CHAPTER 2

Literature Review

2.1 Mannan

Mannan polysaccharides are complicated biopolymers, normally found in plant cell walls where they are nearly related with cellulose and lignin. The polysaccharides are indicated either as structural carbohydrates or as storage carbohydrates in a variety of plant seeds (Puls and Schuseil, 1993). Mannans are composed of mannose molecules linkage. Homo- and heteromannans are derived from variance of β-mannan backbone. Homomannans consist of β-1,4-linked D-mannose (and D-glucose) in the backbone (glucomannan). Heteromannans might be branched with α-1,6-linked D-galactose (galactomannan/galactoglucomannan) (Matheson, 1990).

Mannans are notably different in their structure depending on their origin. These polysaccharides could be categorized into four subfamilies, for example, linear mannan, glucomannan, galactomannan and galactoglucomannan (Moreira and Filho, 2008). The mannose and glucose residues can be acetylated at C-2 or C-3 positions in the backbone according to the polysaccharide source, leading to acetylated galactoglucomannans (Lundqvist *et al.*, 2002).

Linear mannan is particularly found in many plant seeds, for example, coconut kernel (copra), green coffee (*Coffea* spp.), ivory nuts (*Phytelephas* spp.) and the cell walls of some algae (*Codium* spp.). Softwoods contain 25% acetylated galactoglucomannan in contrast to hardwoods comprising 3–5% mannan. Low molecular mass mannans (≤30,000 Da) are present as hemicellulosic mannans. Higher molecular mass mannans are indicated as storage carbohydrates in some plants, such as carob and guar galactomannan gums with β-1,4-mannan backbone including α-1,6-galactose (Moreira and Filho, 2008; Schroder *et al*., 2009). An overview of different types of mannans including their sources, structures and applications is shown in Table 2.1.

Table 2.1 Typical structures, sources, applications of different subfamilies of mannan polysaccharides.

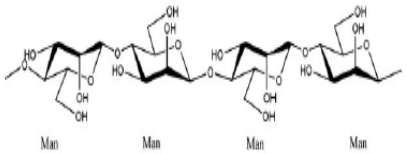
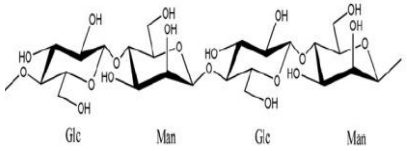
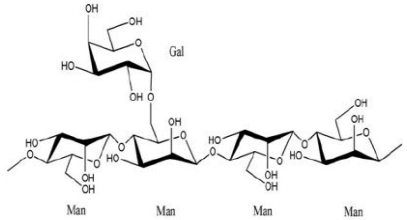
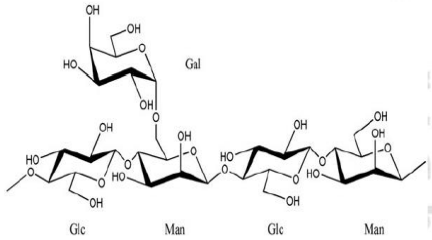
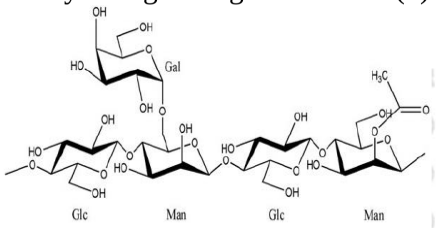
Subfamily of Mannans	DP	Sources	Ratio: Man:Glc:Gal	% Mannan content	Commercial Application	Potential Application
I. Linear mannan (1) 	15–80 (Aspinall, 1959)	Ivory nut, copra meal (Saittagaroon *et al*., 1983), some algae (Mackie and Preston, 1968), aloe vera, coffee	-	25-30 (Sundu *et al*., 2006)	-	Health-promoting effects (Simoes *et al.*, 2009)
II. Glucomannan (2) 	4200 (except eastern white pine; 90, and red wood; 60) (Al-Ghazzewi *et al*., 2007)	Konjac Hardwood	2:1:0 (Millane and Hendrixson, 1994) 1:1:0 (Liu *et al*., 2012b) 2:1:0, 3:1:0, 4:1:0 (Ishurd *et al*., 2006, Northcote, 1972, Willfor *et al*.,2003)	40-60 (Liu, 2004)	Gelling, thickening, suspending and film-forming agent	Prevention of chronic disease (Vuksan *et al*., 1999), and weight-control agent, pre-biotics (Tester *et al*., 2012; Al-Ghazzewi *et al*., 2007)
III. Galactomannan (3) 	n/a	Guar gum Tara gum Locus bean gum Fenugreek gum (Picout *et al*., 2002)	2:0:1 3:0:1 4:0:1 1:0:1 (Moreira and Filho, 2008; de O. Petkowicz *et al*., 2001)	70-80 (Cho and Samuel, 2010; Bouzouita *et al*., 2007; Andrade *et al*., 1999)	Food stabilizer, gel setting, food thickener (Schwartz and Bodie, 1983)	-

Table 2.1 Typical structures, sources, applications of different subfamilies of mannan polysaccharides (continued).

Subfamily of Mannans	DP	Sources	Ratio: Man:Glc:Gal	% Mannan content	Commercial Application	Potential Application
IV Galactoglucomannan Galactoglucomannan (4)  	15–100 (Willfor *et al*., 2003)	Seed endosperm (Kollarova *et al*., 2010) Softwood (Lundqvist *et al*., 2002) Aloe vera bulk water soluble extract (BSW) (Tai-Nin Chow *et al*., 2005)	3:1:1 3:1:1; 4:1:0.1 15:1:1 (Pu *et al*., 2008; Timell, 1964; Timell, 1965) 10:1.9–2.6: (Hannuksela and Herve du Penhoat, 2004)	n/a	-	Immuno modulation (Tai-Nin Chow *et al*., 2005) Biofuel resource (Lavoie *et al*., 2011)
Acetylated galactoglucomannan (5)  	100–150 (Timell, 1967) 10–20 (Lundqvist *et al*., 2002)	Softwood Hardwood (Teleman *et al*., 2003)	3:1:1 3:1:0.1 (Timell, 1967) 4:1:0.1 (Lundqvist *et al*., 2002)	n/a	-	-

(1) Linear mannan, a main chain of β-1,4-linked D-mannose (Man) units; (2) Glucomannan, a main chain of β-1,4-linked D-mannose (Man) and D-glucose (Glc) units; (3) Galactomannan, a linear backbone of β-1,4-linked D-mannose (Man) units with α-1,6-linked D-galactose (Gal) units attached to some D-mannose (Man) residues; (4) Galactoglucomannan, a backbone of β-1,4-linked D-mannose (Man) and D-glucose (Glc) units, with α-1,6-linked D-galactose (Gal) residues attached to some D-mannose (Man) residues; (5) Acetylated galactoglucomannan, galactoglucomannan with β-1,4-linked O-acetyl groups (Ac) attached to C-2 positions of D-mannose (Man) residue

Source: Scheller and Ulvskov, 2010

2.2 Mannan degrading enzymes

The mannans are completely hydrolyzed by a lot of various catalytic activities. β-Mannanase (1,4-β-D-mannan mannohydrolase, EC 3.2.1.78) is an endo-acting enzyme that catalyzes the random hydrolysis of the 1,4-β-D-mannosidic linkages in mannans. β-Mannosidase (β-D-mannopyranoside hydrolase, EC 3.2.1.25) is exoacting hydrolase that releases mannose from the oligosaccharides by attacking the terminal linkage, non-reducing β-D-mannose residues in β-D-mannosides. α-galactosidase (α-galactoside galactohydrolase, EC 3.2.1.22) removes the α-1,6-D-galactose side chains of galactomannan (Halstead *et al*., 2000).

2.2.1 Source of β-mannanase

The characteristic of mannan hydrolysis is extensive in the microbial world. Dhawan and Kaur (2007) have previously summarized the work on microbial mannanases.

Many researches have been studied on β-mannanase from different bacterial and fungal sources as presented in Table 2.2. The most bacteria producing β-mannanase are Gram-positive bacteria, especially *Bacillus* species (Mabrouk and Ahwany, 2008; Singh *et al*., 2010), nevertheless, some Gram-negative bacteria as *Klebsiella oxytoca* have also produced β-mannanase (Titapoka *et al*., 2008). The fungi, such as the genus *Penicillium* (Blibech *et al*., 2010), *Aspergillus* sp. (Kote *et al*., 2009) and *Trichoderma* sp. (Eneyskaya *et al*., 2009), have also been reported to be β-mannanase producers. Furthermore, some actinomycetes like *Streptomyces* sp. have also been investigated as the β-mannanase producers (Bhoria *et al*., 2009).

Table 2.2 β-Mannanase producing microorganisms.

Sources	References
Bacteria	
Bacillus sp. AM-001	Akino *et al*., 1988
Bacillus sp. KK01	Hossain *et al*., 1996
Bacillus licheniformis	Feng *et al*., 2003
Bacillus stearothermophilus	Talbot *et al*., 1990
Bacillus subtilis	Ratto and Poutanen, 1988
Vibrio sp. MA-138	Tamaru *et al*., 1995
Vibrio sp. MA-128	Araki *et al*., 1992
Vibrio sp. MA-129	Araki *et al*., 1992
Cellulomonas fimi	Stoll *et al*., 1999
Cadocellum saccharolyticum	Bicho *et al*., 1991; Gibbs *et al*., 1992
Caldicellulosiruptor sp. RT8B4	Gibbs *et al*., 1992
Clostridium butyricum strain Antonie Leewenhoek	Xiuzhu *et al*., 1991
Streptomyces sp.	Takahashi *et al*., 1984
Streptomyces scabies	Monitel *et al*., 1999
Streptomyces ipomoea	Monitel *et al*., 1999
Streptomyces lividans 66	Areand *et al*., 1993
Enterococcus casseliflavus FL 2121	Oda *et al*., 1993
Pseudomanas sp.	Yamamura *et al*., 1990
Pseudomanas fluorescens supsp. *Cellulose*	Braithwaite *et al*., 1993; Bolam *et al*., 1996; McCutchen *et al*., 1996
Thermotiga neapolitana 5068	Duffaud *et al*., 1997
Thermotiga maritime	Brown *et al*., 1993
Thermomonospora fusca	Hilge *et al*., 1998
Thielavia terrestris	Araujo *et al*., 1990
Polyporus versicolor	Johnson and Ross, 1990
Acromonas sp.	Arald, 1983

Table 2.2 β-Mannanase producing microorganisms (continued).

Sources	References
Fungi	
Aspergillus niger	Tsujisaka *et al*., 1972; Ademark *et al*., 1998; Ademark *et al*., 2001; Yamazaki *et al*., 1976
Aspergillus niger NCH-189	Lin and Chen, 2003
Aspergillus carbonarius	Ghareib and Nour-el-Dien, 1994
Penicillium simplicissimum	Luonteri *et al*., 1998
Penicillium ochrochloron	Dey *et al*., 1993
Rhodothermus marinus	Politiz *et al*., 2000
Aspergillus fumigatus	Puchart *et al*., 2003
Penicillium purpurogenum	Park *et al*., 1987
Penicillium sp. 23	Varbancts *et al*., 2001
Rhizopus niveus	Hashimoto *et al*., 1969
Trichoderma reesei RUT C-30	Zeilinger *et al*., 1993
Trichoderma reesei C-30	Arisan-atac *et al*., 1993
Trichoderma harzianum E58	Torrie *et al*., 1990
Thermomyces lanuginosus	Puchart *et al*., 1999
Trichoderma reesei	Stalbrand *et al*., 1993; Stalbrand *et al*., 1995; Margolles-Clark *et al*., 1996; Harjunpaa *et al*., 1995; Tenkanen *et al*., 1997; Buchert *et al*., 1993
Sporotichum cellulophilum	Araujo *et al*., 1991
Sclerotium rolfsii	Sachslehner and Haltrich, 1999; Sachslehner *et al*., 1998; Gubitz *et al*., 1996

Source: Sa-nguansook (2002)

2.2.2 β-Mannanase production conditions and properties

Most of β-mannanases produced by microbes are extracellular and inducible enzyme (Dhawan and Kaur, 2007). An inducer of β-mannanase, extensively used in many researches, is locust bean gum (LBG) as galactomannan-rich substrate (Kote *et al.*, 2009). Other substrates, such as copra meal, konjac powder and wheat bran are also used for the similar aim due to their low cost and plentiful availability (Zhang *et al*., 2009).

The β-mannanase production is highly influenced by nutritional and physicochemical factors, for instance, carbon and nitrogen sources, inorganic salts, pH, temperature, incubation time, agitation and dissolved oxygen concentration (Aziz *et al*., 2008; Moreira and Filho, 2008). Different microorganisms require varied incubation times for the production of the maximum β-mannanase. In regard to bacteria, it requires 24 h in case of *Acinetobacter* sp. ST 1-1 (Titapoka *et al*., 2008) to 96 h in *Bacillus* sp. MG-33 (Singh *et al.*, 2010). However, fungi range from 3 days in *Streptomyces* sp. PG-08-03 (Bhoria *et al*., 2009) to 11 days in *Aspergillus* ATCC 20114 (Mohamad *et al*., 2011). In case of incubation temperature, it has been reported in the mesophilic range for β-mannanase production. Bacteria normally grow and produce β-mannanase best at neutral to alkaline pH, reversely, fungi preferring acidic pH (Mabrouk and Ahwany 2008).

The most studies have produced β-mannanases by submerged fermentation (Kote *et al.*, 2009). However, the β-mannanase production by solid state fermentation (SSF) has been experimented a little bit. Palm kernel cake has been applied for the increasing β-mannanase production by SSF (Abdeshanian *et al*., 2009; Rashid *et al*., 2010). Optimization of the factors by response surface methodology has been used for enhancing many folds of the β-mannanase production (Lin *et al*., 2007).

The β-mannanase from bacteria commonly exhibited the pH optima of the neutral range (Mabrouk and Ahwany, 2008) and β-mannanase from fungi in the acidic range (Kote *et al*., 2009), nevertheless, optimum pH for activity of alkalophilic *Bacilus* sp. N16-5 has been reported at pH 9.5 (Ma *et al*., 2004), and the beneficial alkaline β-mannanases are expected to apply in the pulp and paper industry. The β-mannanase from microbes were reported to be active at various temperatures, ranging from 37 to 70°C (Eneyskaya *et al*. 2009; Ma *et al*., 2004). Generally, the bacterial β-mannanases

are more thermostable than the enzymes from fungi. This is an essential property for applications in the industries, especially pulp bleaching process.

Kinetics of the β-mannanase, namely, Michaelis–Menten constant (K_m) and the maximum velocity (V_{max}) values have been stated for various bacterial and fungal β-mannanases. Among bacterial β-mannanases, K_m and V_{max} values for *Bacillus* sp. MG-33 are 0.2 mg/ml and 60 U/min/mg, and for *Bacillus* sp. MSJ-5 are 11.67 mg/ml and 3.33×10^3 mM/min/mg on locust bean gum as substrate (Singh *et al*., 2010; Zhang *et al*., 2009). In case of fungal β-mannanases, K_m and V_{max} values for *Penicillium occitanis* Pol6 using locust bean gum as substrate are 17.94 mg/ml and 93.52 U/mg, and for *Aspergillus oryzae* NRRL 3448 are 5.5 mg/ml and 275 U/mg (Blibech *et al*., 2010; Fattah *et al*., 2009). A summary of the production conditions and properties of various microbial β-mannanases is shown in Table 2.3.

2.2.3 Applications of β-mannanase

The wide-ranging substrate specificities of β-mannanases are employed the numerous applications. The following part would mention some of the most recent applications of β-mannanases.

1) Nutraceutical production

1.1) Production of manno-oligosaccharides

Many soluble dietary fibers are reported to function as prebiotics, beneficially affecting on intestinal bacteria and enhancing human health. Konjac glucomannan has lately been reported to increase probiotics (Fan *et al.*, 2009) with glucomannan hydrolysis indicating more potential than unhydrolyzed glucomannan (Chen *et al.*, 2007). Konjac mannan, which is produced from the roots of *Amorphophallus konjac*, is employed as a thickening agent in foods and beverages. mannooligosaccharides (MOS) production from konjac flour hydrolysis by bacterial β-mannanase is expected for high value-added konjac flour (Al-Ghazzewi *et al.*, 2007). As a result of MOS prebiotic effects (Fan *et al.*, 2009), β-mannanases applied for MOS production as functional food ingredients shows commercially potential application. Moreover, clinical trials show the positive effects of MOS on human health. Consumption of MOS derived from coffee galactomannans 3 g/day, represents beneficially human health, i.e., low fat usage and higher fat excretion (Yeh *et al.*, 2009).

Table 2.3 Production conditions and characteristics of mannanases from different microorganisms.

Name of organism	Carbon source/ fermentation conditions	Temp. optima (°C) of activity	Temp. stability	pH optima of activity	pH stability	Molecular weight of protein (KDa)	Reference
Bacteria							
Acinetobacter sp. ST 1-1	CM[a]/ LBG[b], SF[c], 37°C,24 h, 150 rpm	40	100%/40-50°C/30 min	6.0	>80%/pH 3–10 / 24 h	NR[q]	Titapoka *et al.*, 2008
Bacillus amyloliquefaciens I0A 1	GM[d], SF[c], 35°C, 24 h, 150 rpm, pH 7	50	NR[q]	7.0	NR[q]	NR[q]	Mabrouk and Ahwany, 2008
Bacillus circulans M-21	GG[e], SF[c], 32°C, 36 h, 180 rpm, pH 8	50	>80/40°C/4h	7.0	>80%/pH 6–9/ 1 h/30°C	33.4	Mou *et al*., 2011
Bacillus sp. N16-5	KM[f], SF[c], 37°C,34 h, 230 rpm, pH 9.5–10	70	NR[q]	9.6	NR[q]	NR[q]	Lin *et al.*, 2007
Bacillus sp. MSJ-5	KP[g], SF[c], 32°C, 32 h, 220 rpm, pH 7	50	>50%/35–65°C/ 1 h	5.5	>70%/pH 6–9/ 1 h	40.5	Zhang *et al*., 2009
Bacillus sp. MG-33	WB[h]/Wheat straw rich soda pulp,SSF[i],30°C,	65	100%/50–60°C/2 h	6.5	100%/pH 6.5/ 4 h	NR[q]	Singh *et al.*, 2010
Bacillus subtilis SUT1	BIM[j],SF[c],30°C, 24 h, 150 rpm	NR[q]	NR[q]	NR[q]	NR[q]	NR[q]	Rattanasuk and Cairns, 2009

Table 2.3 Production conditions and characteristics of mannanases from different microorganisms (continued).

Name of organism	Carbon source/ fermentation conditions	Temp. optima (°C) of activity	Temp. stability	pH optima of activity	pH stability	Molecular weight of protein (KDa)	Reference
Bacillus subtilis strain (CD-3, CD-6, CD-9, CD-10, CD-23, CD-25)	BFM[k], SF[c], 37°C, 24 h, 180 rpm	45-65	50–70%/70°C/ 30 min	5.0-6.5	60–80%/pH 4.5/1h/ 37°C	NR[q]	Bo *et al*., 2009
Cellulosimicrobium sp. HY-13	LBG[b]/ M9 liquid medium, SF[c], 37°C, 48 h, 180 rpm	50	100%/37°C/ 1 h	7.0	>90%/pH 6–9/1h/ 4°C	34.9	Kim *et al*., 2011
Chryseobacterium indologenes	BIM[j], SF[c], 30°C, 24 h, 150 rpm	NR[q]	NR[q]	NR[q]	NR[q]	NR[q]	Rattanasuk and Cairns, 2009
Klebsiella oxytoca CW23	CM[a]/LBG[b], SF[c], 37°C, 18 h, 150 rpm	50	100%/50°C/ 30 min	7.0	100%/pH 3–6	NR[q]	Titapoka *et al.*, 2008
Paenibacillus sp. MSL –9	GG[e], SF[c], 30°C, 48 h, 160 rpm, pH 8	40	NR[q]	8.0	NR[q]	NR[q]	Manjula *et al.*, 2010
Paenibacillus sp. DZ3	LB[l] with Glucomannan, SF[c], 37°C,120 h, 200	60	100%/60°C/ 1 h	6.0	>70%/pH 5–7/1h/	39	Chandra *et al*., 2011

Table 2.3 Production conditions and characteristics of mannanases from different microorganisms (continued).

Name of organism	Carbon source/ fermentation conditions	Temp. optima (°C) of activity	Temp. stability	pH optima of activity	pH stability	Molecular weight of protein (KDa)	Reference
Fungi							
Aspergillus niger gr	LBG[b], SF[c], 37°C, 168 h, 180 rpm	65	> 50%/60°C/ 8 h	5.5	>80%/pH 4–8/ 16 h/4°C	NR[q]	Kote *et al.*, 2009
Aspergillus flavus gr	LBG[b], SF[c], 37°C, 168 h, 180 rpm	60	>50%/60°C/ 6 h	6.0	>80%/pH 4–8/ 16 h/4°C	NR[q]	Kote *et al.*, 2009
Aspergillus niger LW-1	PP[n]/LBG[b], SSF[i], 32°C, 96 h	NR[q]	NR[q]	NR[q]	NR[q]	NR[q]	Zhang *et al*., 2008
Aspergillus niger ATCC 20114	Peptone, SF[c], 30°C, 264 h, 150 rpm	50	NR[q]	4.0	NR[q]	NR[q]	Mohamad *et al.*, 2011
Aspergillus niger FTCC 5003	PKC[o], SF[c], 30°C, 150 rpm, 192 h, pH 5.5	NR[q]	NR[q]	NR[q]	NR[q]	NR[q]	Aziz *et al.*, 2008
Aspergillus niger FTCC 5003	PKC[o], SSF[i], pH 5	50	NR[q]	5.3	NR[q]	NR[q]	Abdeshanian *et al*., 2009
Aspergillus niger USM F-4	Molasses/PKC[o], SSF[i], 30°C, 120 h	60	NR[q]	4.0	NR[q]	NR[q]	Rashid *et al.*, 2010

Table 2.3 Production conditions and characteristics of mannanases from different microorganisms (continued).

Name of organism	Carbon source/ fermentation conditions	Temp. optima (°C) of activity	Temp. stability	pH optima of activity	pH stability	Molecular weight of protein (KDa)	Reference
Aspergillus niger	GG[e], SF[c], 30°C, 240 h, 150 rpm	50,70	>50%/60°C/ 6 h	3.5	>80%/pH 3.5–7.0/24 h/50°C	NR[q]	Norita *et al.*, 2010
Aspergillus oryzae NRRL 3448	LBG[b], Static culture, 30°C, 168 h	55	100%/55°C/ 15 min	5.5	100%/pH 4–6/ 2 h/45°C	NR[q]	Fattah *et al.*, 2009
Penicillium occitanis Pol 6	CSFL[p], 30°C, 168 h	40	>80%/50°C/ 30 min	4.0	>70%/pH 4–10/ 24 h	18	Blibech *et al.*, 2010
Scopulariopsis candida LMK 004	LBG[b], SF[c], 25°C, 150 rpm	50	100%/30–40°C/ 3 h	5.0	>80%/pH 5–6.5/ 24 h/4°C	41	Mudau and Setati, 2008
Trichoderma reesi	NR[q]	37	NR[q]	3.5	NR[q]	NR[q]	Eneyskaya *et al.*, 2009
Streptomyces sp. PG-08-03	GG[e], SF[c], 37°C, 72 h, 200 rpm, pH 8.0	75	NR[q]	8.0	NR[q]	NR[q]	Bhoria *et al.*, 2009

[a] Copra meal; [b] Locust bean gum; [c] Submerged fermentation; [d] Galactomannan; e Guargum; [f] Konjac mannan; [g] Konjac powder; [h] Wheat bran; [i] Solid state fermentation; [j] Bacterial isolation medium; [k] Bacterial fermentation medium; [l] Luria broth; [m] Defatted copra meal; [n] Potato peel; [o] Palm kernel cake; [p] Carob seed flour liquid medium, [q] Not reported (Chauhan *et al.*, 2012)

Guar gum, consisting mainly of galactomannans, is derived from the endosperm of guar seeds (*Cyamopsis tetragonoloba*). Partially hydrolysed guar gum (PHGG) is used to relieve irritable bowel syndrome (IBS) symptoms (Zhang *et al.*, 2009). This factor supports the application of PHGG as a fiber supplement. In the same way, supplementation of a commercial PHGG to oral rehydration solution was indicated to relieve acute noncholera diarrhoea in children (Kumao *et al.*, 2006).

The preparation of MOS is simple and does not require the application of special β-mannanases because MOS is prepared from natural raw material mannan hydrolysis by only a β-mannanase as opposed to a cocktail of enzymes.

1.2) Pharmaceutical applications

D-Mannose, obtained from birch and beech wood hydrolysis, is an added ingredient. Due to high solubility of mannose, it is taken for the fast dissolving tablets production (Parisi *et al.*, 2002). Intake of mannose is a general treat for urinary system infections. In addition, several mannose-added products are obtainable on the market. Recently sulphite spent liquors separation by chromatography obtains mannose (Alam *et al.*, 2000). Low-priced sources of mannose are represented mannan-rich substrates like copra meals and palm kernel. Mannose-added animal feeds using crude enzyme from *A. niger* have been reported (Fu *et al.*, 2006). The method relates the addition of a mixture of mannan-degrading enzymes and cellulases to complete hydrolysis. However, hydrolysate extraction and purification of mannose or MOS show a high-priced method for acquiring these products.

Accomplished mannan-containing substrates hydrolysis would require a combination of mannan-degrading enzymes, cellulases and proteases. However, palm kernel press cake hydrolysis with various mixtures of enzymes provided few proofs of cooperation between the enzymes (Heikkila, 1986). Higher β-mannosidase activity coherent β-mannanase enhanced mannose yields when solid loadings increased (Yokomizo, 2009). The supplementation of a β-galactosidase to the β-mannanase/β-mannosidase combination did not remarkably increase mannose yields. The substrate appearing proteins did not restrictively entry of the enzymes to the polysaccharides

while protease supplementation did not improve mannose yields (Jorgensen *et al.*, 2010).

2) Food and feed applications

2.1) Hydrolysis of mannan in coffee spent ground

Efficient degradation of coffee mannan reduces significantly viscosity (Nicolas *et al*., 1998). As a result of the enzymatic reaction, the coffee extract could be concentrated by a low-priced method like evaporation. β-Mannanases from fungi are appropriate to this application due to a suitable pH of coffee spent ground as pH 5 (Van Zyl *et al*., 2010). The effective hydrolysis of mannans in the coffee extract has applied both crude and partially purified mannanase (Nunes *et al*., 2006).

2.2) Animal feeds

Monogastric animals, like poultry pigs, and humans, cannot efficiently digest fiber-rich feeds. High protein and oil feeds, added soya, guar, palm and coconut kernel meals, are carried out. Balancing feeds is challenging. However, a lot of supplements cannot be added in feeds due to bad digestion in chicken, flatulency in pigs and undegradation the mannans or MOS in feeds (Chong *et al*., 2008). The high mannan meals treated with β-mannanases has been reported to improve the body weight in broiler and also reduce the flatulency in pigs (Kim *et al*., 2006; Wu *et al*., 2005).

Laying hens are frequently suffered privation for food or water to motivate egg laying and enhance egg quality. This leads to their weakened health to infect *Salmonella enteritidis* (Gutierrez *et al*., 2008). Chickens, fed with MOS or palm kernel meal, reduced *S. enteritidis* numbers, while the increase of probiotic bacterial numbers was observed (Fernandez *et al*., 2002). Laying hens, fed guar meal with β-mannanase, was demonstrated the positive effects of natural molting with better resistance to *S. enteritidis* (Gutierrez *et al*., 2008).

3) Commodity production

3.1) Paper and pulp production

β-Mannanase and other mannan-degrading enzymes can cleave the mannan composition in the pulp without effecting cellulose. An important step for pulps bleaching is the removal of lignin from pulp. Alkaline pulp pretreatment degrades

hemicelluloses, which are covalently bound to lignin and easy to remove lignin. The main disadvantage of alkaline treatment is an environmental pollution due to chlorinated compounds. Therefore, β-mannanases with other enzymes as xylanases could remove equally lignin in pulp bleaching compared to alkaline pretreatment (Dhawan and Kaur 2007).

Softwood pulps contain 15–20% hemicelluloses as galactomannan. Mannanases, specific to galactomannan, would be selected for softwood pulp bleaching (Gubitz *et al*., 1997). Furthermore, the thermostable and alkaline β-mannanases are more beneficial for the pulping process (He *et al*., 2008).

3.2) Detergent formulations

Alkaline β-mannanases, lately found in laundry portion, are stain removal aiders. Mannans are commonly found in gums, applied as thickening agents in sauces, ice-creams, tooth-pastes, hair gels, shampoos and conditioners. From adsorption mannans to cellulose fibers, mannan stains are difficultly removed. β-Mannanases, degrading the gum polymer into smaller molecules, reduce stains appearing. These smaller molecules, water soluble, can remove during the washing. These constituents are also applied as sanitization products, beauty and health care products, hard surface cleaners and contact lens cleansers (Bettiol *et al*., 2000).

3.3) Biofuel production

The second generation bioethanol production from lignocellulosic materials has more attended. Residues from several processes could be raw materials for bioethanol production. The β-mannans are presented in low level in hardwood and herbaceous lignocellulosic residues, but higher in softwoods. However, the bioethanol production from lignocellulosic materials has been ignored (Gírio *et al*., 2000). It is important to many commercial enzyme cocktails including low β-mannanases (Berlin *et al*., 2007). The application of β-mannanases for mannan hydrolysis is as essential as the application of xylanases. In spite of softwood hemicelluloses presented high galactoglucomannan, relation between β-mannanase efficiency on pretreated softwood and levels of enzyme is insignificant (Berlin *et al*., 2005). Recently, Yokomizo (2009) reported that palm kernel press cake (PKC) containing 50% hexose sugars in form of glucan and galactomannan. In theory, it was probable to gain 88% of mannose yields

without thermochemical pretreatment. An optimal combination of β-mannanases, β-mannosidases and cellulases hydrolyzed effectively PKC polysaccharides, and when integrated with a simultaneous saccharification and fermentation, obtained ethanol yields (200 g ethanol/kg PKC).

In the case of PKC, after fermentation of oil cake residues contain less fiber and higher protein (17 - 28%). To produce high value added products from mannan-rich substrates is possible. Firstly, PKC could be applied for the mannose and MOS production. Secondly, PKC could be raw materials for bioethanol production. Finally, the protein-rich residues could be supplemented to animal feeds. This case focused on a combination of enzymes to hydrolyze for various applications. In the case of biofuel production, all polysaccharides hydrolysis by mannan-degrading enzymes and cellulases showed a 5-fold glucose yields (Jorgensen *et al*., 2010).

2.3 Enzyme purification

"Do not waste the clean thinking on dirty enzyme." is a reminding of Efraim Racker (Deutscher, 1990). The combination of thousands of various enzymes released from a broken yeast, or bacterial cell. To study enzyme catalysis usually wastes time until the enzyme has been purified away from a crude cell extract. Enzyme purification led to the explanation of a catalytic activity, reaction pathway, property of enzyme and its reactivity to regulatory molecules that increase or decrease activity. Protein purification procedures are generally operated by many varied techniques (Upathanpreecha, 2003).

2.3.1 Protein precipitation

Enzyme precipitation using some salts can separate the enzyme from other compositions. Two main methods of protein precipitations are salting-out and reduction of protein solubility. Salting-out of proteins is carried out by enhancing the ionic strength of a protein solution by adding inorganic salts like Na_2SO_4 or $(NH_4)_2SO_4$. The ions of the salts, reacting with water more powerfully, cause molecules of protein to precipitate. Organic reduction is achieved by adding organic solvents like ethanol or acetone. Their effects are the same effects of salting-out; i.e., they reduce the solubility of protein (Deutscher, 1990 cited in Upathanpreecha, 2003).

2.3.2 Dialysis

Dialysis could separate higher molecules (molecular weights more than 15-20 kDa) from the dialysis buffer, using dialysis tube containing semipermeable membrane. Therefore, it is used as a concentration procedure on the laboratory scale. Furthermore, it is extensively used during purification methods for desalting or exchanging buffer of enzyme without concentration. The dialysis is operated by the dissimilar concentration of the solutes in the two sides of membrane; until diffusion of solutes is equal both sides. Concentration of the solutes in the protein solution can reduce by changing buffer for dialysis. This process is only appropriate for of a few liters volumes (Scawen and Melling, 1985 cited in Sa-nguansook, 2002).

2.3.3 Ion exchange chromatography

Ion exchange chromatography (IEC) is the most normally chromatographic procedure of protein purification. The advantage of IEC is its ability, its high capacity, its high resolving force and its direct basic principle.

Proteins take both positive and negative charged groups on their surface because of the side chains of acidic and basic amino acids. Positive groups are lysine, arginine, histidine and N-terminal amines. Negative charges are glutamic, aspartic acids, cysteine residues and C-terminal carboxyl groups. The net charge of protein relies on the numbers of positive and negative charged groups, or varies with pH. Isoelectric point (pI) is the pH that a protein has an equal number of positive and negative charged groups. Below their pI proteins have a net positive charged while above their pI overall charge is negative.

Ion exchange matrices are classified into two kinds: anion exchangers (positively-charged types for the adsorption of anionic proteins) or cation exchangers (negatively-charged types for the adsorption of cationic proteins). Each kind of exchanger is also categorized as strong or weak depending on the ionic strength of the fractional group. The quaternaryaminoethyl (QAE) group is usually used in strong anion exchange and the sulpho group for strong cation exchangers, while weak anion exchangers contain diethylaminoethyl (DEAE) group and the carboxymethyl (CM) group for weak cation exchangers. The ionogenic groups used in ion exchange of

proteins with their structural formula are presented in Table 2.4 (Palmer, 1981; Scopes, 1982; Harris and Angal, 1989; Janson and Rydén, 1989, cited in Urairuj, 2003).

Table 2.4 Ion exchange groups used in the purification of proteins.

Formula	Name	Abbreviation
Strong anion		
$-CH_2N^+(CH_3)_3$	Trimethylaminoethyl	TAM-
$-C_2H_4N^+(C_2H_5)_3$	Triethylaminoethyl	TEAE-
$-C_2H_4N^+(C_2H_5)_2CH_2CH(OH)CH_3$	Quaternaryaminoethyl	QAE-
Weak anion		
$-C_2H_4N^+H_3$	Aminoethyl	AE-
$-C_2H_4NH(C_2H_5)_2$	Diethylaminoethyl	DEAE-
Srong cation		
$-SO_{3-}$	Sulpho	S-
$-CH_2\ SO_{3-}$	Sulphomethyl	SM-
$-C_3H_6\ SO_{3-}$	Sulphopropyl	SP-
Weak cation		
-COO-	Carboxy	C-
$-CH_2COO-$	Carboxymethyl	CM-

Source: Harris and Angal (1989, cited in Urairuj, 2003)

2.3.4 Gel filtration chromatography

Gel filtration chromatography (GFC) is a partial chromatography for separating molecules of various sizes. The fundamental principle of GFC is the stationary phase containing porous beads with a definite range of pore sizes. Four groups of gels are classified as dextran, agarose, polyacrylamide and combined polyacrylamide-dextran. The separation process is achieved using a porous gel matrix which is packed in a column and enclosed by solvent. Realize a sample consisting of a combination of molecules smaller and larger than the pores of the stationary phase matrix, including moderate molecules. The larger molecules are driven from the stationary phase and thus move early from the column. The smaller molecules can entry inside of the matrix pores and elute more slowly through the column, presenting as the last compositions in the chromatogram. Moderate molecules can entry the stationary

phase, but spend less time than smaller molecules. Therefore, these molecules are removed both larger and smaller molecules. A partition coefficient (K_{av}), presenting a molecule entering the matrix pores, is shown by the equation;

$$K_{av} = \frac{(V_e - V_o)}{(V_t - V_o)}$$

Where V_e = volume of solvent required to elute the solute from gel column or bead (experimentally examined)

V_t = total volume of gel column or bead

V_o = elution volume is equal to the void per exclusion volume

This is shown obviously that K_{av} is inversely proportional to the logarithm of the molecular weight of solute. Each group of gel can exclude molecules larger than a specific size, and thus fractionate molecules within a specific range and desalt solutions of proteins (Scopes, 1982; Harris and Angal, 1989; Janson and Rydén, 1989; Deutscher, 1990, cited in Upathanpreecha, 2003).

Table 2.5 Some traditional media for gel filtration of proteins.

Media	Gel type	Company	Particle size of hydrated beads (μm)	Fractionation range for globular protein (kDa)	pH stability
Sephadex	Dextran	Pharmacia			2-10
G-50				1.5-30	
G-75			20-100[a]	3-80	
G-100			100-300[a]	4-100	
G-150				5-300	
G-200				5-600	
Sepharose	Agarose	Pharmacia			4-9
5B			45-165	10-4,000	
4B			45-165	60-20,000	
2B			60-200	70-40,000	
Sepharose CL	Agarose	Pharmacia			3-14
6B			45-165	10-4,000	
4B			45-165	60-20,000	
2B			60-200	70-40,000	
Ultrogel	Agarose	Reactifs IBF			Not stated
A6				25-2,400	
A4			60-140	55-9,000	
A2				120-23,000	

Table 2.5 Some traditional media for gel filtration of proteins (continued).

Media	Gel type	Company	Particle size of hydrated beads (μm)	Fractionation range for globular protein (kDa)	pH stability
Bio-Gel	Agarose	Bio-Rad			
A-0.5 m			40-80	1-500	
A-1.5 m			80-150	1-1,500	
A-5 m			150-300	10-5,000	
A-15 m				40-15,000	
A-50 m				100-50,000	
Bio-Gel	Polyacrylamide	Bio-Rad			4-13
P-10			<40	1.5-20	
P-30				2.5-40	
P-60			<80	3-60	
P-100				5-100	
P-150			<80	15-150	
P-200				30-200	
P-300				60-400	
Glycophase CPG	Silica	Pierce Eurochemie			<8
100		B.V.		1-30	
200			37-74	2.5-100	
460				11-320	

Table 2.5 Some traditional media for gel filtration of proteins (continued).

Media	Gel type	Company	Particle size of hydrated beads (μm)	Fractionation range for globular protein (kDa)	pH stability
Ultrogel	Agarose/	Reactifs IBF			3-10
ACA 202	Acrylamide			1-15	
ACA 54				5-70	
ACA 44			60-140	10-130	
ACA 34				20-350	
ACA 22				100-1,200	
Sephacryl	Dextran/	Pharmacia			Not stated
S-200	Bisacrylamide		40-105	5-250	
S-300				10-1,500	
S-200 HR			40-105	20-8,000	
S-300 HR					
S-400 HR			25-75		

[a] Calculated from the stated dry bead diameter through : $d_{p,wet} = d_{p,dry}(1 + Wr \times d)^{1/3}$
Where Wr = water regain of bead and d = density of matrix.
Source: Janson and Rydén (1989 cited in Urairuj, 2003)