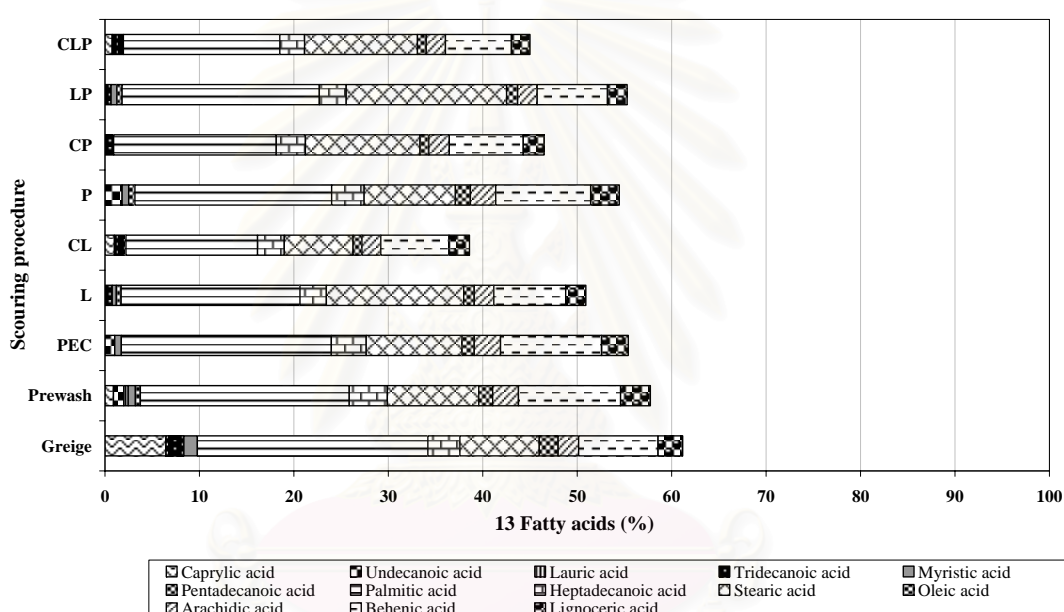
Figure 4.19 Presence of 13 fatty acids being extracted from unscoured and scoured fabrics.

Figure 4.19 shows the presence of 13 fatty acids extracted from scoured fabric, compared with those extracted from greige and prewashed fabrics. In the other words, Figure 4.19 shows the content of wax left on the fabric after scouring. The fabric scoured with the two-step processes using lipase/protease/cellulase showed lower fatty acid content than the pectinase scoured fabric. This result is consistent with the wax content shown in Table 4.17 in which the cellulase scoured fabric showed the lowest wax content of all. This is because cotton wax was partially removed by the first step of lipase and/or protease scouring before performing the second step of

cellulase scouring. Results from the solvent extraction also show that three major fatty acids; palmitic acid, stearic acid, and behenic acid were found on greige and scoured fabrics (see Figure 4.19).

Results from this test indicate that all these enzymes can be used to remove cotton wax to various degrees. Comparing the efficiency of the wax removal between the pectinase; protease; and lipase scouring, the lipase scouring shows the best performance as the lipase scoured fabric contained the lowest fatty acid content or the lowest wax content. In conclusion, lipase performed the highest hydrolysis of cotton wax.

4.4.5 Water absorbency of fabric

In general, scoured fabric is required to have adequate water absorbency such as to absorb water immediately and uniformly once dropping water on the fabric. In this work, the nonionic wetting agent "Ecowet DA" was tested for its scouring effect and found that it did not play a role in scouring. Scouring with the nonionic wetting agent in the absence of enzyme could not effectively treat the fabric to increase water absorbency (see section 3.3 in Table 3.3).

Greige and prewashed fabrics did not absorb water due to the presence of hydrophobic substances on the fabrics. To increase their absorbency, sufficient amounts of impurities such as waxes, oils, pectins, protein, etc. should be removed from the fabrics.

In this research, although various scouring processes and various enzymes were used to treat cotton fabric, all scoured fabrics showed adequate water absorbency. These enzymes could act directly on impurities or could help removing impurities indirectly from the fabric and thus increase the fabric absorbency.

4.4.6   Fabric weight loss

Prewashed and scoured fabrics were tested for % weight loss after prewashing and scouring and the results are shown in Table 4.18 and Figure 4.20.

Table 4.18 % weight loss of prewashed and scoured fabrics.

Fabric	Scouring	Weight loss (%)
Greige fabric		0
Prewashed fabric		2.69
1-step scoured fabric	PEC	1.09
2-step scoured fabric	L	0.02
	CL	0.29
2-step scoured fabric	P	0.13
	CP	0.25
2-step scoured fabric	LP	0.40
	CLP	0.22
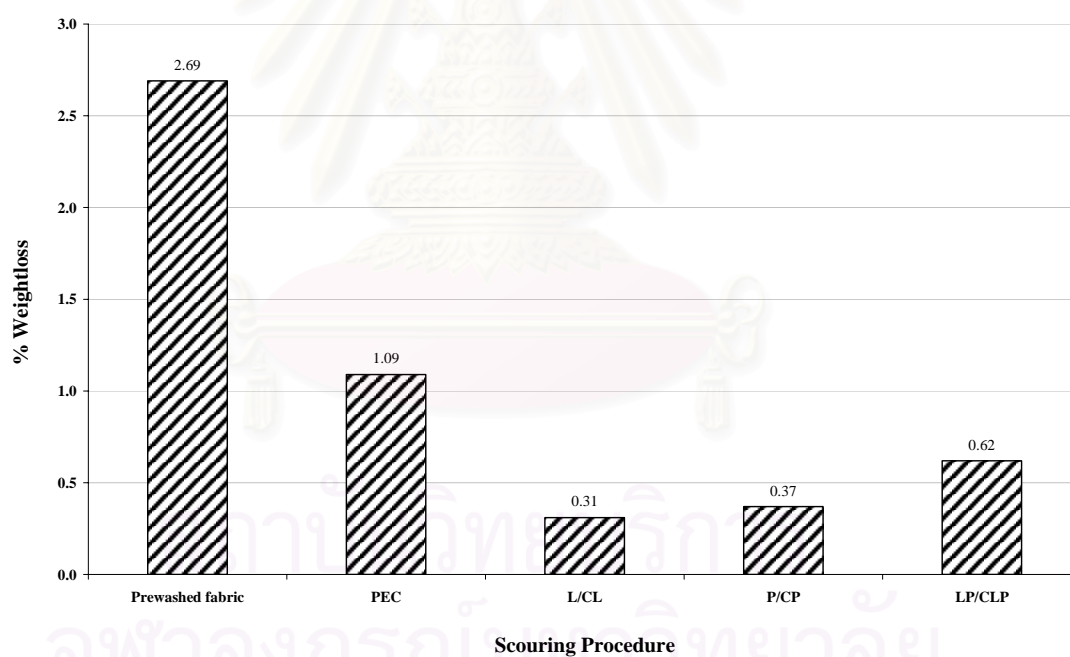
Figure 4.20 % Weight loss of prewashed and scoured fabrics, compared with greige
fabric.

It was found that prewashed fabric lost significant weight of 2.69% after
prewashing with boiling water for 10 min due to the loss of water soluble substances
and other hydrolyzed components. Fabrics scoured with various processes showed
various % weight loss. The fabric scoured with the two-step scouring using

lipase/protease/cellulase showed a lower weight loss (0.3-0.6%) than the fabric scoured with pectinase (1.09%). The loss of fabric weight after scouring could have come from the loss of cotton impurities as water soluble and insoluble substances and the loss of fiber from the fiber breakage and the fiber hydrolysis. Pectinase scoured fabric lost the highest weight when compared with other enzymes scoured fabrics and this could be because the amount of pectinase required for complete scouring was 10 times higher than that of other enzymes and the time required for scouring was 2 hr while other enzymes required a half an hour.

4.4.7   Fabric whiteness and yellowness

Unscoured and scoured fabrics were tested for whiteness and yellowness. Results are shown in Table 4.19 and Figure 4.21. Greige fabric had a light cream color and thus showed a negative whiteness and a yellowness of 31.9. Upon prewashing, the fabric turned whiter, showing a whiteness of 14 and a yellowness of 23 and this could mean that water soluble substances or hydrolyzed components on the cotton surface may contain natural coloring matters which could be removed through a prewashing with boiling water. Upon scouring, the fabric whiteness increased especially when cellulase was used. Enzymatic scouring could also remove natural coloring matters existing everywhere on the fiber.

Table 4.19   Whiteness and yellowness of unscoured and scoured fabrics.

Fabric	Scouring	Whiteness	Yellowness
Greige fabric		undetectable	31.89
Prewashed fabric		13.99	23.23
1-step scoured fabric	PEC	16.51	22.50
2-step scoured fabric	L	19.46	21.58
	CL	21.46	20.92
2-step scoured fabric	P	17.58	22.20
	CP	19.78	21.29
2-step scoured fabric	LP	16.34	22.46
	CLP	19.45	21.43
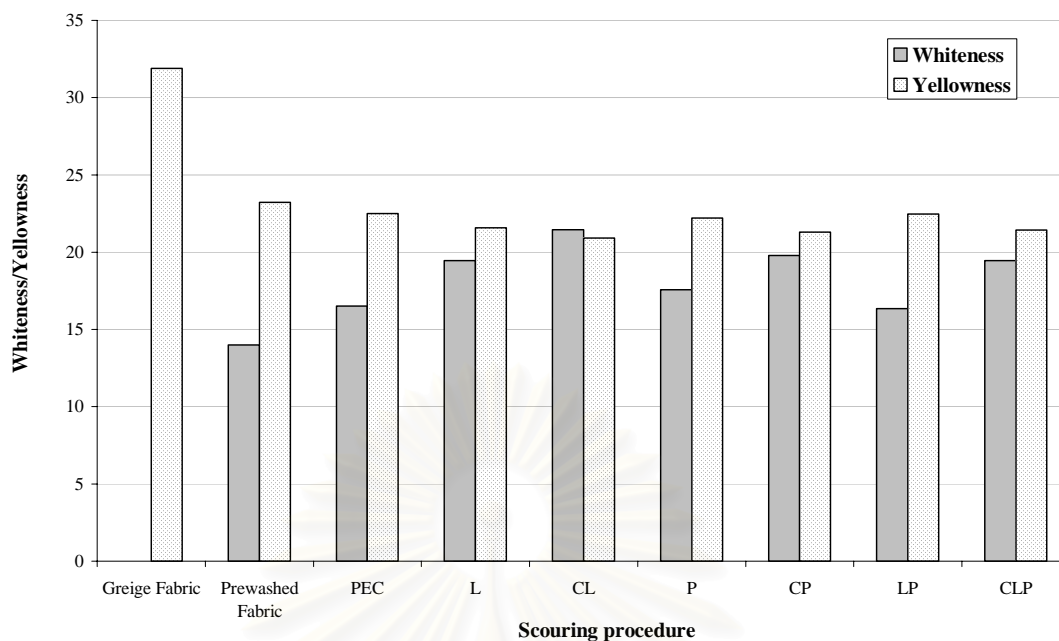
Figure 4.21 Whiteness and yellowness of unscoured and scoured fabrics.

4.4.8 Fabric strength

Unscoured and scoured fabrics were tested for bursting strength. Results are shown in Table 4.20 and Figure 4.22. It was found that bursting strength of the fabric before and after scouring were similar (5.9 - 6.2 kg/cm^2) as enzymatic scouring took place at the fabric surface and left the bulk property untouched.

Table 4.20 Bursting strength of unscoured and scoured fabrics.

Fabric	Scouring	Bursting strength (kg/cm^2)
Greige fabric		6.2
Prewashed fabric		6.1
1-step scoured fabric	PEC	6.2
2-step scoured fabric	L	5.9
	CL	6.0
2-step scoured fabric	P	6.1
	CP	6.2
2-step scoured fabric	LP	6.0
	CLP	6.2
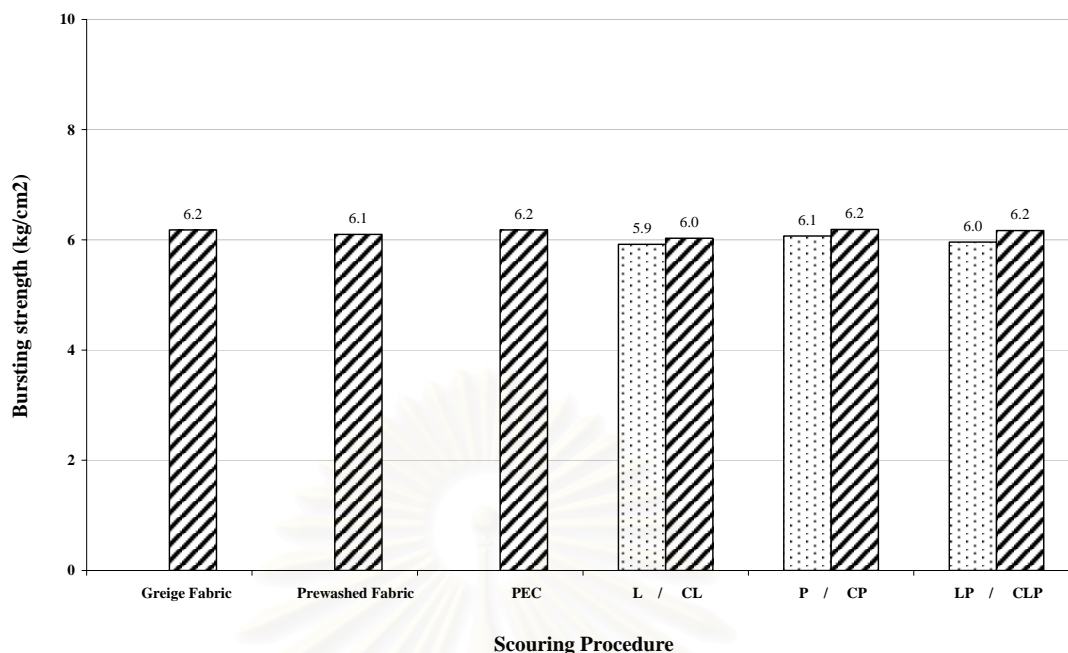
Figure 4.22 Bursting strength of unscoured and scoured fabrics.

4.4.9 Crystallinity index

An empirical method for determining the crystallinity of native cellulose concerns a study with an x-ray diffractometer using the focusing and transmission techniques in which "crystallinity index (CrI)" the percentage of crystalline portion in cellulose can be calculated from the intensity of the specific peaks in the X-ray diffractogram. Figure 4.23 shows X-ray diffractograms of unscoured and scoured fabrics as patterns of cellulose I or native cellulose.

Crystallinity index (CrI) was calculated using the following equation 4.4.

$$CrI = \frac{(I_{002} - I_{AM})}{I_{002}} \times 100 \qquad \text{(Equation 4.4)}$$

Where I_{002} is maximum intensity of crystalline peak at $2\theta = 23°$

I_{AM} is base line of amorphous peak at $2\theta = 19°$.

Table 4.21 shows CrI of unscoured and scoured fabrics. It can be seen that all fabrics showed similar crystallinity index in the range of 75 - 79. In addition, all X-ray diffractograms of unscoured and scoured fabrics represented cellulose I

polymorph or native cellulose. Enzymatic scouring only took place at the fabric surface. The fabric bulk property was left untouched.
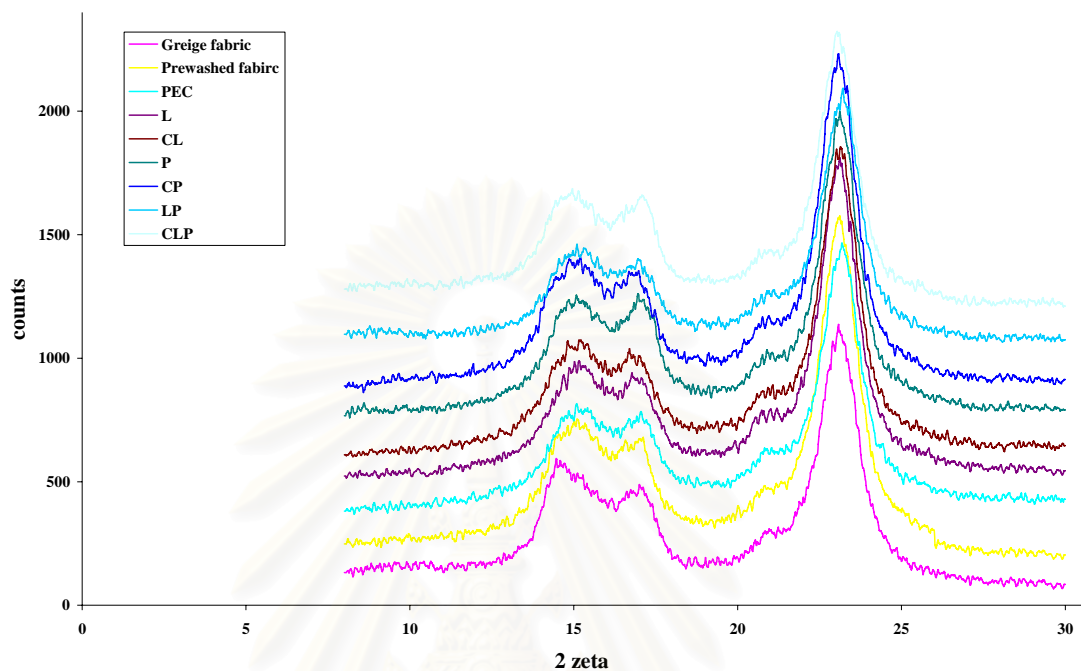
Figure 4.23 X-ray diffractograms of unscoured and scoured fabrics.

Table 4.21 Crystallinity index of unscoured and scoured fabrics.

Fabric	Scouring	Crystallinity Index
Greige fabric		75.4
Prewashed fabric		75.4
1-step scoured fabric	PEC	77.0
2-step scoured fabric	L	79.0
	CL	78.4
2-step scoured fabric	P	78.5
	CP	78.8
2-step scoured fabric	LP	79.4
	CLP	78.3

4.4.10 Surface appearance

Unscoured and scoured fabrics were observed for the appearance of the fiber surface using the SEM technique. SEM micrographs of samples are shown in Figure 4.24 (a) – (j). Greige and prewashed fabrics show clear evidence of impurities on the fibers (Figure 4.22 (a) – (b)). No crack mark on fibers was observed on these fabrics because there were impurities covering the fiber surface. Upon scouring with pectinase (Figure 4.24 (c)), clean fibers were shown with some crack marks because impurities were removed and fiber surface was damaged by the pectinase. Fabric scoured with lipase and/or protease (Figure 4.24 (d), (f), and (h)) still contained small particles of impurities on fibers while those scoured with cellulase showed clean fiber surface (Figure 4.24 (e), (g), and (i)). Scouring with cellulase could also produce fibrils on the scoured fabric due to a direct action or hydrolysis from cellulase (Figure 4.24 (j)).
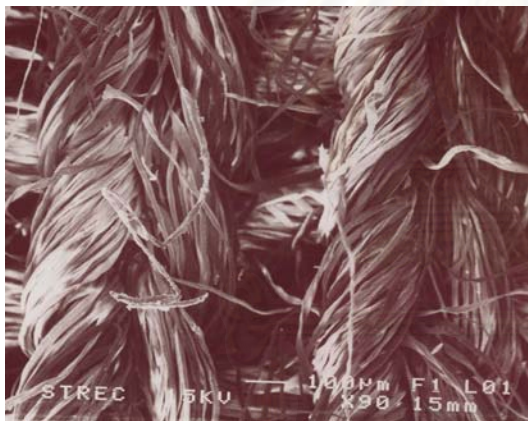
(a)   Greige fabric.
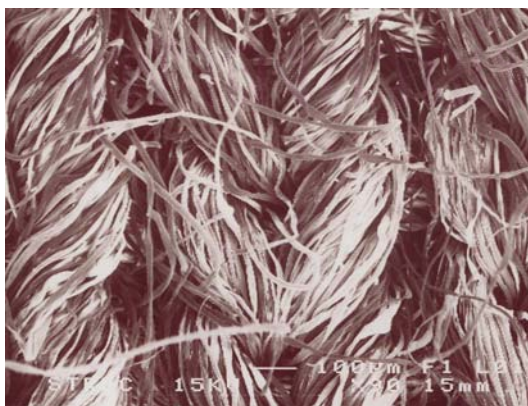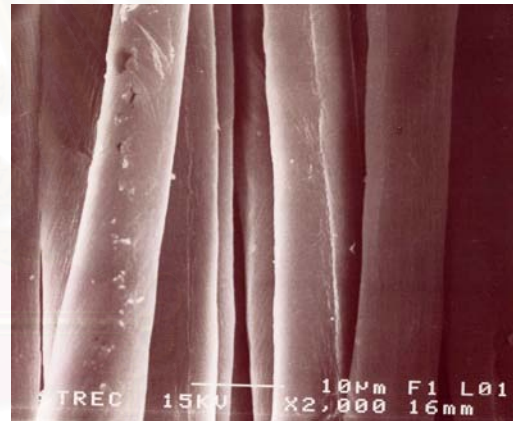
(b)   Prewashed fabric.
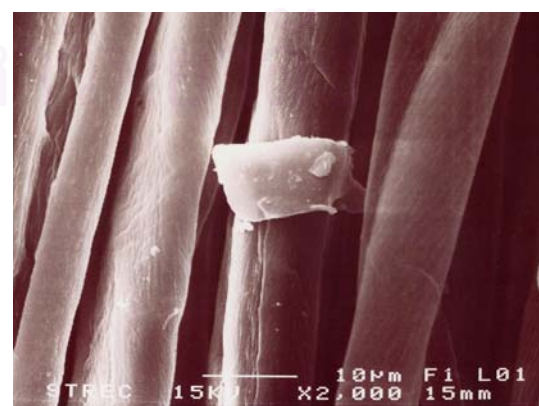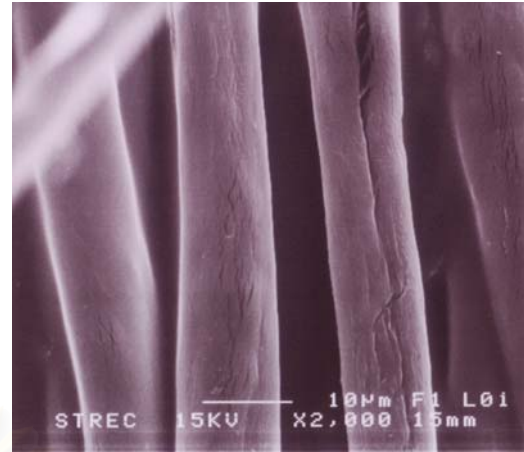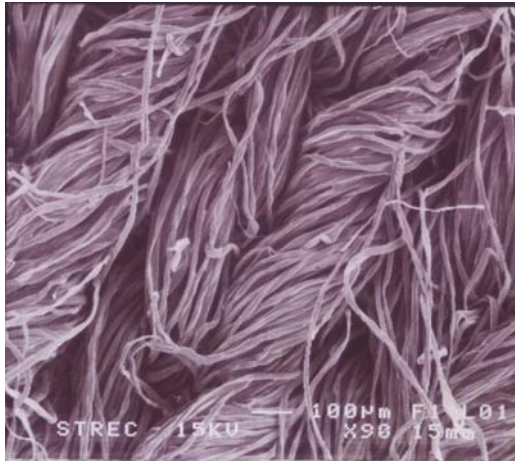
(c)   Fabric scoured with pectinase.
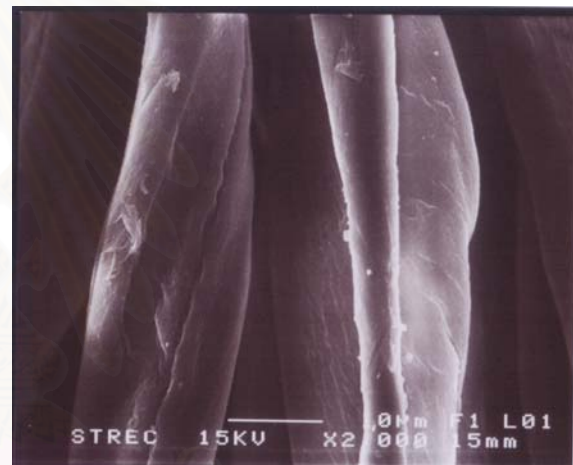
(d)   Fabric scoured with lipase.
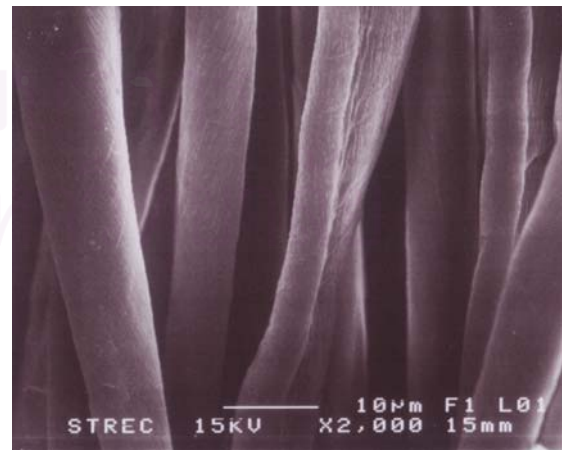
(e)   Fabric scoured with lipase then cellulase.

(f)   Fabric scoured with protease.

(g)   Fabric scoured with protease then cellulase.

(h)   Fabric scoured with lipase/protease.

(i)   Fabric scoured with lipase/protease then cellulase.

(j)   Fabric scoured with cellulase.

Figure 4.24 SEM micrographs of (a) greige fabric (x90, x2000), (b) prewashed fabric (x90, x2000), (c) fabric scoured with pectinase (x90, x2000), (d) fabric scoured with lipase (x90, x2000), (e) fabric scoured with lipase then cellulase (x90, x2000), (f) fabric scoured with protease (x90, x2000), (g) fabric scoured with protease then cellulase (x90, x2000), (h) fabric scoured with lipase/protease (x90, x2000), (i) fabric scoured with lipase/protease then cellulase (x90, x2000), and (j) fabric scoured with cellulase (x3500).

4.5   Structural profile of impurities on cotton fabric

Overall results obtained from this work can be used to establish the structural profile of cotton impurities as follows, they suggest that cotton impurities may be more likely to locate on the fiber surface as waxes on top and maybe some small amount of protein. Mixed portions of proteins, pectins, and small bits of cellulose are located in the middle between the waxes and the main cellulose layers (see Figure 4.25).

This hypothesis can be explained as follows; the lipase scouring process did not produce reducing sugars and galacturonic acid (see Tables 4.4 and 4.8) and this may be because lipase did not act on cellulose and pectins and/or there was no cellulose and pectins on the outermost part of the cotton fiber. However, the lipase scouring process produced amino acid (see Table 4.12) and this could be because lipase used in this work contained residual amount of protease that could act on protein. Or there could be protein in the waxes on the outermost part of cotton fiber. Tables 4.15 and 4.17 show that prewashed fabric lost some protein while wax content remained intact. Protein could be removed by lipase along with the removal of the waxes. At the wax layer, there could be micropores large enough for other enzymes (protease, pectinase, and cellulase) to diffuse through to inner layers and to act on pectins, protein, and cellulose either directly or indirectly (building block removal).
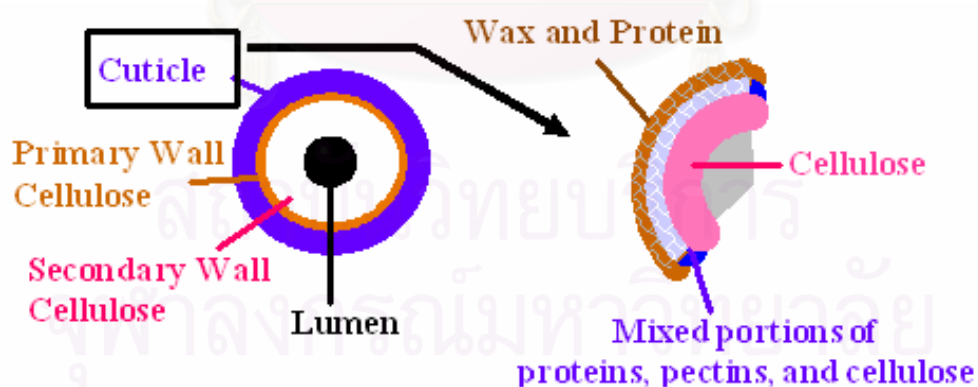
Figure 4.25 An idealized illustration of raw cotton structure in cross section and speculation on the cuticle structure.

4.6 Mechanism of enzymatic scouring of cotton fabric

Data obtained from the analyses of the scouring solutions and the scoured fabrics can also help to establish the mechanism of the enzymatic scouring of cotton fabric using pectinase, lipase, protease, and cellulase.

Upon prewashing greige cotton fabric with boiling water, several large and small substances on the fabric surface could be partially removed from the fabric such as proteins, anionic components and other soluble and hydrolyzed substances including natural coloring matters. Boiling water could dissolve and hydrolyze these substances to smaller particles releasing from the fabric (Diagram 4.1). Waxes could not be removed by boiling water due to their water insoluble property. They were left intact on the fabric after prewashing and thus greige and prewashed fabrics remained an insufficient water absorbency. Waxes have shown a major impact on fabric water absorbency because only the fabric with low wax content (0.001 – 0.005% wax) could absorb water adequately.
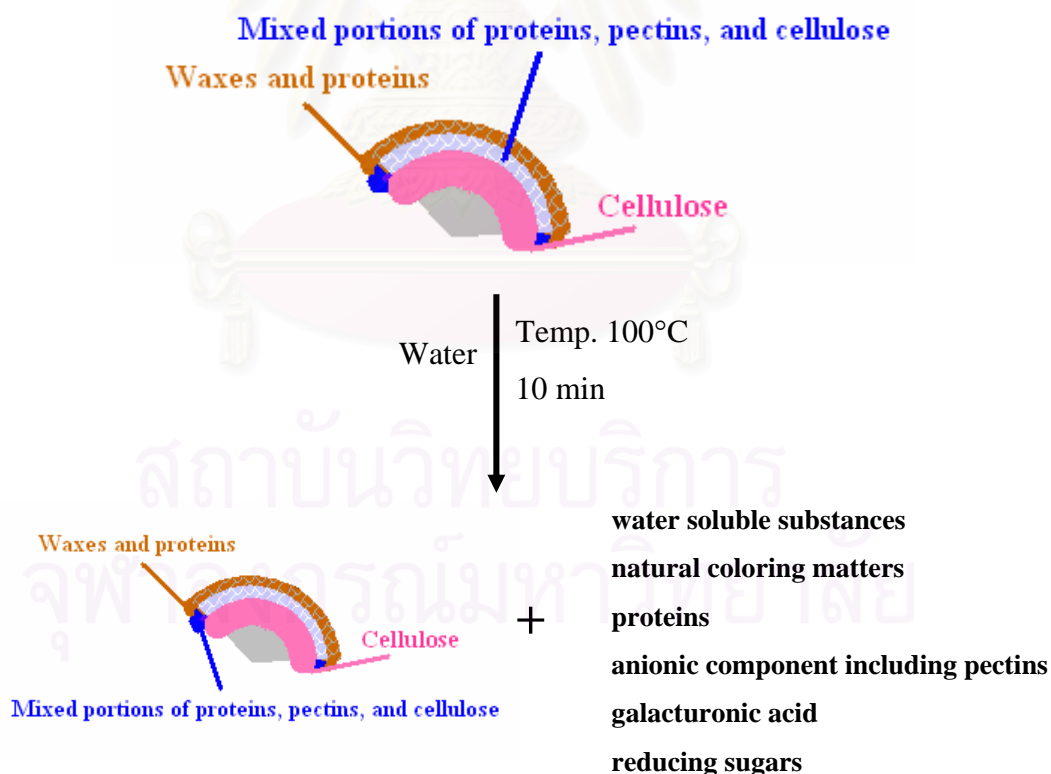
Diagram 4.1 Product released from cotton fabric upon prewashing.

Theoretically, three stages of phenomena were expected to take place during enzymatic souring of cotton fabric as follows.

1. Enzyme molecules were transferred from the scouring solution to the surface of cotton fabric.

2. Enzyme molecules were adsorbed onto the surface of cotton fabric depending on the enzyme types, the concentration of enzymes, pH, temperature, and scouring time.

3. The reaction products were transferred to the scouring solution.

4.6.1 Pectinase scouring

Previous results from this Chapter indicated that during pectinase scouring, pectinase could directly hydrolyze both pectins (producing galacturonic acid and small molecules of pectins) and cellulose (producing reducing sugars) on cotton surface. Pectinase also could indirectly hydrolyze proteins (producing amino acids and small molecules of proteins) and waxes (producing fatty acids and small molecules of waxes) on cotton fabric. All products released from cotton fabric during pectinase scouring are concluded and shown in Diagram 4.2.

Ghose-Walseth kinetic equation was used to express the production of reducing sugars and galacturonic acid from pectinase scouring. Kinetic model of reducing sugars was $P = 218.1217t^{0.3122}$ and kinetic model of galacturonic acid was $P = 589.6783t^{0.2102}$. The production rate of reducing sugars and galacturonic acid showed a decrease with an increase in pectinase scouring time in a quadratic relationship.
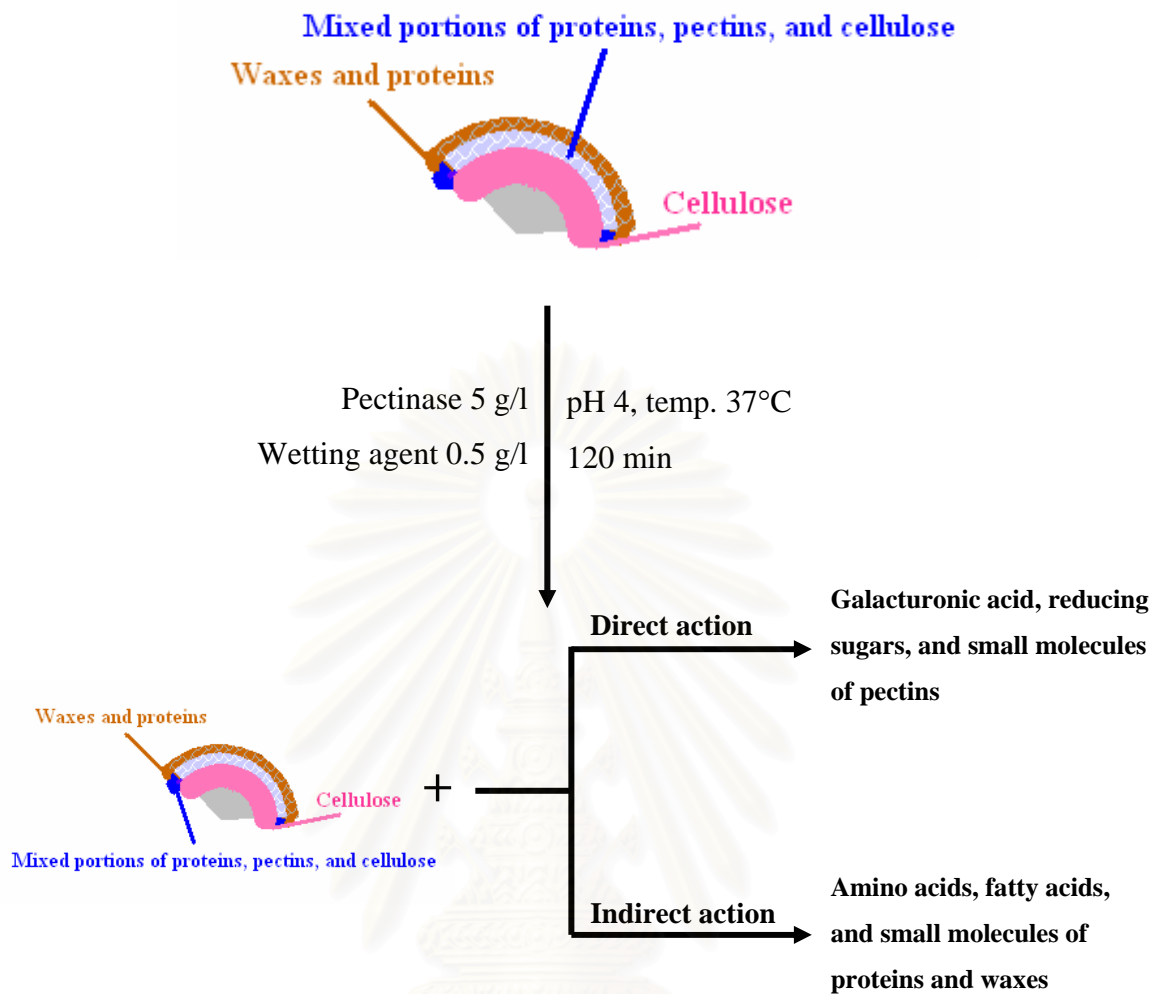
Diagram 4.2 Products released from cotton fabric during pectinase scouring.

4.6.2   Lipase scouring

Previous results from this Chapter indicated that during lipase scouring, the lipase enzyme used in this work could directly hydrolyze waxes (producing fatty acids and small molecules of waxes) and proteins (producing amino acids and small molecules of proteins) on cotton fabric (see Diagram 4.3). Lipase did not act on cellulose and pectins to produce reducing sugars and galacturonic acid respectively.
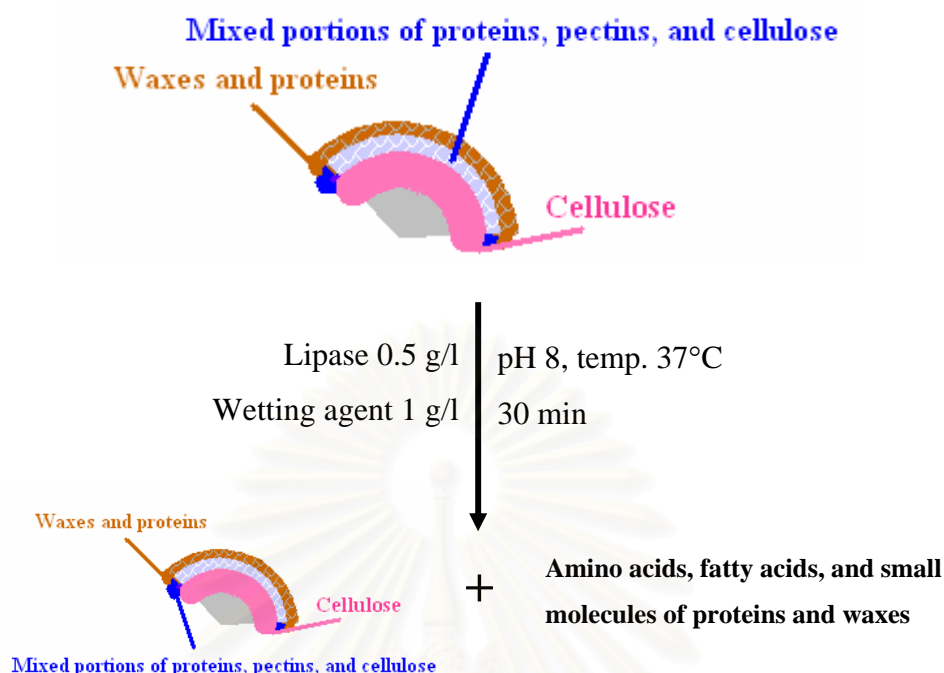
Diagram 4.3 Products released from cotton fabric during lipase scouring.

4.6.3 Protease scouring

Previous results from this Chapter indicated that during protease scouring, protease enzyme could directly hydrolyze proteins (producing amino acids and small molecules of proteins) on cotton surface. Protease also could indirectly hydrolyze pectins (producing galacturonic acid and small molecules of pectins), cellulose (producing reducing sugars) and waxes (producing fatty acids and small molecules of waxes) on cotton fabric. Diagram 4.4 shows products released from cotton fabric during protease scouring.

Ghose-Walseth kinetic equation was used to express the production of reducing sugars and galacturonic acid from protease scouring. Kinetic model of reducing sugars was $P = 8.1310t^{0.3688}$ and kinetic model of galacturonic acid was $P = 14.0408t^{0.3713}$. The production rate of reducing sugars and galacturonic acid showed a decrease with an increase in protease scouring time in a quadratic relationship.
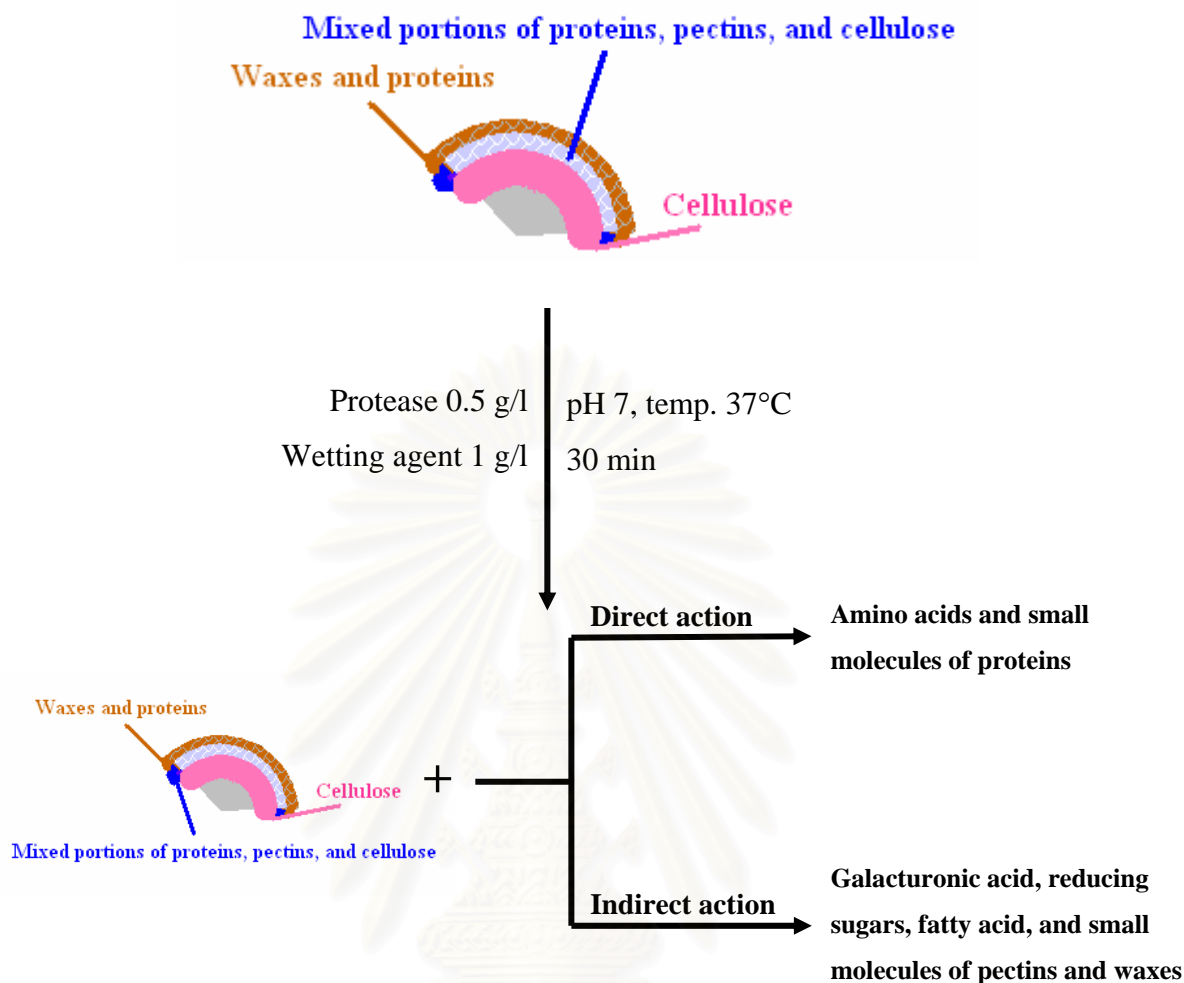
Diagram 4.4 Products released from cotton fabric during protease scouring.

4.6.4   Lipase/protease scouring

Previous results from this Chapter indicated that during lipase/protease scouring, lipase/protease could directly hydrolyze waxes (producing fatty acids and small molecules of waxes in scouring solution) and proteins (producing amino acids and small molecules of proteins in scouring solution) on cotton fabric. Lipase/protease also could indirectly hydrolyze pectins (producing galacturonic acid and small molecules of pectins) and cellulose (producing reducing sugars) on cotton fabric. Diagram 4.5 shows products released from cotton fabric during lipase/protease scouring.

Diagram 4.5 Products released from cotton fabric during lipase/protease scouring.

Ghose-Walseth kinetic equation was used to express the production of reducing sugars and galacturonic acid from lipase/protease scouring. Kinetic model of reducing sugars was $P = 0.6569t^{0.8364}$ and kinetic model of galacturonic acid was $P = 0.8393t^{0.8919}$. The production rate of reducing sugars and galacturonic acid showed a constant production rate over time.

4.6.5 Cellulase scouring

Previous results from this Chapter indicated that during cellulase scouring after the first step of lipase and/or protease scouring, cellulase could directly hydrolyze both cellulose (producing reducing sugars) and pectins (producing

galacturonic acid and small molecules of pectins) on cotton surface. Cellulase also could indirectly hydrolyze proteins (producing amino acids and small molecules of proteins) and waxes (producing fatty acids and small molecules of waxes) on cotton fabric. All released products are shown in Diagram 4.6.
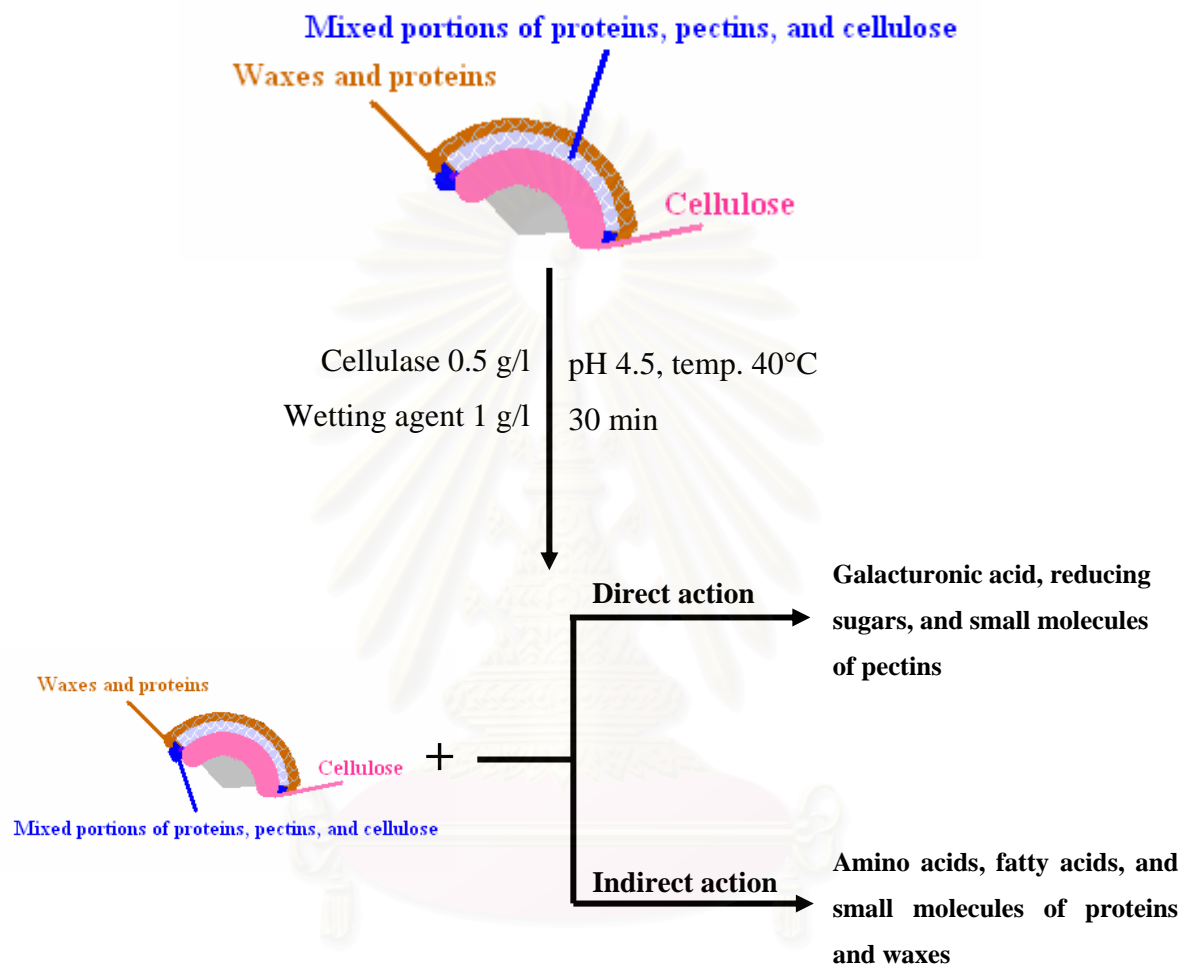
Diagram 4.6 Products released from cotton fabric during cellulase scouring.

Ghose-Walseth kinetic equation was used to express the production of reducing sugars and galacturonic acid from cellulase scouring. Kinetic model of reducing sugars was $P = 5.2896t^{0.6010}$ and kinetic model of galacturonic acid was $P = 10.5727t^{0.5561}$. The production rate of reducing sugars and galacturonic acid showed a decrease with an increase in cellulase scouring time in a quadratic relationship

Although various types and contents of impurities were removed from cotton fabric during enzymatic scouring processes, all scoured fabrics showed adequate water absorbency because they all had very low content of waxes left after scouring (0.001 – 0.005%) and therefore wax content played a major role on cotton absorbency.

CHAPTER V

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

From the results and discussion shown in Chapter 4, some conclusions can be drawn as follows.

1. Prewashing the greige fabric with boiling water could partially help removing impurities from the fabric such as proteins, anionic components and other soluble and hydrolyzed substances including natural coloring matters while waxes could not be removed by boiling water. Waxes were left intact on greige and prewashed fabrics and thus these fabrics showed a hydrophobic property.

2. Waxes have shown a major impact on fabric water absorbency because only the fabric with low wax content (0.001 – 0.005% wax) could absorb water adequately. In addition, three major fatty acids; palmitic acid, stearic acid, and behenic acid were found on greige and scoured fabrics.

3. Pectinase and cellulase showed activities on unspecific substrates having similar chemical structure, cellulose and pectins respectively. On the contrary, lipase and protease showed activities only on specific substrates. During scouring, it was found that the enzyme activity tended to decrease as the scouring time increased.

4. The one-step scouring with pectinase produced approximately 18 fold higher amounts of reducing sugars and galacturonic acid than the two-step scouring with lipase/protease/cellulase. On the contrary, lipase scouring did not produce reducing sugars or galacturonic acid. In addition, it was found that the lipase/protease/cellulase scouring produced approximately 5 fold higher amounts of 17 amino acids than the pectinase scouring.

5. It was found that Ghose-Walseth kinetic equation could be used to explain the production of reducing sugars and galacturonic acid from these enzymatic scouring processes.

6. The production rate of reducing sugars and galacturonic acid from most enzymatic scouring processes showed a decrease with an increase in scouring time in a quadratic relationship. A rapid drop of the production rate was found after the first

half of the total scouring time and the second half of the scouring time showed a slow decrease to plateau, while the lipase/protease scouring process showed a constant production rate over time.

7. All enzymatic scouring processes used in this work could either directly or indirectly generate amino acids from the hydrolysis of proteins, generate fatty acids from the hydrolysis of waxes (fats), and generate anionic components.

All enzymes could be effectively used to scour cotton either alone or in combination as pectinase could be used alone in the process while lipase and/or protease were required to be used in combination with cellulase in which lipase and/or protease were used in the first step and then followed by cellulase in the second step. Although these various enzymes showed various efficiencies for the removal of cotton impurities, they all performed an effective scouring.

8. All enzymatic scouring processes did not affect the bulk property of the fabric such as the physical strength and the crystalline. Unscoured and scoured fabrics showed similar crystallinity index with cellulose I polymorph. Scoured fabric was whiter than unscoured fabric because cotton's natural coloring matters were removed through enzymatic scouring or a prewashing with boiling water.

9. Overall results obtained from this work can be used to establish the structural profile of cotton impurities as follows, they suggest that cotton impurities may be more likely to locate on the fiber surface as waxes on top and maybe some small amount of protein. Mixed portions of proteins, pectins, and small bits of cellulose are located in the middle between the waxes and the main cellulose layers. Waxes/fats and proteins were required to be removed from cotton fabric before cellulase enzyme could reach the cellulose inner layers.

5.2 Suggestions

1. It would be interested to kinetically study the effectiveness of the conventional scouring using NaOH compared to the enzymatic scouring.

2. It would be interested to study for the kinetics of the enzymatic scouring of other fibers.

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APPENDIXES

APPENDIXES

Table A1   Pectinase activity in the presence and in the absence of the nonionic wetting agent.

Substrate	with wetting agent (U/ml)			without wetting agent (U/ml)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Polygalecturonic acid	492.33	498.95	449.16	486.35	482.92	487.28
Filter paper	10.83	9.21	8.50	9.59	11.01	11.93
pNPP	0.00	0.00	0.00	0.00	0.00	0.00
Casein	0.00	0.00	0.00	0.00	0.00	0.00

Table A2   Lipase activity in the presence and in the absence of the nonionic wetting agent.

Substrate	with wetting agent (U/ml)			without wetting agent (U/ml)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Polygalecturonic acid	0.00	0.00	0.00	0.00	0.00	0.00
Filter paper	0.00	0.00	0.00	0.00	0.00	0.00
pNPP	35.54	37.00	37.14	43.72	36.78	35.09
Casein	20.23	17.80	19.17	22.04	16.62	15.62

Table A3   Protease activity in the presence and in the absence of the nonionic wetting agent.

Substrate	with wetting agent (U/ml)			without wetting agent (U/ml)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Polygalecturonic acid	0.00	0.00	0.00	0.00	0.00	0.00
Filter paper	0.00	0.00	0.00	0.00	0.00	0.00
pNPP	0.00	0.00	0.00	0.00	0.00	0.00
Casein	250.77	249.54	254.23	258.67	250.46	245.77

Table A4   Cellulase activity in the presence and in the absence of the nonionic wetting agent.

Substrate	with wetting agent (U/ml)			without wetting agent (U/ml)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Polygalecturonic acid	367.37	364.37	363.84	369.41	350.76	369.84
Filter paper	16.37	17.99	15.29	14.55	14.27	14.82
pNPP	0.00	0.00	0.00	0.00	0.00	0.00
Casein	0.00	0.00	0.00	0.00	0.00	0.00

Table A5   Enzyme activity after scouring.

Enzyme	Enzyme solution after (U/ml)			Enzyme solution + fabric after (U/ml)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
PEC	308.95	299.57	325.11	205.18	210.37	215.23
L	35.05	35.61	35.31	28.92	30.98	31.51
CL	254.04	245.83	241.13	245.28	240.19	242.92
P	14.89	15.33	19.13	14.34	14.00	13.25
CP	14.89	15.33	19.13	10.92	13.68	13.64
CLP	14.89	15.33	19.13	12.17	12.35	11.58

Table A6   Presence of reducing sugars in pectinase (PEC) scouring solution at various scouring times.

Scouring Time (min)	Reducing sugars (μg/ml$\cdot$g cotton)					
	Trial 1	Trial 2	Trial 3	Trial 4	average	Stdev
0	0	0	0	0	0	0
30	638.43	657.70	652.98	640.35	647.37	9.44
60	738.85	749.93	774.81	754.32	754.48	15.04
80	857.95	842.79	851.04	860.10	852.97	7.81
90	907.66	892.20	895.30	908.42	900.89	8.35
100	863.83	904.74	1000.84	907.52	919.23	57.96
110	889.17	902.26	1019.65	937.30	937.10	58.67
120	969.79	944.73	1011.64	1016.22	985.60	34.33

Table A7   Presence of reducing sugars in cellulase (CL) scouring solution at various scouring times.

Scouring	Reducing sugars (µg/ml•g cotton)						
Time (min)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	13.73	14.67	9.04	12.48	9.88	11.96	2.43
10	25.03	18.82	15.71	20.08	17.10	19.35	3.58
15	28.57	25.77	20.20	28.02	25.54	25.62	3.31
20	35.16	34.70	24.23	30.98	27.60	30.53	4.67
25	41.32	36.36	35.28	34.63	33.10	36.14	3.13
30	43.92	38.84	36.87	38.52	39.72	39.57	2.64

Table A8   Presence of reducing sugars in protease (P) scouring solution at various scouring times.

Scouring	Reducing sugars (µg/ml•g cotton)						
Time (min)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	8.22	12.05	13.78	16.97	14.92	13.19	3.30
10	17.06	19.14	21.14	22.11	21.63	20.21	2.10
15	21.54	20.68	20.36	25.91	24.11	22.52	2.40
20	25.34	24.17	23.71	28.66	24.85	25.34	1.95
25	23.51	26.08	28.90	28.61	26.55	26.73	2.18
30	26.08	23.29	27.24	32.40	27.86	27.37	3.31

Table A9   Presence of reducing sugars in cellulase (CP) scouring solution at various scouring times.

Scouring	Reducing sugars (µg/ml•g cotton)						
Time (min)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	14.81	14.30	16.35	19.25	20.34	17.01	2.68
10	18.87	20.12	24.88	26.51	24.03	22.88	3.25
15	25.68	23.67	29.51	24.99	32.78	27.32	3.75
20	30.49	25.97	38.36	27.97	37.58	32.07	5.62
25	41.43	34.25	39.59	40.11	42.24	39.52	3.13
30	47.58	44.35	45.16	41.73	45.47	44.86	2.11

Table A10   Presence of reducing sugars in lipase/protease (LP) scouring solution at various scouring times.

Scouring Time (min)	Reducing sugars (μg/ml•g cotton)						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	2.15	2.24	2.13	4.23	3.42	2.84	0.95
10	3.33	3.30	3.39	5.80	6.79	4.52	1.65
15	5.04	7.35	3.42	7.69	5.48	5.79	1.76
20	6.86	6.85	3.56	8.33	6.18	6.36	1.75
25	10.86	8.93	11.16	9.55	9.81	10.06	0.93
30	13.25	9.60	11.66	10.60	9.82	10.98	1.50

Table A11   Presence of reducing sugars in cellulase (CLP) scouring solution at various scouring times.

Scouring Time (min)	Reducing sugars (μg/ml•g cotton)						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	18.08	3.90	18.41	20.68	16.21	15.46	6.65
10	27.96	10.33	22.55	26.48	25.02	22.47	7.07
15	30.86	5.18	28.66	35.20	26.24	25.23	11.68
20	37.71	5.76	32.68	43.88	33.57	30.72	14.64
25	41.59	2.11	32.36	40.81	42.49	31.87	17.12
30	47.17	37.44	40.28	45.34	41.54	42.35	3.91

Table A12   Presence of galacturonic acid in pectinase (PEC) scouring solution at various scouring times.

Scouring Time (min)	Galacturonic acid (μg/ml•g cotton)					
	Trial 1	Trial 2	Trial 3	Trial 4	average	Stdev
0	0	0	0	0	0	0
30	1193.95	1169.75	1125.69	1135.35	1156.19	31.49
60	1482.35	1502.44	1465.67	1422.78	1468.31	33.87
80	1486.48	1534.41	1490.81	1512.61	1506.08	22.08
90	1548.66	1514.98	1521.53	1542.44	1531.90	16.18
100	1560.98	1508.38	1558.26	1500.49	1532.03	32.04
110	1583.84	1514.37	1609.53	1519.51	1556.81	47.26
120	1621.04	1574.96	1630.80	1564.42	1597.81	32.99

Table A13   Presence of galacturonic acid in cellulase (CL) scouring solution at various scouring times.

Scouring	Galacturonic acid (µg/ml·g cotton)						
Time (min)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	22.05	26.78	15.94	17.47	14.16	19.28	5.11
10	42.58	31.24	27.79	35.62	38.37	35.12	5.82
15	48.68	43.56	38.31	48.57	47.76	45.38	4.47
20	61.45	59.17	45.92	54.10	50.92	54.31	6.26
25	68.12	60.99	60.72	59.18	60.06	61.81	3.59
30	74.78	66.06	63.67	66.06	61.33	66.38	5.09

Table A14   Presence of galacturonic acid in protease (P) scouring solution at various scouring times.

Scouring	Galacturonic acid (µg/ml·g cotton)						
Time (min)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	14.09	20.44	23.42	30.35	25.22	22.71	6.01
10	30.27	28.38	36.70	38.29	38.76	34.48	4.81
15	38.05	39.67	35.12	45.33	41.28	39.89	3.80
20	44.40	46.85	39.43	49.20	43.26	44.63	3.70
25	41.16	49.55	46.80	49.24	46.30	46.61	3.37
30	46.46	39.00	45.49	56.15	48.27	47.07	6.17

Table A15   Presence of galacturonic acid in cellulase (CP) scouring solution at various scouring times.

Scouring	Galacturonic acid (µg/ml·g cotton)						
Time (min)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	23.63	7.80	17.64	37.99	35.25	24.46	23.63
10	32.98	21.95	38.82	57.12	42.75	38.73	32.98
15	45.27	27.00	51.35	58.91	59.76	48.46	45.27
20	52.67	35.10	69.19	50.59	66.40	54.79	52.67
25	49.48	59.63	56.49	68.90	99.30	66.76	49.48
30	56.70	78.22	68.65	70.34	78.54	70.49	56.70

Table A16   Presence of galacturonic acid in lipase/protease (LP) scouring solution at various scouring times.

Scouring Time (min)	Galacturonic acid (µg/ml•g cotton)						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	4.61	3.66	1.66	5.28	6.55	4.35	1.84
10	5.70	5.63	4.51	6.68	11.04	6.71	2.54
15	8.87	12.19	8.90	14.17	9.46	10.72	2.37
20	10.78	10.63	13.49	13.50	10.61	11.80	1.55
25	13.51	13.08	12.71	16.70	17.10	14.62	2.11
30	15.81	13.53	12.41	20.52	16.89	15.83	3.17

Table A17   Presence of galacturonic acid in cellulase (CLP) scouring solution at various scouring times.

Scouring Time (min)	Galacturonic acid (µg/ml•g cotton)						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average	Stdev
0	0	0	0	0	0	0	0
5	32.03	6.58	39.86	35.71	37.36	30.31	13.57
10	55.89	16.69	50.44	46.48	53.45	44.59	15.99
15	62.00	7.19	63.93	60.66	63.16	51.39	24.74
20	71.10	7.39	67.11	73.81	62.64	56.41	27.73
25	77.10	1.32	68.42	68.76	76.29	58.38	32.16
30	79.05	62.71	83.85	76.93	74.73	75.45	7.88

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Table A18   17 amino acids (µg/ml•g cotton) presented in the scouring solution.

Amino acid	PEC	L	CL	P	CP	LP	CLP
Aspartic acid	0.0189	0.1941	0.0261	0.1983	0.0437	0.2164	0.0460
Serine	0.0424	0.7617	0.0405	0.9326	0.0919	0.5497	0.0849
Glutamic acid	0.0000	0.4330	0.0477	0.6968	0.0895	0.4559	0.0991
Glycine	0.0651	0.3190	0.0170	0.3697	0.0512	0.2599	0.0455
Histidine	0.0000	0.2686	0.0000	0.7080	0.0190	0.1919	0.0110
Arginine	0.0277	0.3729	0.0244	0.7629	0.1422	0.2808	0.1148
Threonine	0.0537	0.4251	0.0083	0.4892	0.0212	0.2622	0.0169
Alanine	0.0138	0.3468	0.0249	0.6281	0.0823	0.3099	0.0395
Proline	1.1765	0.0000	0.0024	0.2185	0.0471	0.3003	0.0282
Cystine	0.0000	0.1030	0.0022	0.0000	0.0234	0.8214	0.0112
Tyrosine	0.0000	0.6796	0.0004	0.4798	0.0108	0.2213	0.0057
Valine	0.0042	0.4583	0.0110	0.5573	0.0384	0.3246	0.0316
Methionine	0.0000	0.0000	0.0000	0.0713	0.0065	0.1161	0.0077
Lysine	0.0000	0.2500	0.0030	0.0163	0.0897	0.2302	0.0527
Isoleucine	0.0000	0.3579	0.0000	0.5291	0.0124	0.2857	0.0092
Leucine	0.0000	0.6063	0.0099	1.0721	0.0450	0.5054	0.0326
Phenylalanine	0.0000	0.7179	0.0033	0.7027	0.0170	0.2763	0.0145

Table A19   Protein content in scouring solution.

	Protein content (mg/ml)								
Enzyme	Enzyme solution (before)				Enzyme solution + fabric (after)				
	Trial 1	Trial 2	Trial 3	average	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	average
PEC	135.67	137.56	136.25	136.49	127.25	126.81	127.63			127.23
L	521.69	521.79		521.74	500.19	508.33	510.97	504.43		505.98
CL	158.79	164.85		161.82	161.57	161.16	156.85	158.89	158.01	159.30
P	338.59	340.89		339.74	341.68	343.85	339.59	331.75	344.20	340.21
CP	158.83	157.83	160.08	158.91	153.28	159.47	159.96			157.57
LP	423.45	424.68	421.96	423.36	411.46	415.57	411.32			412.79
CLP	174.62	176.08	174.33	175.01	170.98	170.44	169.68	170.53		170.41

Table A20   Protein content on unscoured and scoured fabrics.

Fabric	% nitrogen				% protein			
	Trial 1	Trial 2	average	stdev	Trial 1	Trial 2	average	stdev
Greige fabric	0.1849	0.1743	0.1796	0.0074	1.1555	1.0897	1.1226	0.0233
Prewashed fabric	0.1334	0.1362	0.1348	0.0020	0.8335	0.8511	0.8423	0.0062
PEC	0.1198	0.1167	0.1183	0.0022	0.7488	0.7293	0.7391	0.0069
L	0.1070	0.1079	0.1074	0.0007	0.6685	0.6745	0.6715	0.0021
CL	0.1087	0.1045	0.1066	0.0030	0.6794	0.6533	0.6663	0.0093
P	0.1013	0.0995	0.1004	0.0013	0.6333	0.6218	0.6275	0.0041
CP	0.1003	0.1000	0.1001	0.0002	0.6267	0.6249	0.6258	0.0006
LP	0.1072	0.1060	0.1066	0.0009	0.6697	0.6622	0.6660	0.0027
CLP	0.1023	0.1050	0.1037	0.0019	0.6396	0.6562	0.6479	0.0058

Table A21   Presence of methylene blue (pectins) on unscoured and scoured fabrics.

Fabric	Methylene blue (g/kg cotton)				
	Trial 1	Trial 2	Trial 3	average	stdev
Greige fabric	8.58	8.86	-	8.72	0.20
Prewashed fabric	6.61	6.80	7.09	6.83	0.24
PEC	6.51	5.86	-	6.19	0.45
L	6.65	6.74	-	6.69	0.07
CL	6.46	5.74	-	6.10	0.50
P	6.89	6.32	6.47	6.56	0.30
CP	6.35	6.04	-	6.19	0.22
LP	6.73	6.15	-	6.44	0.41
CLP	5.89	5.73	-	5.81	0.11

Table A22   Water soluble materials on unscoured fabric.

Fabric	Water soluble materials (%)				Enzyme extractable materials (%)			
	Trial 1	Trial 2	Trial 3	average	Trial 1	Trial 2	Trial 3	average
Greige fabric	2.0624	2.0623	2.1124	2.0790	0.0036	1.94E-14	0.0000	0.0012
Prewashed fabric	0.4071	0.4162	0.4013	0.4082	0.0000	0	0.0041	0.0014

Table A23   Waxes content on unscoured and scoured fabric.

Fabric	Waxes content (%)			
	Trial 1	Trial 2	Trial 3	average
Greige fabric	0.3115	0.2990	0.3181	0.3095
Prewashed fabric	0.3188	0.3090	0.2981	0.3086
PEC	0.0039	0.0082	0.0040	0.0054
L	0.0078	0.0076	2.06E-14	0.0051
CL	0.0038	2.07E-14	0	0.0013
P	0.1450	0.1298	0.0993	0.1247
CP	0.0036		0.0037	0.0037
LP	0.1694	0.1578	0.1263	0.1512
CLP	0.0038		2.13E-14	0.0019

Table A24   Fatty acid content

Fatty acid (%)	Greige fabric	Prewashed fabric	PEC	L	CL	P	CP	LP	CLP
Caprylic acid	6.45	0.89			0.99				0.75
Undecanoic acid		1.09	1.03			1.81			
Lauric acid		0.48							
Tridecanoic acid	1.90			0.75	1.23		0.91	0.64	1.20
Myristic acid	1.43	0.76	0.70	0.46		0.69		0.61	
Pentadecanoic acid		0.52		0.49		0.67		0.55	
Palmitic acid	24.40	22.13	22.22	18.93	13.92	20.82	17.23	20.89	16.55
Heptadecanoic acid	3.39	4.02	3.70	2.80	2.84	3.44	3.08	2.85	2.64
Stearic acid	8.41	9.68	10.13	14.55	7.29	9.66	12.14	16.97	11.93
Oleic acid	1.99	1.48	1.32	1.12	0.93	1.60	0.93	1.17	0.95
Arachidic acid	2.17	2.73	2.77	2.06	2.00	2.68	2.17	2.07	2.03
Behenic acid	8.41	10.82	10.70	7.62	7.22	10.05	7.83	7.46	6.98
Lignoceric acid	2.61	3.13	2.84	2.13	2.17	3.04	2.20	2.08	1.97

Table A25   % weight loss of prewashed and scoured fabrics.

Fabric	Weight loss (%)			
	Trial 1	Trial 2	Trial 3	average
Prewashed fabric	2.6771	2.7041	2.6948	2.69
PEC	1.0353	1.0099	1.2308	1.09
L	0.0000	0.0000	0.0623	0.02
CL	0.1913	0.5300	0.1390	0.29
P	0.1201	0.1889	0.0682	0.13
CP	0.3225	0.1680	-	0.25
LP	0.3788	0.4375	0.3901	0.40
CLP	0.1879	0.1812	0.3036	0.22

Table A26   Whiteness and yellowness of unscoured and scoured fabrics.

Fabric	Whiteness				Yellowness			
	Trial 1	Trial 2	Trial 3	average	Trial 1	Trial 2	Trial 3	average
Greige fabric	-13.78	-13.99	-13.22	-13.66	31.66	32.22	31.80	31.89
Prewashed fabric	13.98	14.15	13.86	13.99	23.21	23.20	23.28	23.23
PEC	16.63	17.26	15.64	16.51	22.54	22.31	22.65	22.50
L	19.80	19.17	19.41	19.46	21.38	21.71	21.66	21.58
CL	21.46	21.75	21.18	21.46	20.92	20.89	20.94	20.92
P	17.11	17.87	17.75	17.58	22.38	22.08	22.15	22.20
CP	20.94	18.78	19.61	19.78	21.12	21.02	21.72	21.29
LP	16.52	16.25	16.25	16.34	22.38	22.57	22.44	22.46
CLP	19.73	18.71	19.91	19.45	21.38	21.67	21.24	21.43

Table A27   Bursting strength of unscoured and scoured fabrics.

Fabric	Trial (kg/cm^2)										
	1	2	3	4	5	6	7	8	9	10	average
Greige fabric	6.6	6.2	6.2	6.0	6.2	5.8	6.2	6.2	6.4	6.0	6.2
Prewashed fabric	6.5	6.5	6.1	6.3	5.9	5.5	6.4	6.1	5.8	5.9	6.1
PEC	6.3	5.9	5.5	6.7	6.7	6.3	6.1	6.1	6.1	6.1	6.2
L	5.7	5.9	5.8	5.0	5.9	6.1	6.4	5.9	6.3	6.2	5.9
CL	6.2	6.1	5.8	5.6	6.0	6.2	6.2	5.9	6.5	5.8	6.0
P	5.9	6.2	5.8	5.5	5.5	6.0	6.2	6.4	6.5	6.7	6.1
CP	6.4	5.9	6.5	6.1	6.3	6.4	5.9	6.1	6.5	5.8	6.2
LP	6.0	6.2	6.2	5.6	5.4	5.7	6.2	6.2	5.8	6.3	6.0
CLP	5.9	5.6	5.4	6.4	6.6	5.8	6.8	6.6	5.7	6.9	6.2

Table A28 Crystallinity index of unscoured and scoured fabrics.

Fabric	I_{am}	I_{002}	CrI
Prewashed fabric	316	1286	75.4
PEC	318	1292	75.4
L	292	1267	77.0
CL	317	1512	79.0
P	313	1446	78.4
CP	312	1449	78.5
LP	335	1582	78.8
CLP	256	1240	79.4

A29   Ghose-Walseth kinetic model of the production of reducing sugars from pectinase (PEC) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	8057260858	200.000000	1.00000000
1.1	182799489.2	45.7211444	.960453485
2	182799489.2	45.7211444	.960453485
2.1	15766074.56	51.9630456	.766560230
3	15766074.56	51.9630456	.766560230
3.1	165007.2272	93.3585841	.489447032
4	165007.2272	93.3585841	.489447032
4.1	1196960.417	180.373059	.294382784
4.2	94467.03212	119.388779	.439440844
5	94467.03212	119.388779	.439440844
5.1	170118.9241	175.503644	.342057472
5.2	60254.75159	137.530063	.412811963
6	60254.75159	137.530063	.412811963
6.1	53241.70475	172.476621	.358495614
7	53241.70475	172.476621	.358495614
7.1	43688.87838	214.113750	.310856100
8	43688.87838	214.113750	.310856100
8.1	31349.08431	218.136387	.312230345
9	31349.08431	218.136387	.312230345
9.1	31348.72524	218.121532	.312216225
10	31348.72524	218.121532	.312216225
10.1	31348.72524	218.121719	.312216031

Run stopped after 22 model evaluations and 10 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable R5

Source	DF	Sum of Squares	Mean Square
Regression	2	20882002.8358	10441001.4179
Residual	30	31348.72524	1044.95751
Uncorrected Total	32	20913351.5611	
(Corrected Total)	31	2927574.90017	

R squared = 1 - Residual SS / Corrected SS =   .98929

Asymptotic 95 %

Parameter	Asymptotic Estimate	Std. Error	Confidence Interval Lower	Upper
k	218.12171862	18.775350307	179.77733783	256.46609941
m	.312216031	.019254286	.272893533	.351538530

A30 Ghose-Walseth kinetic model of the production of reducing sugars from cellulase (CL) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	3149.621006	1.00000000	1.00000000
1.1	9615.599909	3.23408995	.438029390
1.2	733.5011534	1.69412153	.924033917
2	733.5011534	1.69412153	.924033917
2.1	927.7632856	2.90804435	.726628371
2.2	460.7072640	2.20804054	.857618870
3	460.7072640	2.20804054	.857618870
3.1	448.1594775	3.21612130	.723662798
4	448.1594775	3.21612130	.723662798
4.1	285.2303014	3.78881740	.693044074
5	285.2303014	3.78881740	.693044074
5.1	278.0301251	4.21742110	.660236243
6	278.0301251	4.21742110	.660236243
6.1	277.1978359	4.22747827	.661355020
7	277.1978359	4.22747827	.661355020
7.1	277.1978297	4.22765252	.661336922
8	277.1978297	4.22765252	.661336922
8.1	277.1978297	4.22765000	.661337117

Run stopped after 18 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable R5

Source	DF	Sum of Squares	Mean Square
Regression	2	24886.59818	12443.29909
Residual	33	277.19783	8.39993
Uncorrected Total	35	25163.79601	
(Corrected Total)	34	6145.31618	

R squared = 1 - Residual SS / Corrected SS =   .95489

Asymptotic 95 %

Parameter	Asymptotic Estimate	Std. Error	Confidence Interval Lower	Upper
k	4.227649996	.584390346	3.038698897	5.416601095
m	.661337117	.044890964	.570005764	.752668471

A31 Ghose-Walseth kinetic model of the production of reducing sugars from protease (P) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	1494.235520	1.00000000	1.00000000
1.1	10303.49255	4.11096781	.056043902
1.2	1117.699775	1.41449489	.908452545
2	1117.699775	1.41449489	.908452545
2.1	931.3196374	2.25682172	.740466802
3	931.3196374	2.25682172	.740466802
3.1	556.5331310	3.22657194	.647075833
4	556.5331310	3.22657194	.647075833
4.1	502.5147335	5.18051062	.473751514
5	502.5147335	5.18051062	.473751514
5.1	224.6558201	6.20483604	.454783149
6	224.6558201	6.20483604	.454783149
6.1	211.5769762	8.03852998	.360608599
7	211.5769762	8.03852998	.360608599
7.1	192.0735927	8.11999070	.369434884
8	192.0735927	8.11999070	.369434884
8.1	192.0661909	8.13174094	.368739643
9	192.0661909	8.13174094	.368739643
9.1	192.0661870	8.13097996	.368771835
10	192.0661870	8.13097996	.368771835
10.1	192.0661870	8.13101575	.368770335

Run stopped after 21 model evaluations and 10 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable r

Source	DF	Sum of Squares	Mean Square
Regression	2	15949.63333	7974.81667
Residual	33	192.06619	5.82019
Uncorrected Total	35	16141.69952	
(Corrected Total)	34	3052.37971	

R squared = 1 - Residual SS / Corrected SS =   .93708

Asymptotic 95 %

Parameter	Asymptotic Estimate	Std. Error	Confidence Interval Lower	Upper
k	8.131015754	.930359414	6.238185294	10.023846214
m	.368770335	.038476638	.290489027	.447051644

A32   Ghose-Walseth kinetic model of the production of reducing sugars from cellulase (CP) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	5507.955977	1.00000000	1.00000000
1.1	16915.67712	3.86614497	.287302622
1.2	1169.526040	1.83172953	.929041977
2	1169.526040	1.83172953	.929041977
2.1	1461.187923	3.24915009	.716121621
2.2	762.4142133	2.43321197	.857271035
3	762.4142133	2.43321197	.857271035
3.1	702.8063766	3.61968131	.715237184
4	702.8063766	3.61968131	.715237184
4.1	428.1395600	4.29889266	.684906113
5	428.1395600	4.29889266	.684906113
5.1	416.8393206	5.60665231	.590845657
6	416.8393206	5.60665231	.590845657
6.1	374.6976532	5.84250810	.589647633
7	374.6976532	5.84250810	.589647633
7.1	374.6970454	5.84375078	.589623683
8	374.6970454	5.84375078	.589623683
8.1	374.6970454	5.84377504	.589622316

Run stopped after 18 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable R5

Source	DF	Sum of Squares	Mean Square
Regression	2	30748.25393	15374.12697
Residual	33	374.69705	11.35446
Uncorrected Total	35	31122.95098	
(Corrected Total)	34	7026.81138	

R squared = 1 - Residual SS / Corrected SS =   .94668

Asymptotic 95 %

Parameter	Asymptotic Estimate	Asymptotic Std. Error	Confidence Interval Lower	Upper
k	5.843775036	.799318957	4.217548389	7.470001682
m	.589622316	.044743370	.498591246	.680653386

A33   Ghose-Walseth kinetic model of the production of reducing sugars from lipase/protease (LP) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	3424.875443	1.00000000	1.00000000
1.1	118.8376168	.597834586	.935008824
2	118.8376168	.597834586	.935008824
2.1	37.46842976	.630173778	.856295299
3	37.46842976	.630173778	.856295299
3.1	36.81579701	.654351539	.837558881
4	36.81579701	.654351539	.837558881
4.1	36.81518329	.656834367	.836491627
5	36.81518329	.656834367	.836491627
5.1	36.81518276	.656931049	.836446195
6	36.81518276	.656931049	.836446195
6.1	36.81518276	.656935055	.836444222

Run stopped after 12 model evaluations and 6 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable R5

Source	DF	Sum of Squares	Mean Square
Regression	2	1439.94726	719.97363
Residual	29	36.81518	1.26949
Uncorrected Total	31	1476.76244	
(Corrected Total)	30	463.31441	

R squared = 1 - Residual SS / Corrected SS =   .92054

Asymptotic 95 %

Parameter	Asymptotic Estimate	Asymptotic Std. Error	Confidence Interval Lower	Upper
k	.656935055	.157297262	.335226032	.978644078
m	.836444222	.076983887	.678994495	.993893949

A34 Ghose-Walseth kinetic model of the production of reducing sugars from cellulase (CLP) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	K	M
1	2782.622366	1.00000000	1.00000000
1.1	10740.98861	3.72843210	.284026370
1.2	756.5841740	1.71763482	.918667731
2	756.5841740	1.71763482	.918667731
2.1	841.9792616	2.98883196	.716694105
2.2	468.9315960	2.32366324	.839452497
3	468.9315960	2.32366324	.839452497
3.1	427.3712717	3.53589302	.688500558
4	427.3712717	3.53589302	.688500558
4.1	200.8364428	4.22435914	.659806366
5	200.8364428	4.22435914	.659806366
5.1	198.3582520	5.68412712	.552273069
6	198.3582520	5.68412712	.552273069
6.1	154.3003708	5.96681865	.551931374
7	154.3003708	5.96681865	.551931374
7.1	154.3003232	5.96713933	.551929560
8	154.3003232	5.96713933	.551929560
8.1	154.3003232	5.96714103	.551929465

Run stopped after 18 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable R5

Source	DF	Sum of Squares	Mean Square
Regression	2	20426.51665	10213.25833
Residual	26	154.30032	5.93463
Uncorrected Total	28	20580.81697	
(Corrected Total)	27	4432.78454	

R squared = 1 - Residual SS / Corrected SS =   .96519

Asymptotic 95 %

Parameter	Asymptotic Estimate	Std. Error	Confidence Interval Lower	Upper
K	5.967141032	.703010550	4.522082150	7.412199914
M	.551929465	.038697472	.472385673	.631473258

A35 Ghose-Walseth kinetic model of the production of reducing sugars from cellulase (C*) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	K	M
1	2298.925276	1.00000000	1.00000000
1.1	7853.046940	3.60934870	.336514245
1.2	495.2272489	1.76851315	.917448293
2	495.2272489	1.76851315	.917448293
2.1	625.2180363	3.12498663	.709038594
2.2	265.1821494	2.36703122	.844915263
3	265.1821494	2.36703122	.844915263
3.1	247.6530716	3.54459348	.700231066
4	247.6530716	3.54459348	.700231066
4.1	100.3408728	4.21364016	.670615494
5	100.3408728	4.21364016	.670615494
5.1	91.35712165	5.17454598	.600930351
6	91.35712165	5.17454598	.600930351
6.1	83.63318928	5.28889180	.601006827
7	83.63318928	5.28889180	.601006827
7.1	83.63318887	5.28884838	.601007874

Run stopped after 16 model evaluations and 7 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable RS

Source	DF	Sum of Squares	Mean Square
Regression	2	16190.48609	8095.24304
Residual	19	83.63319	4.40175
Uncorrected Total	21	16274.11928	
(Corrected Total)	20	3647.23140	

R squared = 1 - Residual SS / Corrected SS =   .97707

Asymptotic 95 %

Parameter	Asymptotic Estimate	Std. Error	Confidence Interval Lower	Upper
K	5.288848376	.626242742	3.978107252	6.599589499
M	.601007874	.038686318	.520036480	.681979268

A36   Ghose-Walseth kinetic model of the production of galacturonic acid from pectinase (PEC) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	7506018757	200.000000	1.00000000
1.1	496837115.0	93.1222335	.915979552
2	496837115.0	93.1222335	.915979552
2.1	36601000.06	122.954360	.683100424
3	36601000.06	122.954360	.683100424
3.1	2080588.509	249.448115	.360860819
4	2080588.509	249.448115	.360860819
4.1	4865028.653	519.620869	.164944221
4.2	1120061.720	415.870797	.257627566
5	1120061.720	415.870797	.257627566
5.1	228855.8532	582.472492	.200961307
6	228855.8532	582.472492	.200961307
6.1	62063.49356	588.626241	.210945794
7	62063.49356	588.626241	.210945794
7.1	61937.69961	589.722040	.210209516
8	61937.69961	589.722040	.210209516
8.1	61937.69680	589.677223	.210226847
9	61937.69680	589.677223	.210226847
9.1	61937.69680	589.678342	.210226418

Run stopped after 19 model evaluations and 9 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable G5

Source	DF	Sum of Squares	Mean Square
Regression	2	61685584.5881	30842792.2940
Residual	30	61937.69680	2064.58989
Uncorrected Total	32	61747522.2849	
(Corrected Total)	31	8195358.42325	

R squared = 1 - Residual SS / Corrected SS =   .99244

Asymptotic 95 %

Parameter	Asymptotic Estimate	Std. Error	Confidence Interval Lower	Upper
k	589.67834241	38.721370857	510.59875323	668.75793158
m	.210226418	.014778343	.180045016	.240407821

A37   Ghose-Walseth kinetic model of the production of galacturonic acid from cellulase (CL) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	28700.22852	1.00000000	1.00000000
1.1	62883.79132	5.99241857	-.10216217
1.2	4875.322867	2.86609330	.883503992
2	4875.322867	2.86609330	.883503992
2.1	7465.197327	6.22185374	.595721662
2.2	1445.984136	4.28130428	.810646266
3	1445.984136	4.28130428	.810646266
3.1	1901.394516	6.94842038	.632981883
3.2	962.7294536	5.31935873	.754398675
4	962.7294536	5.31935873	.754398675
4.1	960.1287754	7.37117359	.638593461
5	960.1287754	7.37117359	.638593461
5.1	715.8119082	7.97519809	.631667565
6	715.8119082	7.97519809	.631667565
6.1	715.6603395	7.96863166	.632398702
7	715.6603395	7.96863166	.632398702
7.1	715.6603248	7.96946200	.632364222
8	715.6603248	7.96946200	.632364222
8.1	715.6603248	7.96942368	.632365804

Run stopped after 19 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable G5

Source	DF	Sum of Squares	Mean Square
Regression	2	74121.76219	37060.88110
Residual	33	715.66032	21.68668
Uncorrected Total	35	74837.42252	
(Corrected Total)	34	17921.17708	

R squared = 1 - Residual SS / Corrected SS =   .96007

Asymptotic 95 %

Parameter	Asymptotic Estimate	Std. Error	Confidence Interval Lower	Upper
k	7.969423677	1.003001556	5.928801668	10.010045685
m	.632365804	.040988309	.548974463	.715757145

A38   Ghose-Walseth kinetic model of the production of galacturonic acid from protease (P) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	15032.61290	1.00000000	1.00000000
1.1	46148.72083	7.18615471	-.65318656
1.2	10081.02650	2.84450457	.718030081
2	10081.02650	2.84450457	.718030081
2.1	1841.124332	4.91473315	.703550458
3	1841.124332	4.91473315	.703550458
3.1	2521.038064	8.78071248	.457796951
3.2	1442.377462	6.25784744	.622798101
4	1442.377462	6.25784744	.622798101
4.1	1149.645712	9.00482374	.491928679
5	1149.645712	9.00482374	.491928679
5.1	765.3889448	11.8267427	.415252181
6	765.3889448	11.8267427	.415252181
6.1	670.9996696	14.0489781	.366128679
7	670.9996696	14.0489781	.366128679
7.1	660.6806405	14.0278939	.371621345
8	660.6806405	14.0278939	.371621345
8.1	660.6785253	14.0416691	.371249640
9	660.6785253	14.0416691	.371249640
9.1	660.6785206	14.0408334	.371270085

Run stopped after 20 model evaluations and 9 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable G

Source	DF	Sum of Squares	Mean Square
Regression	2	48263.01538	24131.50769
Residual	33	660.67852	20.02056
Uncorrected Total	35	48923.69390	
(Corrected Total)	34	9348.72321	

R squared = 1 - Residual SS / Corrected SS =   .92933

Asymptotic 95 %

Parameter	Asymptotic Estimate	Asymptotic Std. Error	Confidence Interval Lower	Upper
k	14.040833433	1.716329486	10.548934838	17.532732027
m	.371270085	.041087953	.287676017	.454864153

A39   Ghose-Walseth kinetic model of the production of galacturonic acid from cellulase (CP) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	30045.35775	1.00000000	1.00000000
1.1	66988.46830	8.00500490	-.67554878
1.2	19019.43544	3.69247644	.681189292
2	19019.43544	3.69247644	.681189292
2.1	2575.323426	6.58561517	.749765540
3	2575.323426	6.58561517	.749765540
3.1	3255.505454	11.7025255	.471485863
3.2	1527.612532	8.60609431	.621498720
4	1527.612532	8.60609431	.621498720
4.1	1879.781472	13.3650445	.455133962
4.2	1303.427652	10.1567155	.574968208
5	1303.427652	10.1567155	.574968208
5.1	1244.335285	13.2560398	.481179083
6	1244.335285	13.2560398	.481179083
6.1	1141.867332	14.3502050	.467013562
7	1141.867332	14.3502050	.467013562
7.1	1141.457863	14.3690360	.467388574
8	1141.457863	14.3690360	.467388574
8.1	1141.457862	14.3687797	.467393785

Run stopped after 19 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable G5

Source	DF	Sum of Squares	Mean Square
Regression	2	68670.74689	34335.37344
Residual	25	1141.45786	45.65831
Uncorrected Total	27	69812.20475	
(Corrected Total)	26	16851.42741	

R squared = 1 - Residual SS / Corrected SS =   .93226

Asymptotic 95 %

Parameter	Asymptotic Estimate	Confidence Interval Std. Error	Lower	Upper
k	14.368779749	2.607445288	8.998645654	19.738913845
m	.467393785	.059601327	.344642556	.590145015

A40   Ghose-Walseth kinetic model of the production of galacturonic acid from lipase/protease (LP) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	k	m
1	1402.664583	1.00000000	1.00000000
1.1	80.44975141	.790228996	.938876986
2	80.44975141	.790228996	.938876986
2.1	55.82695243	.823489777	.898979964
3	55.82695243	.823489777	.898979964
3.1	55.78792513	.837579700	.892568740
4	55.78792513	.837579700	.892568740
4.1	55.78774109	.839161527	.892003550
5	55.78774109	.839161527	.892003550
5.1	55.78774011	.839291146	.891954224
6	55.78774011	.839291146	.891954224
6.1	55.78774011	.839302380	.891949904

Run stopped after 12 model evaluations and 6 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable G5

Source	DF	Sum of Squares	Mean Square
Regression	2	3013.70384	1506.85192
Residual	26	55.78774	2.14568
Uncorrected Total	28	3069.49158	
(Corrected Total)	27	969.56455	

R squared = 1 - Residual SS / Corrected SS =   .94246

Asymptotic 95 %

Parameter	Asymptotic Estimate	Asymptotic Std. Error	Confidence Interval Lower	Upper
k	.839302380	.200948061	.426247726	1.252357034
m	.891949904	.076854601	.733973009	1.049926799

A41 Ghose-Walseth kinetic model of the production of galacturonic acid from cellulase (CLP) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	K	M
1	30722.73160	1.00000000	1.00000000
1.1	68427.28783	8.07684678	-.69980099
1.2	19811.91274	3.73835059	.669683040
2	19811.91274	3.73835059	.669683040
2.1	2359.603045	6.69354015	.752597199
3	2359.603045	6.69354015	.752597199
3.1	2302.304743	11.7513978	.473262822
4	2302.304743	11.7513978	.473262822
4.1	354.0593554	14.5352958	.460407769
5	354.0593554	14.5352958	.460407769
5.1	350.7157140	14.5513585	.462338239
6	350.7157140	14.5513585	.462338239
6.1	350.7156660	14.5499328	.462363393
7	350.7156660	14.5499328	.462363393
7.1	350.7156660	14.5499121	.462363872

Run stopped after 15 model evaluations and 7 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable G5

Source	DF	Sum of Squares	Mean Square
Regression	2	71028.76794	35514.38397
Residual	26	350.71567	13.48906
Uncorrected Total	28	71379.48361	
(Corrected Total)	27	14124.84414	

R squared = 1 - Residual SS / Corrected SS =   .97517

Asymptotic 95 %

Parameter	Asymptotic Estimate	Asymptotic Std. Error	Confidence Interval Lower	Upper
K	14.549912070	1.292071495	11.894021075	17.205803066
M	.462363872	.029482658	.401761401	.522966343

A42   Ghose-Walseth kinetic model of the production of galacturonic acid from cellulase (C*) scouring process using non-linear regression (NLR) in SPSS 12.0 for windows.

All the derivatives will be calculated numerically.

Iteration	Residual SS	K	M
1	20197.05357	1.00000000	1.00000000
1.1	45274.52088	6.95659456	-.37008077
1.2	8319.204214	3.36082764	.764508840
2	8319.204214	3.36082764	.764508840
2.1	583.3766931	5.75238449	.742414432
3	583.3766931	5.75238449	.742414432
3.1	1247.818120	9.65084217	.534792438
3.2	401.7500175	6.92142480	.686904217
4	401.7500175	6.92142480	.686904217
4.1	339.8007870	9.27834206	.583270136
5	339.8007870	9.27834206	.583270136
5.1	224.8765882	10.5523552	.554311829
6	224.8765882	10.5523552	.554311829
6.1	222.1926505	10.5709954	.556176771
7	222.1926505	10.5709954	.556176771
7.1	222.1926102	10.5715974	.556149272
8	222.1926102	10.5715974	.556149272
8.1	222.1926102	10.5715904	.556149493

Run stopped after 18 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-08

Nonlinear Regression Summary Statistics   Dependent Variable GALAC

Source	DF	Sum of Squares	Mean Square
Regression	2	49322.90596	24661.45298
Residual	19	222.19261	11.69435
Uncorrected Total	21	49545.09857	
(Corrected Total)	20	10760.28140	

R squared = 1 - Residual SS / Corrected SS =   .97935

Asymptotic 95 %

Parameter	Asymptotic Estimate	Asymptotic Std. Error	Confidence Interval Lower	Upper
K	10.571590391	1.128836775	8.208907868	12.934272914
M	.556149493	.035056828	.482774708	.629524278

BIOGRAPHY

Ms. Porntip Sae-be was born on August 27, 1977 in Bangkok, Thailand. She received her Bachelor of Science degree in Polymer and Textiles in 1999 and Master of Science degree in Applied Polymer Science and Textile Technology in 2002 from the Department of Materials Science, Faculty of Science, Chulalongkorn University. During her study for MS degree, she received a one-year teaching assistantship from the Graduate School of Chulalongkorn University. She started her Ph.D. program in Materials Science, in June 2002 and completed it in May, 2007 at Chulalongkorn University.