Applied Environmental Science and Engineering for a Sustainable Future

Zainul Akmar Zakaria
Cristobal N Aguilar
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Parameswaran Binod *Editors*

Valorisation of Agro-industrial Residues – Volume II: Non-Biological Approaches

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Zainul Akmar Zakaria • Cristobal N Aguilar •
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Parameswaran Binod
Editors

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Editors
Zainul Akmar Zakaria
School of Chemical and Energy
Engineering, Faculty of Engineering
Universiti Teknologi Malaysia
Johor Bahru, Malaysia

Cristobal N Aguilar
Facultad de Ciencias Quimicas
Universidad Autonoma de Coahuila
Saltillo, Coahuila, Mexico

Ratna Dewi Kusumaningtyas
Department of Chemical Engineering,
Faculty of Engineering
Universitas Negeri Semarang
Semarang, Indonesia

Parameswaran Binod
Microbial Processes and Technology Division
CSIR-National Institute for Interdisciplinary
Science and Technology (CSIR-NIIST)
Thiruvananthapuram, Kerala, India

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Contents

Contributors

Asha P. Antony Department of Biotechnology and Microbiology, Kannur University, Kannur, Kerala, India

Erna Astuti Department of Chemical Engineering, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

Nur Izyan Wan Azelee School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

R. Baeza-Jiménez Centro de Investigación en Alimentación y Desarrollo, Delicias, Chihuahua, Mexico

Parameswaran Binod Microbial Processes and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram, Kerala, India

Arief Budiman Department of Chemical Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia

J. J. Buenrostro-Figueroa Centro de Investigación en Alimentación y Desarrollo, Delicias, Chihuahua, Mexico

Florencia Cattaneo Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Achmad Chafidz Department of Chemical Engineering, Universitas Islam Indonesia, Yogyakarta, Indonesia

Daniel Joe Dailin School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia
Institute of Bioproduct Development, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

Daniyanto PT Perkebunan Nusantara (PTPN) XI, Surabaya, Indonesia

Mousumi Das M Department of Biotechnology and Microbiology, Kannur University, Kannur, Kerala, India

Rizqy Romadhona Ginting Department of Chemical Engineering, Graduate School of Engineering, Hiroshima University, Hiroshima, Japan

Dhoni Hartanto Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia

María Inés Isla Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Ratna Dewi Kusumaningtyas Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia

L. X. López-Martínez CONACyT—Centro de Investigación en Alimentación y Desarrollo, Culiacán, Sinaloa, Mexico

Ajeng Y. D. Lestari Department of Chemical Engineering, Universitas Islam Indonesia, Yogyakarta, Indonesia

Khoirun Nisa Mahmud School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia

Nor Hasmaliana Abdul Manas School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

Megawati Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia

Mitamaytawati Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia

María Alejandra Moreno Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Zahrul Mufrodi Department of Chemical Engineering, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

Noor Idayu Nashiruddin School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

María Eugenia Orqueda Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Jorgelina Pérez Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Ashok Pandey Centre for Innovation and Translational Research, CSIR- Indian Institute for Toxicology Research (CSIR-IITR), Lucknow, Uttar Pradesh, India

Agus Prasetya Department of Chemical Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia

Nur Qudus Civil Engineering Department, Faculty of Engineering, Universitas Negeri Semarang, Gunungpati, Semarang, Indonesia

Roshanida A. Rahman School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

Rochmadi Department of Chemical Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia

Ivana Fabiola Rodríguez Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Hasan Abdul Rohman Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia

E. Rojo-Gutiérrez Centro de Investigación en Alimentación y Desarrollo, Delicias, Chihuahua, Mexico

Sabu Abdulhameed Department of Biotechnology and Microbiology, Kannur University, Kannur, Kerala, India

Wahyudi Budi Sediawan Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia

D. R. Sepúlveda Centro de Investigación en Alimentación y Desarrollo, Delicias, Chihuahua, Mexico

Shalyda Md Shaarani Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Kuantan, Pahang, Malaysia

Raveendran Sindhu Microbial Processes and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram, Kerala, India

Supranto Department of Chemical Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia

Sutijan Department of Chemical Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia

Swapna Kunhiraman Department of Biotechnology and Microbiology, Kannur University, Kannur, Kerala, India

Sebastián Torres Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Florencia María Correa Uriburu Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Zainul Akmar Zakaria School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia

Iris Catiana Zampini Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

Chapter 1
Pretreatment and Enzymatic Hydrolysis of Lignocellulosic Biomass for Reducing Sugar Production

Noor Idayu Nashiruddin, Nor Hasmaliana Abdul Manas, Roshanida A. Rahman, Nur Izyan Wan Azelee, Daniel Joe Dailin, and Shalyda Md Shaarani

Abstract Conversion of lignocellulosic biomass into reducing sugar has contributed to an alternative use of lignocellulose source, especially in the production of value-added products such as amino acids, biofuels, and vitamins. In the bioconversion process, pretreatment of lignocellulosic biomass is important to enhance the accessibility of enzyme hydrolysis, thus increasing the yield of reducing sugar. Lignocellulosic biomass has a very complex arrangement of structure that needs a proper study in pretreatment and enzymatic hydrolysis process to obtain an optimum yield of reducing sugar. This chapter discusses chemical and enzymatic pretreatment methods that are commonly applied to effectively modify the chemical structures of lignocellulosic biomass. Acid pretreatment using dilute sulfuric acid (H_2SO_4) is the most commonly employed for chemical pretreatment while sodium hydroxide (NaOH) is the most commonly applied for alkaline pretreatment because of its ability to delignify biomass. Then, enzymatic hydrolysis of lignocellulosic biomass for the production of reducing sugar is discussed in detail. The kinetics and optimization of hydrolysis which are the key parameters that determine the yields of reducing sugar are also presented. The right pretreatment method combined with

N. I. Nashiruddin · N. H. A. Manas · R. A. Rahman (✉) · N. I. W. Azelee
School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia
e-mail: hasmaliana@utm.my; r-anida@utm.my; nur.izyan@utm.my

D. J. Dailin
School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

Institute of Bioproduct Development, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia
e-mail: jddaniel@utm.my

S. M. Shaarani
Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Kuantan, Pahang, Malaysia
e-mail: shalyda@ump.edu.my

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an efficient hydrolysis process will ensure successful conversion of lignocellulosic biomass into reducing sugar, thus providing a sustainable production of reducing sugar from biomass for various applications.

Keywords Lignocellulosic biomass · Pretreatment · Enzymatic hydrolysis · Reducing sugar · Kinetic

1.1 Introduction

Lignocellulosic biomass is known as a potential renewable and inexpensive energy source because of the abundant material in most countries, especially in Asia (Chapla et al. 2011). Large amounts of lignocellulose generated through forestry, agricultural, and agro industries are categorized as waste that causes environmental pollution problem because of the large accumulation in nature. The utilization of biomass as a renewable source of raw material for various industrial applications is a great alternative to solve the issue. For example, conversion of biomass to bioenergy is an environmental friendly and safe process and could replace fossil fuels in a sustainable manner due to process that release low carbon dioxide and sulfur dioxide that become a serious threat to human health (Roberto et al. 2003; Van Wyk 2001). The degradation or bioconversion of biomass contributes not only to the production of potential products, but also eliminates the environmental pollution caused by their accumulation and abundance in nature (Howard et al. 2003).

Recently, a lot of research have been performed to find the source of biomass, mainly lignocellulosic material that can potentially be converted into many value-added products such as chemicals, biofuels, cheap energy sources for fermentation, human nutrients, and improved animal feeds. The major source of lignocellulosic biomass are agricultural wastes, such as wheat straw, rice straw, wood (hardwood), by-products of leftover from the corn milling process, annual and perennial crops, waste paper, and sweet sorghum (Visioli et al. 2014). Lignocellulosic biomass has been known as a material that is considered as the only sustainable source of organic carbon in the earth and suitable to produce fuels and fine chemicals with zero net of carbon emission (Ragauskas et al. 2006; Zhou et al. 2011). Many studies have shown that lignocellulosic biomass holds enormous potential for the sustainable production of biofuels, biomolecules, and biomaterials, which can decrease CO_2 emissions and atmospheric pollution (Mäki-Arvela et al. 2010; Sun et al. 2011; Barakat et al. 2013).

Lignocellulosic biomass mainly composed of cellulose, hemicellulose, lignin, and ash (Kumar et al. 2009). Lignocellulose compositions play an important role in the performance of the conversion of biomass into high-value products (Mood et al. 2013). The chemical properties of these components make them substrate of enormous biotechnological value (Howard et al. 2003). Cellulose is the main composition in lignocellulosic biomass (35–30% (w/w)) followed by hemicellulose (20–35% (w/w)) and lignin (15–20% (w/w)). Other components of lignocellulose are ash and others (15–20% (w/w)). The compositions of these constituents are different from

Table 1.1 Cellulose, hemicellulose and lignin contents in common agricultural residues and waste

Lignocellulosic material	Cellulose % (w/w)	Hemicellulose % (w/w)	Lignin % (w/w)	References
Pineapple leaves fiber (PALF)	66.2	19.5	4.2	Daud et al. (2014)
Corn cobs	33.7	31.9	6.1	Nigam et al. (2009)
Wheat straw	30.2	18.7	17	Ballesteros et al. (2006)
Switchgrass	39.5	20.3	17.8	Li et al. (2010)
Sugarcane bagasse	43.1	31.1	11.4	Martin et al. (2007)
Rice straw	31.1	18.7	13.3	Chen et al. (2011)
Kenaf	37–49	18–24	15–21	Li et al. (2007)
Jute	61.1–71.5	13.6–20.4	12–13	Taj et al. (2007)
Hemp	70–74	17.9–22.4	3.7–5.7	Taj et al. (2007)
Abaca	56–63	15–17	12–13	Taj et al. (2007)
Fiber flax	71	18.6–20.6	2.2	Li et al. (2007)
Seed flax	43–47	24–26	2.2	Li et al. (2007)

one plant species to another as illustrated in Table 1.1. The compositions depend on many factors such as genetic variability, environmental influences, soil type, and climate (Kshirsagar et al. 2015).

Cellulose ($(C_6H_{10}O_5)_n$) is a linear polymer of D-glucose linked through β-(1,4)-glycosidic bond, wrapped by hemicellulose and lignin (Chen 2014). The complete hydrolysis of cellulose in the presence of acid solution gives glucose, which affirms the way that cellulose is made of glucose unit. Cellulose is a biodegradable, cheap, and renewable polymer, which is fibrous, intense, and water insoluble and helps in sustain the cell wall structure of plants, oomycetes, and algae (Suhas et al. 2016). The total annual amount of cellulose is several billion tons, revealing the large economic value of it. This type of polymer usually contains carbon (44.44%), hydrogen (6.17%), and oxygen (49.39%) (Chen 2014).

Hemicellulose ($(C_5H_8O_4)_m$), was located in the secondary walls, which are heterogenous branched biopolymers that contain pentose (β-D-xylose, α-L-arabinose), hexose (β-D-mannose, β-D-galactose, β-D-glucose), and organic acids (α-D-4-*O*-methyl-galacturonic, α-D-glucuronic, and α-D-galacturonic acids) (Mood et al. 2013). Its monomer units can be fermented to the production of biofuel (Jacobsen and Wyman 2000). In biomass, hemicellulose is connecting to lignin and cellulose by covalent bonds and hemicellulose much easier to breakdown compared to crystalline cellulose because it is involved in a few hydrogen bonds. Based on the main sugar residue in the backbone, different classification of hemicellulose has been identified which are xylan, glucans, β-glucans, xyloglucans, glucuronoxylans, arabinoxylans, glucomannans, galactomannans, and galactoglucomannans. Xylan is the most abundant hemicellulose (Badal 2003).

Lignin ($C_9H_{10}O_2$, $C_{10}H_{12}O_3$, $C_{11}H_{14}O_4$) is an aromatic polymer synthesized from phenyl propanoid precursors (Mood et al. 2013). Lignin is a phenolic polymer with a three-dimensional network that gives a hydrophobic surface to water conducting and gives rigidity of structure that needed for mechanical support (Wilfred 2008). Lignin made out of entangled phenylpropane unit nonlinearly and randomly connected where is the main monomers are coniferyl alcohol, coumaryl alcohol, and sinaphyl alcohol and because of this three different monomers, these polymers, lignin, are divided into three types which are guaiacyl lignin polymerized by guaiacyl popanen, syringyl lignin polymerized by syringyl propane and hydroxyl-phenyl lignin polymerized by hydroxyl-phenyl propane (Chen 2014). Strong intermolecular and intramolecular hydrogen bonds are made from hydroxyl and many polar groups that exist in lignin structure, hence make the lignin insoluble in any solvent.

The major difference between hemicellulose and cellulose is those hemicelluloses contain branches with short lateral chains that consist different sugars while cellulose contains easily hydrolyzable oligomers. On the other hand, lignin is linked to both cellulose and hemicellulose and forming a physical seal that is an impenetrable barrier in plant cell wall (Sanchez 2009; Singh and Bishnoi 2011a).

In the middle of the twentieth century, plant fibers (biofibers) are widely used in industries for numerous applications from many resources (Asim et al. 2015). The important property of natural biofibers is biodegradability and noncarcinogenic which makes it suitable for many applications including automobiles, building construction, furniture for machinery used and packaging, railway coach, and partition wall cabinet (Asim et al. 2015). The potential of natural fiber-based composite as reinforcing fibers in both thermoplastic resins and thermosetting receive high attention all over the world among scientist because of their excellent specific properties (Mishra et al. 2004). In Malaysia, natural biofibers are important agricultural biomass that contributes to the Malaysian economy (Shawkataly et al. 2006). Large area of plantation of oil palm, coir, banana, and pineapple which were 3.87 million ha, 147 thousand ha, 34 thousand ha, and 15 thousand ha, respectively, can generated large quantities of cellulosic and noncellulosic raw materials during harvesting (Malaysia Palm Oil Board (MPOB) 2011; MAO (Ministry of Agriculture) 2006). Large quantities of raw materials in this country have generated a large amount of vegetable waste, thus create problems such as replanting operations. Therefore, economic utilization of this natural biofiber will be beneficial. Abundance of biofibers in Malaysia provides an opportunity to explore the possibility of their utilization for various applications (Mishra et al. 2004). Unfortunately, the exploring of utilization of these biofibers is very limited due to lack of knowledge about their process.

1.2 Chemical Pretreatment of Lignocellulosic Biomass

Pretreatment basically refers to the disruption of carbohydrate–lignin complex that limits the enzymatic hydrolysis to cellulose and hemicellulose (Parameswaran and Ashok 2015). There are few methods to enhance the enzymatic hydrolysis, for

example, decreased the particle size which is improving the available surface area. The advantageous impacts of pretreatment of lignocellulosic materials have been perceived for quite a while. Pretreatment should meet a few requirements, for example, enhance the formation of sugar or the ability to subsequently form sugars by hydrolysis, maintain the carbohydrate contents, reduce the formation of by-products that are inhibitory to the subsequent hydrolysis and fermentation process and be profitable (Kumar et al. 2009).

Pretreatment is the most important step in lignocellulosic biomass processing where carbohydrate polymers are converted into simple sugars before fermentation. Due to the heterogenous and very complex nature of the lignocellulosic biomass, enzymatic hydrolysis is not an efficient method for native biomass. Hence, lignocellulosic biomass needs to be pretreated to make in amenable to enzyme action (Parameswaran and Ashok 2015).

In general, there are four different pretreatment methods, which are physical, chemical, physiochemical, and biological (Mood et al. 2013). Among these pretreatments, chemical pretreatments are demonstrated to effectively modify the chemical structures of lignocellulose (Young and Kyoung 2015). The utilization of chemicals in pretreatment, neutralization, and fermentation conditioning should be minimal and cheap. Acid pretreatment involves utilization of sulfuric acid, nitric, or hydrochloric acid to remove hemicellulose and expose cellulose to enzymatic hydrolysis while alkali pretreatment involves alkaline solution, to remove lignin and different uronic acid substitutes on hemicellulose that will lower the accessibility of enzyme to the hemicellulose (Parameswaran and Ashok 2015). Table 1.2 shows the advantages and disadvantages of different pretreatment methods for lignocellulosic biomass.

1.2.1 Dilute Acid Pretreatment

In lignocellulosic pretreatment using concentrated acid, low temperature and high acid concentration are used to obtain high sugar yield without enzymatic hydrolysis as the addition hydrolysis process (Young and Kyoung 2015). In 1819, Henri Braconnot, a French chemist, is the first who found that lignocellulose could be converted into fermentable sugar by using concentrated sulfuric acid (Young and Kyoung 2015). Acid pretreatment using sulfuric acid is the most commonly employed for chemical pretreatment of lignocellulosic biomass where polysaccharide, mostly hemicellulose is hydrolyzed to monosaccharide and leads to higher accessibility of cellulose for enzymatic hydrolysis (Mood et al. 2013). There are several types of acid use in lignocellulose pretreatment including mineral acids which are sulfuric, hydrochloric, hydrofluoric, phosphoric, nitric, and formic acids, in either concentrated or diluted form and organic acids which are maleic, acetic, and oxalic acids (Kumar et al. 2009; Zhang et al. 2016).

Acid pretreatment could be formed either under low acid concentration with high temperature or under high acid concentration with low temperature. Pretreat

Table 1.2 Advantages and disadvantages of different chemical pretreatment methods of lignocellulosic biomass

Pretreatment	Advantages	Disadvantages	References
Dilute acid	• Less corrosion problem • Less formation of inhibitor • Increase cellulose accessibility and max fermentable sugar production	• Generation of degradation products • Low sugar concentration in exit stream	Rabemanolontsoa and Saka (2016) Alvira et al. (2010) Kshirsagar et al. (2015)
Alkali	• Does not require complex reactor • Operated at low temperature • Less sugar degradation • Improve enzyme accessibility	• Long residence time	Rabemanolontsoa and Saka (2016), Mood et al. (2013), Ganesh and Min-Kyu (2015)
Autohydrolysis	• Simple process • No chemical recovery • Environmental friendly • Less degradation product • Less equipment	• Re-localize lignin structure-slightly increase lignin content	Lee et al. (2009, 2010)

lignocellulosic biomass by using concentrated acid has both advantages and disadvantages. Concentrated acid is more economical than dilute acid since it is performed at low temperature while diluted acid needs to perform under high temperature (Mood et al. 2013). However, there are few disadvantages, for example, corroding of the equipment, pose to environmental pollution, consume high acid, energy demand for acid recovery, and production of fermentation inhibitor which is 5-hydroxylmethyl furfural (5-HMF) (Rabemanolontsoa and Saka 2016).

In efforts to overcome the drawbacks of concentrated acid, dilute acid hydrolysis gives an advantage by lowering the acid consumption but required high temperature and applied a strong condition to produce glucose from crystalline cellulose and result in a major degradation of the amorphous hemicellulose (Rabemanolontsoa and Saka 2016). Dilute sulfuric acid (H_2SO_4) pretreatment had the potential to remove lignin and broke down hemicellulose into pentose sugar without any application of enzyme (Thirmal and Dahman 2012) Dilute H_2SO_4 had been employed in many previous works on acid–base pretreatment of various lignocellulosic biomass. Table 1.3 shows the application of dilute H_2SO_4 on various lignocellulosic biomass.

Table 1.3 shows that H_2SO_4 was mostly been used as the acid–base pretreatment for various lignocellulosic biomass. The benefits of using dilute H_2SO_4 is that it can achieve high reaction rates and significantly improve the hydrolysis of cellulose during enzyme hydrolysis (Kumar et al. 2009). From Table 1.4, H_2SO_4 was usually used below 3 wt% as it is inexpensive, effective, and the pretreatment time was

Table 1.3 Classification of acidic catalyst for acid pretreatment of lignocelllosic biomass

	Process condition	Advantages	Disadvantages	References
Concentrated mineral acid	>30% (w/v) at ambient to moderate temperature of several hours	• No need of enzyme • Low temperature and pressure	• Catalyst toxicity • Catalyst corrosiveness	Kumar et al. (2009), Mood et al. (2013), Rabemanolontsoa and Saka (2016)
Dilute mineral acid	0.5–5% (w/v) at high temperature for a few minutes	• High reaction rate • Applicability of combined severity factor	• High temperature • Inhibitor formation	Kumar et al. (2009), Mood et al. (2013), Rabemanolontsoa and Saka (2016)
Organic acid	0.5–5% (w/v) at high temperature for a few minutes	• Less toxicity • Less formation of inhibitory compounds	• Expensive catalyst • Pretretatment effectiveness	Mood et al. (2013), Rabemanolontsoa and Saka (2016)

Table 1.4 Application of dilute H_2SO_4 pretreatment for various lignocellulosic biomass

Raw material	Pretreatment condition	Total reducing sugar yield	References
Rice straw	• 0.5% (w/v) • 60 min • 120 °C (autoclave)	0.359 g g^{-1}	Kshirsagar et al. (2015)
Sugarcane bagasse	• 0.5% (w/v) • 10–20 min • 80–150 °C (water bath and autoclave)	23.49g L^{-1}	Timung et al. (2015)
Wheat straw	• 0.75–2.25% (w/v) • 10–30 min • 120–160 °C (autoclave)	26.74 g L^{-1}	Baboukani et al. (2012)
Rye straw	• 0.6–1.5% (w/v) • 30, 60, 90 min • 121 °C (autoclave)	135–155 mg g^{-1}	Sun and Cheng (2005)

usually used below 60 min. Increase of H_2SO_4 concentration and longer time of pretreatment did not give any beneficial effect to the lignocellulose content after pretreatment since acid concentration and pretreatment time had no significant effect on the glucose yield and resulted in lower yield of Total Reducing Sugar (TRS) (Sun and Cheng 2005; Avila-Lara et al. 2015).

The temperature used for dilute H_2SO_4 pretreatment is summarized in Table 1.4. Zhao et al. (2007) reported that at higher temperatures (>80 °C), dilute acid pretreatment were produced "toxic" intermediates such as furfural and aldehydes which significantly affect enzyme hydrolysis to produce fermentable sugar (Zhao et al. 2007). Selig et al. (2007) and Nlewen and Thrash (2010) also reported the same

statement according to their studies in the pretreatment of maize stems and switchgrass. Selig et al. (2007) reported that at high temperatures (>130 °C) of dilute acid pretreatment of maize stems, a spherical droplets, composed of lignin and possible lignin–carbohydrate complexes, were formed on the surface of residual corn stover that gave a negative effect on the enzymatic saccharification (Selig et al. 2007). The studies of Nlewen and Thrash (2010) reported that running the dilute acid pretreatment of switchgrass at high temperature and high pressure was prohibitive because furfural, a microorganism growth inhibitor, was produced (Nlewen and Thrash 2010). Therefore, autoclaves are not suitable to be used even though it is promising a better pretreatment since it operated at high temperature and high pressure. Instead of autoclave, a water bath was more promisingly to be used for the pretreatment.

1.2.2 Alkaline Pretreatment

Pretreatment is the first step in the bioconversion process. The purpose of pretreatment is to disorganize the crystalline structure of micro- and macrofibrils and to modify pores for enzymatic hydrolysis. Compared to acid pretreatment, alkali pretreatment has different objectives since its ability to remove lignin without degrading the carbohydrates (Raveendran and Ashok 2015). In alkaline pretreatment, no washing is necessary after the pretreatment and any addition of alkaline would not result in a corrosion problem to equipment compared to acid pretreatment (Karunanithy and Muthukumarappan 2011).

Compared to other pretreatment, alkaline pretreatment process utilize under low temperature and pressure. Alkaline pretreatment is carried out at ambient conditions but takes hours and days rather than minutes or seconds for the incubation because some of the alkali solution is converted to irrecoverable salts into biomass by pretreatment process (Mosier et al. 2005). Unlike other pretreatments, lignocellulose undergoes two reactions, solvation and saponification, which cause a swell in lignocellulose structure, decrease in polymerization degree, thus making the lignocellulose components more accessible to enzymatic hydrolysis (Raveendran and Ashok 2015).

The common alkali used in the pretreatment of lignocellulose are sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonia (NH_3), and calcium hydroxide ($Ca(OH)_2$). Calcium hydroxide is the cheapest alkali used in pretreatment to remove lignin and acetyl group that in return enhance the enzymatic saccharification (Chang 2007). However, the removal of lignin by using $Ca(OH)_2$ pretreatment only depends on types of biomass treated as well as the quality of lignin present in biomass. NaOH is the most commonly alkali used in lignocellulosic biomass pretreatment because of its ability to delignify biomass. This treatment causes lignocellulosic biomass swell and leads to an increase in the area of internal surface, reduces cellulose crystallinity and disrupt the structure of lignin, thereby enhancing the reactivity of the remaining

Table 1.5 Application of NaOH as alkaline-based pretreatment on various lignocellulosic biomass

Raw material	Pretreatment condition	Total reducing sugar yield	References
Oil Palm Fronds	• 0.5–10% (w/v) • 15–90 min • 50–100 °C (waterbath)	0.24–2.93 mg/mL	Mohd Sukri et al. (2014)
Corn stalk	• 0.5%–2.0% (w/v) • 60 min • 80 °C.	39.0 g/L	Cai et al. (2016)
Switchgrass	• 0.5%–10% (w/v) • 60 min • 85–90 °C	0.51–0.31 g/g	Nlewen and Thrash (2010)
Corn Stover	• 0.25–0.75% (mol dm^{-3}) • 30, 60, 120 min • 60, 90, 120 °C	0.34–0.29 g/g	Hong et al. (2015)

carbohydrate (Rabemanolontsoa and Saka 2016). Table 1.5 shows the application of NaOH pretreatment on various lignocellulosic biomass.

From Table 1.5, NaOH was used widely to pretreat various lignocellulosic waste. NaOH has been known as one of the strongest base catalysts compared to other alkalines because of the effectiveness in pretreatment reaction where NaOH will be dissociated into sodium ion (Na^+) and hydroxide ion (OH^-) to increase the rate of hydrolysis reaction as the hydroxide ion concentration increase (Kim et al. 2016). Hong et al. (2015) reported that majority of the studies of NaOH pretreatment were conducted under high NaOH concentration (>1.25 mol dm^{-3}) and high temperature (>100 °C). However, they also reported low sugar yield were produces from these studied which maybe because of the attributed to certain reaction condition (Hong et al. 2015).

NaOH was usually used in the concentration of 0.5% (w/v) and the pretreatment temperature was usually used below 100 °C. Increasing NaOH concentration in the pretreatment at elevated temperature did not affect the enzymatic hydrolysis (Hong et al. 2015). The purpose of the best pretreatment was to enhance the effectiveness of enzymatic hydrolysis process. Hong et al. (2015) and Mohd Sukri et al. (2014) reported that increasing NaOH concentration at higher temperature decreased the recovery rate for glucan. Rawat et al. (2013) also reported that increase of NaOH concentration might lower the TRS due to the formation of by-products from the hydrolyzed sugar. On the other hand, pretreatment time for NaOH also had been summarized in Table 1.5. McIntosh and Vancov (2010) and Kim and Han (2012) reported that extending pretreatment time did not give any significant effect on the pretreatment as well as enzymatic hydrolysis process. It was observed that 60 min was an optimum pretreatment time for NaOH concentration and extending the time to 90 min does not give any discernable differences at 60 °C at any alkaline concentration (McIntosh and Vancov 2010). Moreover, as shown in Table 1.5, most of the studied had chosen 60 min as their pretreatment time.

1.2.3 Autohydrolysis Pretreatment

An effective and economical pretreatment for lignocellulosic biomass must meet few requirements which enhance the production of sugar, or the ability to subsequently produce sugars by enzymatic hydrolysis, resist the degradation or loss of hemicellulose and cellulose, minimize the production of inhibitors and lastly must be cost effective (Jeong and Lee 2015). Recently, supercritical water, ionic liquid treatment, and combined pretreatment method have been a great interest because they show an effective effect to remove lignin and hemicellulose as well as enhance the enzymatic hydrolysis by decreasing the cellulose crystallinity (Weerachanchai et al. 2012). Among all pretreatment process, hydrothermal pretreatment, also called autohydrolysis or hot water pretreatment, is the most environmentally friendly pretreatment since it only uses water for a reaction medium without any addition of chemical, compared with chemical pretreatment. This pretreatment also called green technology because of its ability to prevent corrosion of equipment and environment because there is no need for the chemical recycling process after the pretreatment. The major objective in this pretreatment is to solubilize the hemicellulose as well as increase the accessibility of enzyme on cellulose for enzymatic hydrolysis, thereby avoiding the formation of inhibitor (Jeong and Lee 2015).

Autohydrolysis pretreatment is carried out at a relatively high temperature which is around 160–240 °C and pressure 1–3.5 MPa during a few minutes to several hours. In autohydrolysis pretreatment, hydronium ions [H+] generated from water and acetic groups released from hemicellulose and acts as a catalyst in higher concentration at high temperature for acid hydrolysis, thus reduces the pH significantly (Lei et al. 2013). At high temperatures, water will create enough hydrogen ions to drop pH to become acidic (Iwona et al. 2013). Extensive studies have been investigated about the operating condition of autohydrolysis pretreatment on different types of lignocellulosic materials. Table 1.6 shows the studies of autohydrolysis condition on different lignocellulosic materials.

Autohydrolysis pretreatment or known as hot water pretreatment was initially used by Bobleter et al. (1976) to enhance the accessibility of enzymatic hydrolysis of lignocellulosic materials (Goncalves et al. 2015). As shown in Table 1.6, autohydrolysis is carried out by heating the lignocellulosic materials in water at higher temperature and resulting in the depolymerization and solubilization of the hemicellulose in liquid phase (Romaní et al. 2012; Ruiz et al. 2013). Most of the

Table 1.6 Application of autohydrolysis pretreatment on different lignocellulosic materials

Raw material	Temperature (°C)	Time (min)	References
Costal Bermuda grass	150	60	Lee et al. (2009)
Sugarcane bagasse and bamboo	100–150	10–20	Timung et al. (2015)
Switchgrass	100	60	Nlewen and Thrash (2010)
Lignocellulosic materials (LCMs)	160–200	10–50	Goncalves et al. (2015)

studies as shown in Table 1.6 used higher temperature, which means more than 100 °C. Goncalves et al. (2015) reported that they applied an autohydrolysis pretreatment on cactus, mature coconut fiber, green coconut shell, and mature coconut shell at temperature 160–200 °C. Similar observations have been reported by Lee et al. (2009) and Timung et al. (2015) where the pretreatment temperature used for coastal Bermuda grass and sugarcane bagasse and bamboo was 150 °C and 100–150 °C, respectively. Most of the studies shown in Table 1.6 applied autohydrolysis pretreatment at higher temperature with shorter time incubation.

High temperature of autohydrolysis pretreatment would inhibit the production of reducing sugar because it would burn the surface of the biomass. Therefore, Nlewem and Thrash (2010) had applied an autohydrolysis pretreatment temperature at 100 °C for 60 min in water bath for switchgrass biomass. However, the hot water pretreatment at mild condition (100 °C, 60 min) had a negligible impact on the yield of reducing sugar compared to untreated sample, thus suggested that the result might be improved if pretreatment is done at higher temperature or pretreated at the longer time (Nlewen and Thrash 2010).

1.3 Parameters Affecting Chemical Pretreatment of Lignocellulosic Biomass

Among all the pretreatment methods, dilute H_2SO_4 and NaOH pretreatment are the most extensively studied biomass pretreatment in different substrate such as agricultural waste (Kumar et al. 2009; Xu et al. 2010). However, the effectiveness of the pretreatment methods depends on the few operating conditions such as chemical loading concentration, pretreatment temperature, and time. However, if severe conditions are applied several degradation products are formed, thus inhibiting the production of reducing sugar (Avila-Lara et al. 2015). In addition, different substrates have a different operating condition for both pretreatment. Therefore, the need to investigate the condition of this pretreatment method for optimization purpose is important to enhance the effectiveness of the pretreatment method as well as increase the production of reducing sugar.

1.3.1 Chemical Loading Concentration

The concentration of chemical loading plays an important role in the optimization process of pretreatment. A number of chemical pretreatments have been studied to improve the hydrolysis of lignocellulosic biomass. However, different biomass attributed to severe reaction condition and needs to study to establish an effective conversion process of biomass (Hong et al. 2015).

Choi et al. (2014) reported that the majority of studies of NaOH pretreatments were conducted at a high concentration of NaOH (Choi et al. 2014). Cotano et al. (2015), Ganesh and Min-Kyu (2015), and Rawat et al. (2013) reported that the lowest concentration amount of NaOH they used for optimization process of vineyard pruning, whole rice waste, and poplar biomass, respectively, was 0.5% (w/v) while the highest concentration of NaOH was 4.5% (w/v). However, Kim and Han (2012) reported that they obtained the optimum NaOH concentration for rice straw at 2.96% (w/v) while Rawat et al. (2013) found 2.8% (w/v) of NaOH concentration was the optimum alkaline concentration for poplar biomass. Cotano et al. (2015) reported that the optimum NaOH concentration for vineyard pruning was found to be 2.5% (w/v). Uzunlu et al. (2014) found that the optimum NaOH concentration for poppy stalk was 2.4% (w/v). Ganesh and Min-Kyu (2015) reported that the optimum NaOH concentration for whole rice waste was 2.0% (w/v). McIntosh and Vancov (2010) also reported the same result where the optimum NaOH concentration for *Sorghum bicolor* straw was 2.0% (w/v) while Avila-Lara et al. (2015) reported that the optimum NaOH concentration for agave bagasse was found to be 1.87% (w/v). Therefore, it can be concluded from previous work that the optimum NaOH concentration was below 2.5% (w/v) of NaOH concentration for most lignocellulosic biomass. Therefore, the best range of NaOH concentration for optimization can be concluded from 0.5 to 2.5% (w/v).

In acidic pretreatment, acid serves a catalyst to hydrolyze carbohydrate, especially hemicellulose, thus loosening the barrier that made from lignin and hemicellulose that protects cellulose from enzyme accessibility (Young and Kyoung 2015). Dilute acid pretreatment is conducted usually at 120–215 °C with 0.5–5% (w/v) acid below 10 atm for a few minutes and allows almost complete hydrolysis of hemicellulose, disruption of lignin structure, modified the structure of lignocellulosic material and partial solubilization of cellulose. Similar observation also had been reported by Anwar et al. (2012) where they chose the range for dilute acid concentration from 0.5 to 3.0% (w/v) for optimization of pretreatment process of rice polish and found that 1.5% (w/v) was the optimum concentration with 16.52 mg/mL reducing sugar yield while Dussan et al. (2014) reported that the highest glucose extraction efficiency (70%) was observed when the treatment was performed with 2.0% (w/v) H_2SO_4 (Anwar et al. 2012; Dussan et al. 2014). Besides that, Baboukani et al. (2012) reported that the range of dilute H_2SO_4 for optimization pretreatment process of wheat straw was 0.75–2.25% (w/v) and found that the optimum yield of reducing sugar was pretreated at 1.53% (w/v) (Baboukani et al. 2012). The best range of dilute H_2SO_4 concentration for optimization process is concluded from 0.5 to 3.0% (w/v).

1.3.2 Temperature

Other than chemical loading concentration, temperature also gives an impressive effect on the breakdown of hemicellulose–lignin complex structure in order to enhance the cellulose accessibility. Different temperatures will produce various

yields of sugar production. It can be concluded that the reaction takes less time to reach the maximum production of reducing sugar as temperature increases. However, this condition leads to a rapid rate of sugar degradation (Lenihan et al. 2010). Therefore, choosing the best range for optimization plays an important role to obtain an effective production of reducing sugar.

In alkaline pretreatment, Kim and Han (2012) reported that in their studies, the optimum temperature for rice straw was found at 81.79 °C while Cotano et al. (2015) found that the optimum temperature for vineyard pruning was at 100 °C. Rawat et al. (2013) and Uzunlu et al. (2014) also reported the same result where the optimum temperature for poplar biomass and poppy stalk was in the range of 80–100 °C which was 94 and 80 °C, respectively. On the other hand, McIntosh and Vancov (2010) reported that 121 °C of pretreatment temperature produced lower reducing sugar compared to pretreatment at 60 °C, thus it raises the possibility that the optimum temperature for alkaline concentration should be lower than 121 °C. Increasing the temperature (>100 °C) would probably burn the surface of the biomass, thus inhibiting the production of reducing sugar. Most of the studies on alkaline pretreatment shows chose the optimization range for temperature was between 60 and 121 °C. However, the optimum temperature found was in the range 80–100 °C. As a conclusion, the best range of temperature for optimization of NaOH pretreatment are from 80 to 100 °C.

In dilute H_2SO_4 pretreatment, Kumar et al. (2009) reported that the most widely temperature used for pretreatment of lignocellulosic biomass is in the ranging of 80–200 °C (Kumar et al. 2009). Lu et al. (2007) reported that the optimization of dilute H_2SO_4 for corn stover was carried out at range of temperature from 80 to 120 °C and the optimum temperature was found at 120 °C with 8.4% glucose yield (Lu et al. 2007). Similar observation had been reported by Timung et al. (2015) that the optimization of dilute H_2SO_4 for sugarcane bagasse and bamboo were carried out at range of temperature from 80 to 150 °C and the optimum temperature for both sugarcane bagasse and bamboo was found at 100 °C with optimum yield of reducing sugar, 23.49 g/L and 15.6 g/L, respectively. Anwar et al. (2012) reported that the dilute H_2SO_4 pretreatment of cellulosic biomass of rice polish was carried out at a temperature of 100 °C as an optimum temperature (Anwar et al. 2012). Although most of the studies used a higher temperature (>100 °C) for dilute H_2SO_4 pretreatment, there still are a few drawbacks by using high temperature. Nlewem and Thrash (2010), Saha et al. (2005) and Sun and Cheng (2005) reported that running the dilute H_2SO_4 pretreatment of switchgrass, rice hulls, and rye straw at higher temperature (120 °C) had given insignificant increase in the production of reducing sugar because of the production of furfural that reduces the effectiveness of pretreatment process and further process (Sun and Cheng 2005; Nlewen and Thrash 2010; Saha et al. 2005). The best range of temperature for optimization process for dilute H_2SO_4 pretreatment is concluded from 80 to 100 °C.

1.3.3 Incubation Time

Incubation time is one of the important parameters that need to be optimized in the pretreatment methods. Incubation time is important to ensure that all lignin–hemicellulose complexes have been breakdown to increase the enzymatic hydrolysis reaction. However, longer time of incubation will lead to sugar degradation. Cotano et al. (2015) reported that the extending time of pretreatment time for vineyard pruning from 30 to 60 min at constant NaOH concentration and temperature does not give any significant increase in the production of reducing sugar (Cotano et al. 2015). Therefore, choosing the best range for pretreatment time also important to enhance the effectiveness of the pretreatment process.

In alkaline pretreatment, Kim and Han (2012), McIntosh and Vancov (2010), and Avila-Lara et al. (2015) have a same pretreatment time range (30–90 min) of NaOH pretreatment for rice straw, *Sorghum bicolor* straw, and agave bagasse, respectively. The different range was observed reported by Uzunlu et al. (2014), where the range of pretreatment time was at 10–110 min for poppy stalk. However, Uzunlu et al. (2014) found that the optimum pretreatment time for poppy stalk was at 70 min. Kim and Han (2012) and Avila-Lara et al. (2015) found that the optimum pretreatment time for rice straw and agave bagasse were at 56.66 min and 50.3 min, respectively, while Cotano et al. (2015) and Rawat et al. (2013) reported that the optimum pretreatment time for vineyard pruning and poplar biomass was at 40 and 60 min, respectively. From the comparison above, it was found that each biomass obtained different optimum pretreatment time. Since most of the optimum pretreatment time for different biomass is in the range of 30–90 min, it can be concluded that this range is the best range for the optimization process for NaOH pretreatment.

Generally, dilute H_2SO_4 pretreatment is carried out with high temperature (160–180 °C) at short pretreatment time (1–5 min) to obtain maximal production of reducing sugar (Hsu et al. 2010). However, pretreatment of biomass by using dilute H_2SO_4 at high temperature could lead to the production of furfural that inhibits the production of reducing sugar as mentioned previously. Therefore, few researchers have done the pretreatment by using mild conditions of pretreatment temperature (80–100 °C) at the longest time (10–180 min) to improvise the pretreatment process. Idress et al. (2013) reported that the range of pretreatment time for optimization of dilute H_2SO_4 pretreatment for water hyacinth biomass was from 30 to 180 min at 90–130 °C and found that 60 min was the optimum pretreatment time (Idrees et al. 2013). Avila-Lara et al. (2015) also reported that the range of pretreatment time for optimization of dilute H_2SO_4 pretreatment of agave bagasse was from 15 to 90 min and found that 33.8 min was the optimum time (Avila-Lara et al. 2015). However, Timung et al. (2015) reported a slightly lower range of pretreatment time for sugarcane bagasse and bamboo, 10–20 min, at mild condition of temperature 80–100 °C and found the optimum time at 20 min (Timung et al. 2015). Similar range of pretreatment time also reported by Baboukani et al. (2012) for dilute H_2SO_4 pretreatment of wheat straw where the time was chosen from 10 min to 30 min but at slightly higher temperature (120–160 °C) and found

that 30 min was the optimum pretreatment time (Baboukani et al. 2012). Since most of the optimum pretreatment time of different biomass is in the range 20–80 min, it can be concluded that this range is the best range for the optimization process for dilute H_2SO_4 pretreatment.

1.4 Enzymatic Hydrolysis in Production of Reducing Sugar

The process of converting biomass into reducing sugar involves few steps including pretreatment and hydrolysis. The aim of the pretreatment process is to alter the structure of lignocellulosic biomass to make cellulose more accessible for hydrolysis, while the aim of hydrolysis process is to convert the polysaccharide into fermentable sugar. There are two methods of hydrolysis which are acid hydrolysis and enzymatic hydrolysis. However, Banerjee et al. (2010) reported that acid hydrolysis has a few drawbacks including the production of degradation products while enzymatic hydrolysis by using cellulase does not generate any inhibitor and the types of enzymes were very specific to hydrolyze cellulose polymer into its monomer (Banerjee et al. 2010). El-Zawawy et al. (2011) also reported that hydrolysis by using enzyme is the most promising alternative method to compare to acid hydrolysis (El-Zawawy et al. 2011). In addition, cost utilization for enzymatic hydrolysis is low compared to sulfuric and other acid hydrolysis because enzymatic hydrolysis usually conducted at mild conditions (temperature 50 °C at pH 4.8), less energy and no corrosion problem occurred (Sun and Cheng 2002).

Most of the research have been carried out enzymatic hydrolysis process in the production of reducing sugar using different lignocellulosic biomass such as rice straw (Singh and Bishnoi 2011b), wheat straw (Singh and Bishnoi 2011b, 2012; Qi et al. 2009), sweet sorghum bagasse (Umagiliyage et al. 2015), and potato peel residue (Ben Taher et al. 2017). Enzymatic hydrolysis of lignocellulosic biomass is a very complex process because of the numerous structural features makes it harder to hydrolyze and required a combination of several hydrolytic enzymes (Manimaran et al. 2013; Maitan-Alfenas et al. 2015). A group of cellulase enzyme that include endoglucanases are able to hydrolyze the cellulose polymer and exposed the nonreducing and reducing ends of the linear polymer of glucose unit, cellobiohydrolases and exoglucanase, which act on these end to produce cellobiose and cellooligosaccharides, and β-glucosidase (BGL) which hydrolyze the cellobiose unit to produce the end product, glucose (Maeda et al. 2011; Gottschalk et al. 2010).

1.5 Parameters Affecting Enzymatic Hydrolysis

There are few factors that can affect the enzymatic hydrolysis of cellulose process including substrate loading, cellulase activity and condition of the reaction such as temperature, reaction time as well as other parameters (Sun and Cheng 2002). The

effects of these parameters are correlated for the efficiency of the enzymatic hydrolysis process (Ben Taher et al. 2017).

1.5.1 Enzyme Loading

Enzyme loading plays a crucial role in the optimization process of enzymatic hydrolysis to obtain optimum production of reducing sugar. Increasing an enzyme dosage in the reaction could enhance the yield of the reducing sugar but it would significantly increase the cost of the process. However, an increase in enzyme loading up to certain activity also did not give a significant improvement in the production of reducing sugar.

This situation was proven from the result reported by Qi et al. (2009) where the increase of cellulase dosage from 10 to 30 FPU/g substrate increased the reducing sugar yield 72.65%, 90.94% while increasing the cellulase dosage up to 50 FPU/g substrate reduce the yield of reducing sugar to 90.07% (Qi et al. 2009). Chen et al. (2007) also reported that higher cellulase concentration (50–150 FPU/g substrate) resulted in the increase in hydrolysis yield of corncob, although above 100PFU/g substrate the raise was weaker (Chen et al. 2007). The right amount of enzyme doses in the enzymatic hydrolysis process could avoid the use of overdose enzymes as well as reduce the costing of process. Greg and Saddler (1996) reported that cellulase dosage of 10 PFU/g cellulose is often used in laboratory-scale studies to provide high levels of glucose yield at a reasonable time (Gregg and Saddler 1996). Singh and Bishnoi (2011b) reported that at lower cellulase loading, the efficiency of hydrolysis increased with increasing of substrate loading while at higher cellulase loading, the production of reducing sugar increase but become lesser when substrate loading further increases which indicate a probable saturation effect (Singh and Bishnoi 2011b).

1.5.2 Substrate Concentration

Substrate concentration is one of the main important factors that affects the yield and initial rate of enzymatic hydrolysis of cellulose (Qi et al. 2009; Ben Taher et al. 2017). Increasing the yield of reducing sugar and reaction rate of hydrolysis are depended on the low and high of substrate concentrations (Cheung and Anderson 1997). Increasing of substrate concentration would increase the yield of reducing sugar; however, further increase of substrate also could give insignificant effect on the production of sugar because of the substrate inhibition, which substantially lowers the rate of hydrolysis and this phenomenon happens depending on the ratio of total substrate to total enzyme (Sun and Cheng 2002).

According to Qi et al. (2009), increasing substrate concentration in enzymatic hydrolysis process from 20.00 to 80.00 g/L did show any significant increase in the

production of reducing sugar (Qi et al. 2009). Chen et al. (2013) also reported that higher substrate loading in the reaction could lead to the improper mixing that might have hindered enzymatic hydrolysis, thus resulting in lower efficiency of hydrolysis (Chen et al. 2013). A similar observation also has been reported by Kumar and Wyman (2009) where the increasing of substrate concentration from 10 to 50 g/L leads to the decreasing of hydrolysis yield due to production of high-end product concentration inhibiting enzyme activities, Ben Taher et al. (2017) concluded that 10% of substrate concentration was the optimum value to obtain optimum concentration of reducing sugar (Ben Taher et al. 2017; Kumar and Wyman 2009). In conclusion, the increase of substrate concentration could increase the yield of reducing sugar, but further increase does not give any significant effect and caused the decreasing trend of reducing sugar production. The substrate concentration ranges from 1 to 10% is acceptable to be used in optimum enzymatic hydrolysis for higher production of reducing sugar.

1.5.3 Temperature

Temperature is also one of the parameters that affected the activity of the enzyme during hydrolysis. Maitan-Alfenas et al. (2015) reported that the optimal temperature for cellulase is 40–50 °C (Maitan-Alfenas et al. 2015). Ben Taher et al. (2017) reported that the optimum temperature occurred at 45 °C to obtain optimum reducing sugar yield (Ben Taher et al. 2017). Pandiyan et al. (2014) also reported that the efficiency of enzymatic hydrolysis could be decreased on the increasing temperature due to the thermal inactivation (Pandiyan et al. 2014). Meanwhile, Kaur et al. (2009) had chosen the range of temperature from 36.59 to 53.41 °C for the optimization process and found that 43.4 °C was the optimum temperature. Another study by Ferreira et al. (2009) reported that the chosen range of temperature from 30 to 60 °C for optimization process of enzymatic hydrolysis with an incubation time at 72 h shows an increase of hydrolysis yield continuously for studied biomass (Ferreira et al. 2009). Therefore, the higher temperature (>60 °C) does not give any significant effect and caused the decreasing trend of reducing sugar production due to the inactivation of the enzyme. The temperature ranges from 40 to 60 °C is acceptable to be used in optimum enzymatic hydrolysis for higher production of reducing sugar.

1.5.4 Hydrolysis Time

Hydrolysis time is one of the important parameters in enzymatic hydrolysis process to ensure that all substrates have been completely hydrolyzed into simple sugars. The rate of hydrolysis varies depends on the enzyme activity and accessibility of the substrate. Qi et al. (2009) reported that the considerable increase in the enzymatic

hydrolysis yield was observed at 24–72 h period (Qi et al. 2009). Meanwhile, Ruangmee and Sangwichien (2013) reported that the chosen range of hydrolysis time from 24 to 72 h shows an increasing of reducing sugar yield and the peak was started at 24 h process, but further increase of hydrolysis time up to 96 h shows a declined yield of reducing sugar due to the complete hydrolysis of substrate into simple sugars (Ruangmee and Sangwichien 2013). Similar observation had been reported by Qi et al. (2009) that the conversion of carbohydrate polymer increased from 75.39 to 80.04% when increasing the reaction time from 24 to 72 h (Qi et al. 2009). In addition, Saini et al. (2012) and Ben Taher et al. (2017) reported that the optimum hydrolysis time for enzymatic hydrolysis of sweet sorghum bagasse and potato peel residue, respectively, was 48 h while Ferreira et al. (2009) reported that the optimum hydrolysis time for *Cistus ladanifer* and *Cytisus striatus* was found to be 72 h (Ben Taher et al. 2017; Ferreira et al. 2009; Saini et al. 2012). Hence, the higher hydrolysis time up to 96 h gives insignificant effect on the production of reducing sugar, thus the range of hydrolysis time from 24 to 72 h is acceptable to be the best range to be used in optimum enzymatic hydrolysis process for higher production of reducing sugar.

1.6 Optimization Analysis for Pretreatment and Enzymatic Hydrolysis of Lignocellulosic Biomass

Optimization is referring to improve the performances of a process in a system, or a product in order to achieve the maximum benefit from it due to the time consumption and requiring large amounts of chemicals and samples. Optimization of multifactorial system by conventional techniques is generally done with one factor at a time, but unfortunately this method does not reveal the relationship and interaction effects between variables and time consumption (Jeya et al. 2009). Optimization technique of one variable at a time does not include the interactive impact among the parameter studies, thus complete impacts of the independent variables on the response process cannot be achieved (Bezerra et al. 2008).

In order to overcome this issue, the optimization step by utilizing multivariate statistic techniques is crucial. Response surface methodology (RSM) is the suitable experimental design among the most multivariate technique, that can integrate all the independent variables and utilize the data input from the experiment to finally come up with a set of equations and give a theoretical value of an output (Mohamad Said and Mohamed Amin 2015). The optimization for the pretreatment process and enzymatic hydrolysis process is important in order to obtain an optimum production of reducing sugar. The optimization needs to be done since many parameters in the pretreatment and enzymatic hydrolysis processes affect the lignocellulose content and production of reducing sugar.

1.6.1 Application of Response Surface Methodology (RSM) and Box-Behnken

1.6.1.1 Design (BBD) for Optimization Process

Response surface methodology (RSM) is a powerful mathematical approach to analyze the effect of multiple factors or variables, alone or in combination, on a given process rapidly and efficiently with a minimal number of experiment while keeping a high degree of statistical significance in experimental results (Guan and Yao 2008). There are various types of models available in RSM. BBD is a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial design. In addition, BBD is slightly efficient compared to central composite design (CCD) but more efficient than other three-level full factorial design because of the efficiency of one experimental design is defined as the number of coefficients in the estimated model divided by the number of experiments (Ferreira et al. 2007). Moreover, BBD also does not have a combination where all factors are simultaneous at their highest and lowest levels and able to avoid experiments perform under extreme conditions.

There are few research that have been done by using BBD for pretreatment and enzymatic hydrolysis optimization. Kim and Han (2012) and Chittibabu et al. (2012) had used BBD to determine the optimum condition for alkaline pretreatment of rice straw and banana pseudostem, respectively, while Singh and Bishnoi (2012), Qi et al. (2009), and Singh and Bishnoi (2011b) had used BBD to determine the optimum condition for enzymatic hydrolysis for wheat straw, and rice straw, respectively. Table 1.7 shows the application of BBD for the optimization of both pretreatment and enzymatic hydrolysis with their variables.

Table 1.7 Application of BBD for optimization for both pretreatment and enzymatic hydrolysis of lignocellulosic biomass

Process	Independent variables	References
Pretreatment		
Alkaline pretreatment of banana pseudostem for sugar production	NaOH concentration, temperature and pretreatment time	Chittibabu et al. (2012)
Alkaline pretreatment of rice straw for enhancing glucose yield	NaOH concentration, reaction temperature and pretreatment time	Kim and Han (2012)
Enzymatic hydrolysis		
Enzymatic hydrolysis of wheat straw for sugar production	Cellulase dosage, hydrolysis time, temperature and β-glucosidase dosage	Singh and Bishnoi (2012)
Enzymatic hydrolysis of wheat straw for reducing sugar production	Enzyme loading, substrate concentration, surfactant concentration and hydrolysis time	Qi et al. (2009)
Enzymatic hydrolysis of pretreated rice straw for sugar production	Enzyme loading, surfactant concentration, and Tween 80 concentration	Singh and Bishnoi (2011b)

1.6.2 Kinetic Coefficients Determination of Enzymatic Hydrolysis of Lignocellulosic Biomass

In the conversion of lignocellulosic biomass into reducing sugar, enzymatic hydrolysis process is the most challenging step, which determines the overall efficiency of the process (Pratto et al. 2016). The knowledge of hydrolysis kinetics is a crucial part in order to reach high conversion rates of polysaccharides into monomeric sugars and the most challenging task when the bioconversion of polysaccharides takes place in heterogenous system that involves insoluble substrate and soluble enzyme. The difficulties in modeling the enzymatic hydrolysis of lignocellulosic biomass may be grouped into three classes which were the complexity of the substrate, the action of the enzyme, and interaction between enzyme–substrate (Carvalho et al. 2013).

The kinetics of enzyme has been usually studied by the Henri–Michaelis–Menten (MM) equation from initial velocity kinetics (Carillo et al. 2005). Chrastil (1988) reported that Henri–Michaelis–Menten equation was only suitable for the analysis of enzymatic hydrolysis of homogeneous structures which involved a soluble substrate (Chrastil 1988). On the other hand, Baley (1989) introduced an alternative approach of Henri–Michaelis–Menten equation by taking into account that the measurement of initial reaction velocity, v_o, as a function of substrate concentration, should be expressed as a function of the initial enzyme concentration, $[E_o]$ (Baley 1989).

In addition, several kinetic models have been developed concerning enzymatic hydrolysis of cellulose on heterogenous system and were reviewed by Bansal et al. (2009) and Sousa et al. (2011). They included nonmechanistic, semi-mechanistic, functionally, and structurally based models that applied to different types of enzymes and lignocellulosic materials. Table 1.8 shows a list of kinetics studied for heterogenous system.

As shown in Table 1.8, Classical MM kinetic model and Chrastil approach have been applied by most researchers to study the kinetics of enzymatic hydrolysis and pretreatment for heterogenous system. Chrastil approach was developed by Chrastil (1988) as an alternative to MM models in order to provide additional kinetic and structural characteristics for a heterogenous system (Pratto et al. 2016). Chrastil's approach is assed to study the dependent of time curves on the heterogenous rate-limiting phenomena that are present in the substrate–enzyme system including enzyme adsorption and diffusion (Carvalho et al. 2013).

1.7 Summary

Utilization of lignocellulosic biomass into beneficial products needs to be developed in a proper way in order to reduce the environmental pollution problem that leads into increasing carbon dioxide concentration and other greenhouse gases. Lignocellulosic biomass is considered as one of the new renewable material that is abundantly

Table 1.8 List of kinetic models for heterogenous system of insoluble lignocellulosic biomass

Kinetic model	Substrate	Hydrolysis/Pretreatment	References
Classical MM Model (considered solid substrate and soluble enzyme)	Sugarcane straw	Hydrolysis of cellulose to glucose	Pratto et al. (2016)
	Wheat straw	Hydrolysis of cellulose to reducing sugar	Carillo et al. (2005)
	Corn Stover	Hydrolysis of cellulose to reducing sugar	Kadam et al. (2004)
Modified MM Model (Substrate considered soluble reactant)	Sugarcane straw	Hydrolysis of cellulose to glucose	Pratto et al. (2016)
	Sugarcane bagasse	Hydrolysis of cellulose to glucose	Carvalho et al. (2013)
Pseudo-homogenous MM	Cellulosic Pulp	Hydrolysis of cellulose to reducing sugar	Li et al. (2004)
Multireaction Semimechanistic Kinetic Model	Corn Stover	1. Heterogenous reaction (Breakdown of cellulose to cellobiose) 2. Heterogenous reaction (Glucose formation from cellulose) 3. Homogenous reaction (hydrolysing cellobiose to glucose)	Kadam et al. (2004)
Chrastil approach (Diffusion-limited Kinetic model)	Wheat straw	Study reaction of alkaline pretreatment	Carillo et al. (2005)
	Sugarcane straw	Study reaction of alkaline pretreatment	Pratto et al. (2016)
	Sugarcane bagasse	Study reaction of dilute acid pretreatment	Carvalho et al. (2013)

being dumped as waste materials, which has a great potential to be converted into many valuable products including functional food in food industries and biofuel in biofuel industries because of high in cellulose and hemicellulose contents. Different pretreatment methods which are alkaline pretreatment, dilute acid pretreatment, and autohydrolysis, could be utilized to ensure an effective bioconversion process. Optimum operating conditions for pretreatment and enzymatic hydrolysis will ensure the optimum conversion of this biopolymer into simple sugars. The optimum operating condition for both pretreatment and enzymatic hydrolysis plays an important role in the process to improve the production of reducing sugar as well as provide an additional data and information for the future work. Moreover, the studies

of kinetic of enzymatic hydrolysis and pretreatment also play an important role to provide the knowledge and information regarding the reaction of enzyme occur in enzymatic hydrolysis process and pretreatment process due to the complex structure of lignocellulosic biomass.

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Chapter 2
Mangosteen Peel Antioxidant Extraction and Its Use to Improve the Stability of Biodiesel B20 Oxidation

Megawati, Rizqy Romadhona Ginting, Ratna Dewi Kusumaningtyas, and Wahyudi Budi Sediawan

Abstract Antioxidants can be extracted from mangosteen peel with ethanol as solvent using microwave assisted extraction (MAE) efficiently and economically. The mangosteen peel antioxidant can be used to inhibit the biodiesel B20 oxidation. The microwave power gives a great factor of antioxidant conversion in mangosteen peel extraction. At 35 min and 300, 450, 600 W, the antioxidant conversions obtained were 15.45, 17.00, 18.33%, respectively. The total phenolic concentration was about 156–202 mg GAE/g. In addition, the extraction kinetic can be quantitatively described by antioxidant diffusivity from inside the solid to the solid's surface and antioxidant mass transfer from the solid's surface into solution with diffusion coefficient (D_e) of 2.81×10^{-11}, 3.42×10^{-11}, 3.8×10^{-11} cm^2/s, mass transfer coefficient (k_c) of 6.36×10^{-8}, 8.97×10^{-8}, 1.05×10^{-7} cm/s for 300, 450, 600 W, respectively, and Henry equilibrium constant (H) of 0.032. In the oxidation, the mangosteen extract antioxidant can improve 26.32% of the oxidative stability of biodiesel B20. Theoretically, the performance of mangosteen peel extract antioxidants in biodiesel B20 oxidation can be evaluated from its oxidation kinetic which can be approached using the pseudo-homogeneous first-order model. The reaction rate constant follows the Arrhenius equation with activation energy (E_a) of 54.34 and 56.27 kJ/mol as well as collision factors (A) of 348,711 1/min, for the oxidation of biodiesel B20 and the mixture of biodiesel B20 and mangosteen peel extract antioxidant, respectively. The activation energy of the mixture of biodiesel B20

Megawati (✉) · R. D. Kusumaningtyas
Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia
e-mail: megawati@mail.unnes.ac.id; ratnadewi.kusumaningtyas@mail.unnes.ac.id

R. R. Ginting
Department of Chemical Engineering, Graduate School of Engineering, Faculty of Engineering, Universitas Gadjah Mada, Hiroshima, Japan

W. B. Sediawan
Department of Chemical Engineering, Faculty of Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia

Z. A. Zakaria et al. (eds.), *Valorisation of Agro-industrial Residues – Volume II: Non-Biological Approaches*, Applied Environmental Science and Engineering for a Sustainable Future, https://doi.org/10.1007/978-3-030-39208-6_2

and mangosteen peel antioxidant was higher, so that the mixture of biodiesel B20 and antioxidant is more difficult to oxidize.

Keywords Antioxidant · Biodiesel B20 · Mangosteen peel · Microwave assisted extraction · Oxidation

2.1 Introduction

Indonesia is an agricultural country which is rich in various kinds of fruit plants; one of them is mangosteen (*Garcinia mangostana* L.) (BPS 2017). The biggest component of mangosteen lies in the skin, about 70–80%. Accordingly, mangosteen peel waste contains polyphenol compounds with high total phenolic concentration, which can be used as antioxidants (Zarena and Udaya Sankar 2009; Suttirak and Manurakchinakorn 2012; Naczk et al. 2011). Theoretically, antioxidants work by donating one electron to oxidant compounds, so that its oxidation activity can be inhibited (Borsato et al. 2014; Gregorio et al. 2017; Nimse and Pal 2015; Zarena and Udaya Sankar 2011; Walker 2007). Moreover, mangosteen peel extract contains IC_{50} of 44.49 mg/L (<50 mg/L), so the antioxidant activity level is very strong (Wibawanti et al. 2019).

The most commonly used method to extract antioxidants from mangosteen peel is conventional method which still has several disadvantages; it needs a lot of solvents and long extraction process, while the yield obtained is low (Tjahjani et al. 2014). Certainly, to solve this problem, it is mentioned that an underdeveloped method called microwave assisted extraction (MAE) can be used as an alternative (Bagherian et al. 2011; Buanasari et al. 2017; Guo et al. 2012; Megawati et al. 2018). MAE is a nonconventional extraction method that utilizes microwave radiation as a heating medium. This is convenient to extract thermolabile antioxidant compounds, because this method has better temperature control compared to conventional heating methods (Thirugnanasambandham and Sivakumar 2017; Chuyen et al. 2017; Karami et al. 2015).

The most important thing in extraction is the solute mass transfer from solid to solution which can be approached using two main phases; they are solute diffusivity from inside the solid to the solid's surface and solute mass transfer from the solid's surface into solution (Fernando and Soysa 2015). It is important that the data obtained from antioxidant extraction experiment is developed to find out the mass transfer phenomena.

In fact, biodiesel fuel is easily oxidized by oxygen, light, high temperature, and metals (Bouaid et al. 2009; Leung et al. 2006; Kivevele and Huan 2013; Park et al. 2008). Recently, antioxidants added into biodiesel have one function—to capture free radicals formed during oxidation and stop chain reactions in fuel degradations (Spacino et al. 2016). Moreover, natural antioxidants can inhibit biodiesel oxidation (Coppo et al. 2013; Spacino et al. 2015). Mangosteen peel antioxidant extract can inhibit biodiesel oxidation. The performance of mangosteen peel extract as an antioxidant for biodiesel can be investigated through its oxidation kinetics. Pseudo-

homogeneous rate law was used to do the oxidation kinetics. Some researchers have successfully used pseudo-homogeneous first-order model to investigate antioxidant performance in biodiesel oxidation (Gregorio et al. 2017; Xin et al. 2009).

2.2 The Composition of Mangosteen Peel Antioxidant Extract

Mangosteen is one of the plants of the genus *Garcinia* and the Guttiferae family. Mangosteen is an annual fruit plant that grows naturally in tropical forests in the Asian region, such as Indonesia, India, Myanmar, Sri Lanka, and Thailand (Jung et al. 2006). Mangosteen is often called as the "Queen of Fruits" because it contains high antioxidants (Gutierrez-Orozco and Failla 2013). The description of mangosteen can be seen in Fig. 2.1. Mangosteen productivity in Indonesia continues to increase every year. In 2016, the production of mangosteen was 162,864 tons/year (BPS 2017). The biggest component of mangosteen lies in the skin (about 70–80%), so that mangosteen peel waste in Indonesia is about 114,000–130,000 tons/year. Mangosteen skin contains water and high organic compounds (Tjahjani et al. 2014), polyphenol compounds. Mangosteen peel antioxidant extract contains antioxidants (Suttirak and Manurakchinakorn 2012; Naczk et al. 2011). Phenolic compounds in

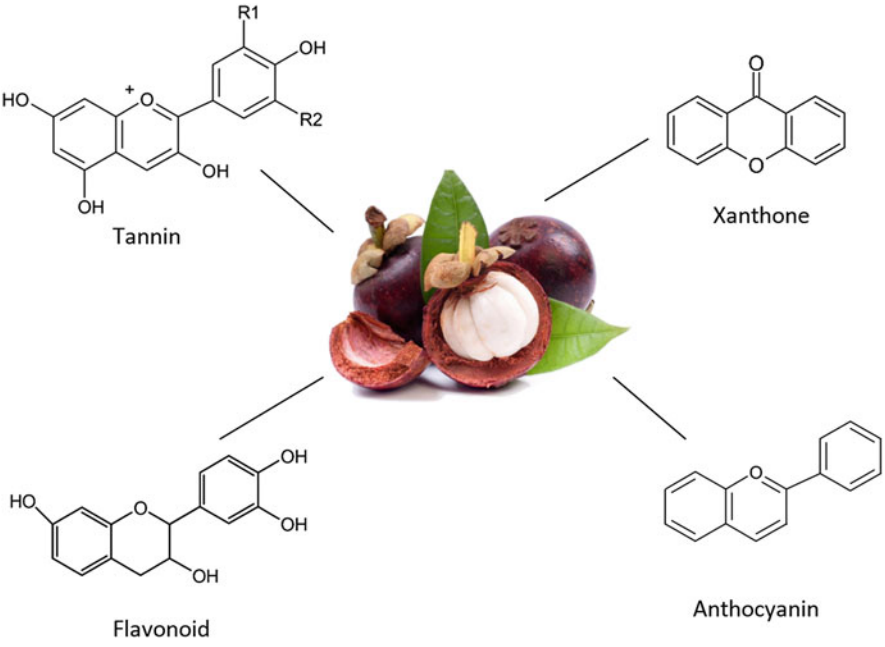


Fig. 2.1 Mangosteen fruit

mangosteen peel antioxidants consist of xanthones, flavonoids, anthocyanins, and tannins (Zarena and Udaya Sankar 2011).

Xanthones are the largest compounds, which include 3-iso mangostin, alpha-mangostin, beta-mangostin, gamma-mangostin, 8-desoxygartanin, gartanin, and garsinon (Walker 2007). Xanthone ($C_{13}H_8O_2$) is a polar compound that has a molecular weight of 196.19 g/mol, boiling point of 351 °C, and melting point of 174 °C. Xanthone's high melting point causes difficulties in its physical transformation even though the environment's temperature is very hot (Palapol et al. 2009). Meanwhile, flavonoids (C6-C3-C6) are polar compounds and included in the group of phenolic compounds, so they will dissolve in polar solvents such as ethanol and methanol (Dai and Mumper 2010). Flavonoids are divided into several groups according to their chemical structure, such as flavones, flavanols, and anthocyanins (Moraes et al. 2013). Whereas tannins are polyphenol compounds found in plants, which taste bitter and chelate and can clump proteins (Paryanto et al. 2017). Tannin monomers are digallic acid and D-glucose. Tannin has the molecular formula $C_{76}H_{52}O_{46}$ (Zalacain et al. n.d.). The last compound found in large quantities in mangosteen peel is anthocyanin. The term "anthocyanin" itself was derived from Greek words, "anthos" which means flower and "kyanos" which means blue. Anthocyanin is a compound that can give red, blue, and purple color to fruits, vegetables, and ornamental plants. Anthocyanin ($C_5H_{11}O$) has a molecular weight of 207.08 g/mol. Most anthocyanins are found in six forms; they are pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (Khoo et al. 2017).

Apart from its utilization as biodiesel additive, antioxidants from mangosteen also have an eminent prospect to be used in the food industry, primarily as a preservative in edible oil. As an additive for edible oil preservation, the mangosteen antioxidants act as lipid peroxidation resistors (Chong et al. 2015). Lipid peroxidation is a process when free radicals (oxidants) invade the double bond carbon-contained lipids such as polyunsaturated fatty acids which leads to food quality deterioration (Ayala et al. 2014). Unlike the use of synthetic antioxidants such as butylated hydroxytoluene (BHT); butylated hydroxy anisole (BHA); and tert-62 butylhydroquinone (TBHQ), which have a potential health risk, natural antioxidants from mangosteen peel are safe to human health (Maisuthisakul et al. 2007). In addition to its safety and ability to stabilize the edible oils and extend the shelf life, antioxidants from mangosteen peel are also known to contribute to the nutritional value of the oil (Chong et al. 2015; Bera et al. 2006).

2.3 Antioxidant Extraction of Mangosteen Peel

Extraction is a method used to separate a component in a material using a solvent (Zhang et al. 2018a; Dean 2009; Ramaswamy and Marcotte 2008). The solvent used must be able to extract the desired component without dissolving the other components. Broadly speaking, extraction is divided into two types; they are solid–liquid extraction and liquid–liquid extraction. In the solid–liquid extraction, like

mangosteen peel antioxidant extraction, contact occurs between two phases that causes the solvent diffusion from solids to liquids or solvents. The mechanism that occurs during the solid–liquid extraction can be described as follows:

(a) Mass transfer of the solvent to the material's surface, so the material's surface is coated with the solvent.
(b) There is a mass diffusion of the solvent from the material's surface into the material's pore.
(c) Solute contained in the material dissolves in the solvent.
(d) The solute solution in the material will be diffused out to the material's surface.
(e) The mass transfer of the solute solution from the material's surface to the solvent.

The extraction methods can be divided into two types, conventional and nonconventional. Conventional extraction is the simplest method because it uses solvents and conventional heaters which still has several disadvantages; it needs a lot of solvents and long extraction time, while the yields obtained are few (Tjahjani et al. 2014). Moreover, in a long period of time, the yield produced is not maximal. Recently, this method can cause thermolabile compounds to be degraded. In contrast to the conventional methods, nonconventional or modern methods have advantages, higher yields, faster extraction times, and fewer solvent volumes. This modern method has been carried out on an industrial scale. An example of a nonconventional method is MAE (Bagherian et al. 2011; Buanasari et al. 2017; Guo et al. 2012; Megawati et al. 2018).

The most commonly used conventional methods are maceration and Soxhlation. Maceration is a simple extraction method that is commonly used. This method is suitable for both laboratory and industry scale. This method can be done by adding materials in solvents with tightly closed state at room temperature (Damayanti and Fitriana 2015). The extraction is stopped when the equilibrium between the concentration of the component (extract) in the solvent and its concentration in the material is reached. To separate the extract solution with material, filtering should be done. A pure extract is obtained by evaporating the solvent and/or liquid–liquid extraction. The disadvantage of this method is that some compounds are difficult to extract at room temperature (Handayani and Nurchayati 2015). Some studies using this method are mangosteen peel antioxidant extraction (Jung et al. 2006), rose essential oil extraction (Damayanti and Fitriana 2015), and zodiac leaf essential oil extraction (Handayani and Nurchayati 2015).

As a matter of fact, recently there is one other choice of method to extract the antioxidants which used biotechnology. This method makes use of enzyme assistance in order to obtain the bioactive compound. Enzymes such as pectinases, cellulases, glucanases, and amylases can be used to disrupt the plant or fruit cell membranes, hence the bioactives can be released more effectively (Arnous and Meyer 2010). In comparison with previously mentioned methods, this enzyme-assisted extraction is far more ecofriendly as it does not use either high amount of solvent or energy (Puri et al. 2012). However, this method also has some drawbacks, for instances, longer extraction time; lower yield obtained; and limited information

about suitable type of enzymes for various kind of plants (Meini et al. 2019). Thus, further studies about enzyme-assisted extraction are still needed to develop a highly efficient and ecofriendly antioxidant method.

There are several factors that can affect the solid–liquid extraction; they are:

Solvent The solvents used in extraction should have the following properties:

(a) The solvent used must be adjusted to the polarity of the compound to be extracted, so that a purer extract can be obtained.
(b) The solvent used should not cause a chemical change in the components of the extract.
(c) Solvents must have a low boiling point, so that the solvent is easily evaporated even in low temperatures.
(d) The solvent used must not be corrosive, so that the equipment used is not corroded.

The solvents that have been used in extracting antioxidants are ethanol and water. Ethanol solvents are used for antioxidant extraction of petai leaves and basil leaves (Buanasari et al. 2017; Warsi and Sholichah 2017). Whereas water solvents were used for extraction of dragon fruit antioxidants (Thirugnanasambandham and Sivakumar 2017) and antioxidant extraction of green tea leaves (Ziaedini et al. 2010).

Particle Size Solid–liquid extraction process will be better if the particle diameter size is smaller. A smaller particle size will expand the contact surface with the solvent, so that the diffusion rate increases. However, it is not desirable that the particle size is too small, because the smaller the particle size, the more expensive the operating costs and the more difficult the separation process will be. Thus, obtaining pure extracts will be difficult (Sun et al. 2012). The particle size used in antioxidant extraction of mangosteen peel was 80 mesh (Ghasemzadeh et al. 2018) and the extraction of antioxidant dragon fruit was 40 mesh (Thirugnanasambandham and Sivakumar 2017).

Extraction Time A longer extraction time can lead to longer contact materials with solvents, so more extracts will be obtained (Handayani and Nurchayati 2015). The extraction has an optimum time, that is the time when the increase in the amount of extract is high, so that the amount of extract is high in an efficient time. The saturated solvent can no longer extract or decrease in its ability to extract because the thrust is getting smaller. As a result, the extraction time is longer and the resulting extract is no longer increasing (Buanasari et al. 2017). The time that has been used in antioxidant extraction using MAE was at least 2 min (Chong et al. 2015) and at the longest was 50 min (Buanasari et al. 2017).

Temperature In general, increasing extraction temperature will increase the amount of substances dissolved in the solvent. The solubility of the extracted material will increase with increasing temperature. In addition, the diffusivity coefficient is also increasing, so reaction rate will also be increasing (Damayanti and Fitriana 2015). In extraction using MAE, the effect of temperature is represented by power.

2.3.1 Antioxidant Extraction of Mangosteen Peel Using Soxhlation Method

Soxhlation is the most commonly used extraction method in a laboratory scale. This method can be done by adding a material on the filter paper placed in the extractor. The solvent used is put into the boiling flask and the temperature of the heater is set below the reflux temperature. This extraction will be done with several cycles determined by the researcher. The advantage of this method is the continuity of the extraction process; it does not require a lot of solvents and time. However, this process has a disadvantage. Thermolabile compounds can be degraded, because the extract is obtained continuously at the boiling point (Zhang et al. 2018b; Handayani and Juniarti 2012). This method was used in some studies on extractions of several components, such as coriander oil, frangipani leaves essential oil, and clove flower essential oil (Handayani and Juniarti 2012; Megawati and Saputra 2012; Hadi 2012).

In the antioxidant extraction of mangosteen peel using Soxhlation, before being used, 1 kg mangosteen peel was dried using an oven (Mammert™) at 50 °C for 24 h. The dried mangosteen peel was blended using a blender (Philips) until it became powder which was then sieved using a 500 μm strainer (Endecotts). Drying mangosteen peel was done to prevent the fungal growth, so the peel will still be in a good shape when it is stored before being used. Moreover, drying the peel also eases the blending process (Zhang et al. 2018b). The mangosteen peel is dried at low temperatures so that the components are not damaged, the quality is not reduced. The purpose of blending the mangosteen peel was to enlarge its contact area with ethanol solvents.

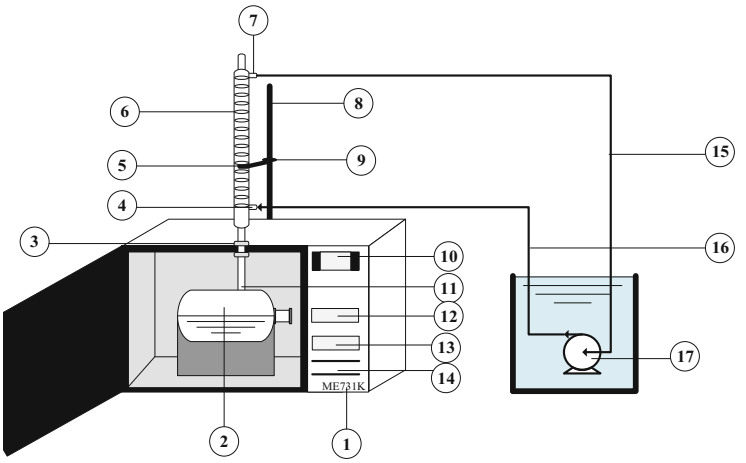
The antioxidant content of mangosteen peel was tested through an extraction using Soxhlet and methanol as solvent (material mass of 40 g and solvent volume of 400 mL). The heater used was a 200 W Electrothermal M575370/03. The extraction was done for 25 cycles (12.5 h). After the extraction, the solvent was recovered using distillation at 68 °C and until the volume was 20 mL. For further purification, the extract obtained was heated in the oven at 68 °C until constant weight was obtained. The phases of the process are depicted in Fig. 2.2. The extract was then measured using a digital scale and its phenolic concentration was tested using UV–Vis spectrophotometer (Genesys 10 UV).

From the experiment, the phenolic content of mangosteen peel extract obtained from Soxhlation method is about 23.16% (9.264 g). Accordingly, mangosteen peel has a phenolic content of 28.88% (Suttirak and Manurakchinakorn 2012). Differences in climate, geography, and other environmental factors affect the phenolic contents of the same material. The energy needed to produce 1 g phenolic compound was about 0.3 kWh. It is assumed that 1 kWh is IDR 1350, so the production cost of 1 g phenolic compound using Soxhlation method is Rp 364.

Fig. 2.2 Phases of change from mangosteen peel to antioxidant extracts

2.3.2 Antioxidant Extraction Using MAE Method

MAE is an extraction method which utilizes microwave radiation as a heating medium. The basic mechanism of heating using MAE involves molecules in dipole material. If a molecule is exposed to microwave radiation, the dipole will align itself with each other. In addition, when microwaves are continuously emitted, there is a movement between molecules that cause heat because of the friction between one molecule and another. This heat serves as a heating to the sample in the microwave (Kapoore et al. 2018). This is convenient to extracting thermolabile antioxidant compounds, because this method has better temperature control compared to conventional heating methods (Thirugnanasambandham and Sivakumar 2017; Megawati et al. 2019). Moreover, MAE is seen to be more advantageous, because the extraction process is shorter and higher yields can be obtained (Chuyen et al. 2017; Karami et al. 2015). MAE has been used to extract the sweet orange peels essential oil (Megawati and Kurniawan 2015), dragon fruit pectin (Megawati and Ulinuha 2015), *Chorella* sp. microalgae oil (Barqi 2015), dragon fruit peel betalain pigment (Thirugnanasambandham and Sivakumar 2017), *Parkia speciose* "petai" leaves antioxidant (Buanasari et al. 2017), basil leaves antioxidant (Dean 2009), tea



1. Microwave oven, 2. Glass extractor, 3. Hanger, 4. Water inlet nozzle, 5. Cast iron clamp, 6. Spiral condenser, 7. Water outlet nozzle, 8. Cast iron static, 9. Cast iron boss head, 10. Monitor, 11. Vapor outlet nozzle, 12. Power regulator, 13. Timer, 14. Power switch, 15. Water outlet hose, 16. Water inlet hose, 17. Cooling water pump

Fig. 2.3 The equipment of microwave-assisted extraction of mangosteen peel antioxidant

leaf caffeine (Pan et al. 2003), and chestnut saponins (Buanasari et al. 2017; Thirugnanasambandham and Sivakumar 2017; Arnous and Meyer 2010; Megawati and Kurniawan 2015; Megawati and Ulinuha 2015; Barqi 2015; Pan et al. 2003; Kerem et al. 2005). MAE has great potential to be a good technique for extracting organic materials (Olalere et al. 2019). Compared to conventional methods, MAE can also accelerate extraction rates (Megawati et al. 2019). The amount of phenolic compounds obtained from the MAE method in 1–3 min is almost the same as that obtained by the maceration method within 15 h (Li et al. 2012). The lowest microwave power used in antioxidant extraction was 100 W (Thirugnanasambandham and Sivakumar 2017) and the highest was 900 W (Zhang et al. 2018b).

The antioxidant extraction of mangosteen peel using MAE method can be performed by putting 40 g mangosteen peel powder into a 600 mL glass extractor, in which 400 mL ethanol was then added. The equipment used is depicted in Fig. 2.3. The microwave oven used was Samsung ME731K and the reflux condenser used was Leibig with a diameter of 4.1 cm and length of 30 cm. The extraction was performed at 300, 450, and 600 W for 5–35 min. Sampling was carried out every 5 min and the phenolic concentration was analyzed using a UV–Vis spectrophotometer. After the extraction was completed (35 min), the antioxidant solution was vacuum filtered to separate the filtrate and residue; the solvent in the filtrate was recovered using distillation at 78 °C until the extract volume was 20 mL; the extract obtained was purified by evaporating the solvent in the filtrate using the oven at

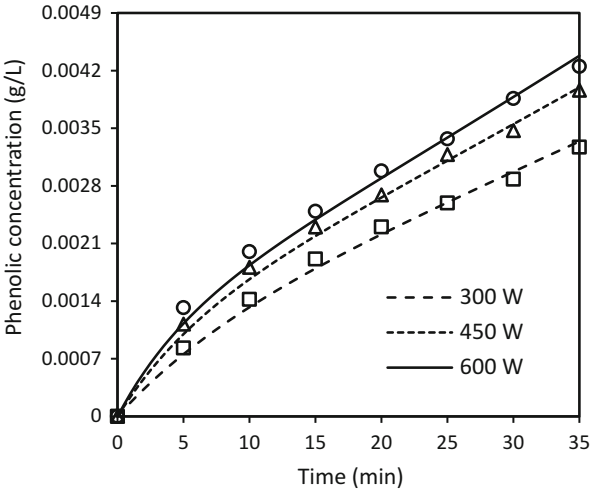


Fig. 2.4 The effect of power and time on antioxidant concentration of mangosteen peel extraction using MAE

78 °C until constant weight was obtained. The extract obtained was used as an antioxidant solution.

The effect of extraction times (5–35 min) on phenolic concentration obtained from mangosteen peel extraction using MAE can be seen in Fig. 2.4. The longer the time, the more the phenolic concentrations of mangosteen peel extraction will be. In addition, until 35 min, the concentrations were still increasing. At 300, 450, and 600 W, the highest increase of phenolic concentrations occurred when the extraction times were 5–10 min, which was 0.59, 0.68, and 0.68 mg/mL, respectively, and after 10 min, the phenolic concentration steadily increased. A similar observation was conducted for alpha-mangostin extraction from mangosteen pericarp, in which with an increase in extraction time from 2 to 4 min, the α-mangostin value increased significantly (Ghasemzadeh et al. 2018).

Phenolic concentration of mangosteen peel extract is also affected by microwave power. At 15 min and 300, 450, 600 W, the phenolic concentrations obtained were 1.91, 2.30, 2.49 mg/mL, respectively. The highest phenolic concentrations were obtained at 35 min; they were 3.28, 3.96, 4.25 mg/mL at 300, 450, 600 W, respectively. The increase in power can provide more heat as a driving force to destroy the pore cells of mangosteen peel, so that antioxidants can be diffused out (Megawati et al. 2019). Extraction conversion can be calculated from the values of phenolic concentrations and the result showed that at 300, 450, 600 W, the conversion values were 15.45, 17.00, 18.33%, respectively. Meanwhile, generally, the greater the microwave power used, the greater the conversion will be (Li et al. 2017). Accordingly, at 40–120 °C, the increase in total vitamin E was 59.63% in rice bran oil extracted using isopropanol as a solvent through microwave assisted method (Zigoneanu et al. 2008).

After the solvent was separated, a very concentrated antioxidant solution was obtained, which would be used for biodiesel B20 oxidation. The concentrated antioxidant solutions had total phenolic concentrations in mg GAE/g extract; they

were 155.77, 188.32, and 202.20 mg GAE/g extract at 300, 450, and 600 W, respectively. These values are in accordance with research conducted by Uslu and Ozcan (Uslu and Ozcan 2017). In their research, the highest phenolic concentration was at 720 W (107 mg GAE/g extract) and the lowest was at 180 W (33.38 mg GAE/g extract).

In 35 min, the phenolic compound produced from extractions using MAE at 300, 450, and 600 W was about 6.18, 6.8, and 7.33 g, respectively. Therefore, the energy needed to produce 1 g phenolic compound at 300, 450, and 600 W were about 0.028, 0.039, and 0.047 kWh. Hence, the production cost to produce 1 g phenolic compound using MAE method at 300, 450, and 600 W are IDR 38; 53; and 65. It can be concluded that MAE method is more economical and effective for mangosteen peel antioxidant extraction, with about 82.14% energy efficiency.

2.4 Kinetics on Antioxidant Extraction of Mangosteen Peel Using MAE

Extraction kinetics is important to facilitate the design of unit operation. In the solid–liquid extraction, the mass transfer of solute from solid to liquid will undergo through two main stages; they are diffusion from the solid to its surface and mass transfer from the surface to the liquid. Both processes go simultaneously. If one of the processes is relatively much faster, then the extraction speed is determined by the slow one. However, if both processes have similar extraction speeds, then the extraction speed is determined by both processes. If the solid is relatively small, the diffusion of solute from solid to its surface will be very fast, so that the extraction speed is determined by the speed of the solute mass transfer from the solid's surface to the liquid. In contrast, when the solid is relatively large, then the diffusion of solute from the solid to the surface will be very slow. Therefore, the extraction speed is determined by the solute diffusion speed from the solid to the surface.

Accordingly, in the case of very small particles, the diffusion of solute in the inner particles was assumed very fast that can be negligible (Sayyar et al. 2009). Hence, the rate-limiting step is only the mass transfer from the particle surface to the bulk of solution. The mass transfer of solute in the solid–liquid extraction can be expressed by homogeneous reaction rate law. In addition, the extraction kinetics of *Jatropha* seeds with hexane and petrochemical ether solvents using MAE has been studied. The kinetic study results stated that the mass transfer from the solid's surface to the solution is controlling the rate of process that followed the second order of homogeneous reaction model. Krishnan dan Rajan (2016) also did the same study on flavonoid extraction on *Terminalia bellerica* using MAE.

In this section, the three models above will be studied, they are:

1. Mathematical model for the intra-particle diffusion-controlled rate (Model 1)
2. Mathematical model for the intra-particle diffusion and mass transfer-controlled rate (Model 2)

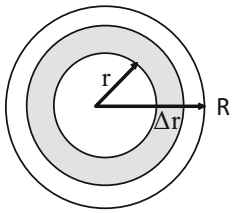


Fig. 2.5 Element of solute volume in a sphere-shaped material item. *Rate of Input – Rate of output = Rate of Accumulation*

3. Mathematical model for mass transfer-controlled rate (Model 3)

Solute diffusion from the inside the material to its surface can be explained as follows.

Intra-particle Diffusion The mass balance *A* in the volume element in Fig. 2.5 is expressed as Eq. (2.1) and is simplified into Eqs. (2.2)–(2.5).

$$\left(-D_e.4.\pi.r^2.\left.\frac{\partial C_A}{\partial r}\right|_r\right) - \left(-D_e.4.\pi.(r+\Delta r)^2.\left.\frac{\partial C_A}{\partial r}\right|_{r+\Delta r}\right) = 4.\pi.r^2.\Delta r.\frac{\partial C_A}{\partial t} \tag{2.1}$$

$$\frac{(r+\Delta r)^2.\left.\frac{\partial C_A}{\partial r}\right|_{r+\Delta r} - r^2.\left.\frac{\partial C_A}{\partial r}\right|_r}{\Delta r} = \frac{1}{D_e}.r^2.\frac{\partial C_A}{\partial t} \tag{2.2}$$

with:

D_e: Effective diffusivity (cm^2/s)
r: radius, cm
t: time, s

If $\Delta r \to 0$, then,

$$\frac{\partial}{\partial r}\left(r^2.\frac{\partial C_A}{\partial r}\right) = \frac{1}{D_e}.r^2.\frac{\partial C_A}{\partial t} \tag{2.3}$$

$$r^2.\frac{\partial^2 C_A}{\partial r^2} + 2.r.\frac{\partial C_A}{\partial r} = \frac{1}{D_e}.r^2.\frac{\partial C_A}{\partial t} \tag{2.4}$$

$$\frac{\partial^2 C_A}{\partial r^2} + \frac{2}{r}.\frac{\partial C_A}{\partial r} = \frac{1}{D_e}.\frac{\partial C_A}{\partial t} \tag{2.5}$$

Mass Transfer in Extraction Mass transfer in extraction is affected by operating conditions. To calculate the mass transfer rate, mass transfer coefficient is needed. Theoretically, several factors can influence the mass transfer rate; they are contact time and operating temperature. For extraction using MAE, the operating

temperature can be represented by power. The speed of mass transfer from the grain surface to the liquid follows Eq. (2.6) (Scott Fogler 2016; Levenspiel 1999).

$$N_A\left(\frac{\text{mass}A}{\text{time} \times \text{area}}\right) = K_C\left(C_f^* - C_f\right) \tag{2.6}$$

with:

K_C: Mass transfer coefficient
C_f^*: The level of A in the liquid which is balanced with the concentration of A on the surface of the grain.
C_f: The level of A in the liquid (mass A/free solvent mass A) at any time

At certain times, the concentration of antioxidants in solids will balance the concentration of antioxidants in the solvent. The equilibrium relationship follows an equation similar to Henry's Law (Eq. 2.7).

$$C_A = H.C_f^* \tag{2.7}$$

with:

C_A: The concentration of solute in solids, g solut/solid's volume
H: Henry's equilibrium constant

2.4.1 *Mathematical Model for Intra-particle Diffusion-Controlled Rate of Antioxidant Extraction of Mangosteen Peel Using MAE (Model 1)*

In extraction, the initial level of A is C_{AO} with radius R, as many as Np pieces that are in the extractor, they will be dissolved in pure solvent with volume V. Equation (2.5) can be solved by finite difference approximation. In this way, the equation is changed to an algebraic equation. Solid radius and time are divided into small intervals as thick as Δr, number N, interval boundaries are indexed $i = 1, 2, 3, \ldots, N$, for radius, while for time divided by Δt, a number of T and given an index $j = 1, 2, 3, \ldots, T$. The approach is done using an explicit method, Eqs. (2.8)–(2.10).

$$\frac{\partial C_A}{\partial r} = \frac{(C_A)_{i+1,j} - (C_A)_{i-1,j}}{2.\Delta r} \tag{2.8}$$

$$\frac{\partial^2 C_A}{\partial r^2} = \frac{(C_A)_{i-1,j} - 2(C_A)_{i,j} + (C_A)_{i+1,j}}{(\Delta r)^2} \tag{2.9}$$

$$\frac{\partial C_A}{\partial t} = \frac{(C_A)_{i,j+1} - (C_A)_{i,j}}{\Delta t} \tag{2.10}$$

Thus, by substituting Eqs. (2.8)–(2.10) to Eq. (2.5), Eq. (2.11) is obtained.

$$\frac{(C_A)_{i-1,j} - 2(C_A)_{i,j} + (C_A)_{i+1,j}}{(\Delta r)^2} + \frac{2}{i.\Delta r} \cdot \frac{(C_A)_{i+1,j} - (C_A)_{i-1,j}}{2.\Delta r} = \frac{1}{D_e} \cdot \frac{(C_A)_{i,j+1} - (C_A)_{i,j}}{\Delta t} \tag{2.11}$$

By multiplying Δr (Zarena and Udaya Sankar 2009) on the left and right segments, Eq. (2.12) is obtained. If M is expressed by Eq. (2.13), the Eq. (2.12) can be simplified to Eq. (2.14).

$$(C_A)_{i-1,j} - 2(C_A)_{i,j} + (C_A)_{i+1,j} + \frac{1}{i}\left((C_A)_{i+1,j} - (C_A)_{i-1,j}\right) = \frac{(\Delta r)^2}{D_e.\Delta t}\left((C_A)_{i,j+1} - (C_A)_{i,j}\right) \tag{2.12}$$

$$M = \frac{(\Delta r)^2}{D_e.\Delta t} \tag{2.13}$$

$$(C_A)_{i,j+1} = \frac{\left(1 - \frac{1}{i}\right)(C_A)_{i-1,j} + (-2 + M)(C_A)_{i,j} + \left(1 + \frac{1}{i}\right)(C_A)_{i+1,j}}{M} \tag{2.14}$$

Eq. (2.14) applies to $i = 1, 2, 3, \ldots, N-1$ and boundary conditions such as Eqs. (2.15)–(2.17). The boundary condition as in Eq. (2.16) was obtained by assuming that the solute mass transfer from the solid's surface to the liquid was relatively quick, so the solute level on the solid surface was in proportion with the solute level in the liquid, and the proportion relationship can be approached by an equation similar to Henry's law.

$$C_A(r, 0) = CAO \tag{2.15}$$

$$C_A(R, t) = C_f\, H \tag{2.16}$$

$$\frac{\partial C_A}{\partial t}(0, t) = 0 \tag{2.17}$$

with:

C_{AO}: The concentration of solute in the original material, g solut/g volume of solids
R: Radius, cm

To solve Eq. (2.14) with limitations as in Eqs. (2.15)–(2.17), one equation was still needed to correlate C_f with C_A. The correlation between C_f and C_A can be

obtained by generating the total mass balance of A (antioxidant) in solid particles during extraction, as depicted in Eq. (2.18). The integral equation can be solved using the Simpson's Rule method.

$$N.\frac{4}{3}.\pi.R^3.C_{AO} = N.\int_0^R 4.\pi.r^2.C_A.dr + V.C_f \tag{2.18}$$

Equations (2.13)–(2.16) and total phenolic concentration data were then applied to curve-fitting method to evaluate the values of D_e and H. For a set of values of D_e and H, the values of C_f can be calculated. The values of C_f were evaluated and the values of D_e and H were optimized. The values chosen were the ones presenting the minimum value of the Sum of Squared Error (SSE). The *SSE* was defined in Eq. (2.19). Optimization was done using a solver tool of Microsoft Excel.

$$\text{SSE} = \sum \left(C_{f(\text{calc})} - C_{f(\text{data})}\right)^2 \tag{2.19}$$

Calculation Steps:

1. Determine C_{AO}, Δt, Δr, R, V, and N.
2. D_e and H values trial.
3. Calculating the value of M.
4. Calculating the value of C_A when $j = 0$.
5. Calculating C_f as $C_{f(\text{calc})}$.
6. Repeat steps 4–5 for $j = 1$ to $j = T$.
7. $C_{f(\text{calc})}$ is compared with $C_{f(\text{data})}$ until the smallest SSE value is obtained.
8. Calculating the average of error using Eq. (2.19).

$$\%\text{Average Error} = \frac{\sum\left(C_{f(\text{calc})} - C_{f(\text{data})}\right)}{C_{f(\text{data})}}\frac{1}{n}100\% \tag{2.20}$$

In the study described in the mangosteen peel extraction using MAE (Fig. 2.4), the solvent volume used was 400 mL; the mass of mangosteen peel powder 40 g; total solids 428; initial antioxidant concentration 0.232 g/cm^3; the material powder size was 50 μm (0.05 cm), so that the radius was 0.025 cm. Experimental data and results of calculations every time can be seen in Table 2.1 and Fig. 2.6. Based on calculations using Model 1 at power variations of 300, 450, and 600 W, it can be concluded that the antioxidant extraction of mangosteen skin using MAE with the Model 1 approach is less appropriate, especially at 300 W. Model 1 is also relatively more difficult to solve.

Table 2.1 Antioxidant concentration with intra-particle diffusion model-controlled rate approach (Model 1) of mangosteen peel extraction using MAE with ethanol as solvent ($V = 400$ mL, $m = 40$ g, $N_p = 428$, $C_{AO} = 0.232$ g/cm^3, $R = 0.025$ cm)

t (min)	Antioxidant concentration (g/cm^3)					
	300 W		450 W		600 W	
	Data	Calculation	Data	Calculation	Data	Calculation
0	0.0000	0.0000	0.00000	0.0000	0.00000	0.0000
5	0.0008	0.0016	0.00112	0.0016	0.00132	0.0016
10	0.0014	0.0018	0.00181	0.0020	0.00200	0.0020
15	0.0019	0.0021	0.00230	0.0024	0.00249	0.0025
20	0.0023	0.0023	0.00269	0.0028	0.00298	0.0030
25	0.0026	0.0026	0.00318	0.0032	0.00337	0.0034
30	0.0029	0.0028	0.00347	0.0036	0.00386	0.0039
35	0.0033	0.0030	0.00396	0.0039	0.00425	0.0043
SSE		1.17×10^{-06}		1.45×10^{-06}		1.03×10^{-06}
Average error (%)		11.51		18.29		13.53

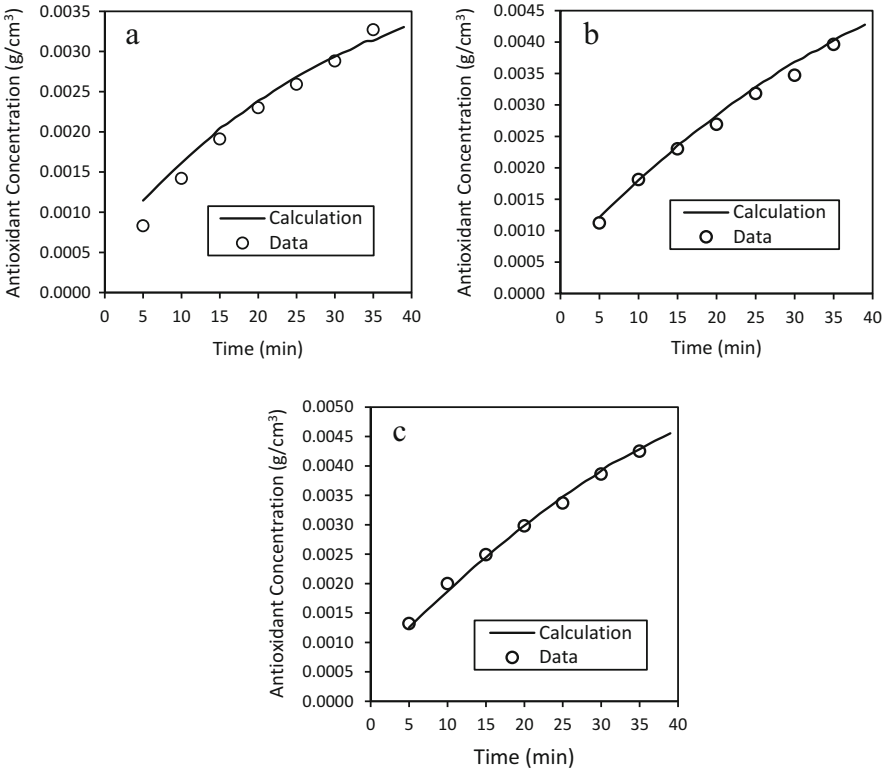


Fig. 2.6 The relationship between antioxidant concentration and extraction time with Model 1 approach on mangosteen peel extraction using MAE (**a** = 300 W, **b** = 450 W, **c** = 600 W)

2.4.2 *Mathematical model for Intra-particle Diffusion and Mass Transfer-Controlled Rate of Antioxidant Extraction of Mangosteen Peel Using MAE (Model 2)*

The extraction kinetics can be approached using solute diffusivity from inside the solid to the solid's surface and solute mass transfer from the solid's surface into solution. The solid was assumed to be the same and spherical with the radius R. During the extraction, the phenolic concentrations in mangosteen peel powder as a function of position and time ($C_A = f(r,t)$) can be derived through Eq. (2.13), with the limitation is as in Eq. (2.14). Equation (2.13) is used to calculate the phenolic concentration when $i = 1, 2, 3, \ldots$, and $N-1$. However, for $i = 0$, the phenolic concentration can be formulated using Eq. (2.21).

$$(C_A)_{0,j+1} = \frac{6(C_A)_{i,j} - (6 - M)\,(C_A)_{0,j}}{M} \tag{2.21}$$

The phenolic concentration for $i = N$ can be obtained by arranging $(C_A)_N = (C_A)_f = C_f$ as well as the mass balance of antioxidants in the solid's surface. Thus, the phenolic concentration when $i = N$, can be expressed in Eq. (2.22), with $\propto$, β, and γ in Eqs. (2.23)–(2.25), respectively.

$$(C_A)_{f,j+1} = \frac{-\alpha + \gamma.M - \frac{\beta}{H}\left[(C_A)_{f,j}\right] + \alpha.(C_A)_{f-1,j} + \beta C_f}{\gamma.M} \tag{2.22}$$

$$\left(R - \frac{\Delta r}{2}\right)^2 = \alpha \tag{2.23}$$

$$\frac{K_c.R^2.\Delta r}{D_e} = \beta \tag{2.24}$$

$$\frac{1}{2}\left(R - \frac{\Delta r}{4}\right)^2 = \gamma \tag{2.25}$$

Equations (2.13), (2.14), (2.18), (2.21)–(2.25), and total phenolic concentration data were then applied to curve-fitting method to evaluate the values of D_e, H, and K_c. For a set of values of D_e, H, and K_c the values of C_f can be calculated. The values of C_f were evaluated and the values of D_e, H, and K_c were optimized. The values chosen were the ones presenting the minimum value of the Sum of Squared Error (SSE). The SSE was defined in Eq. (2.19). Optimization is done using a solver tool of Microsoft Excel.

Calculation steps:

1. Determined C_{AO}, Δt, Δr, R, V, and Np.
2. K_c, D_e, and H values trial.
3. Calculating the value of M, α, β, and γ.

Table 2.2 Antioxidant concentration with intra-particle diffusion and mass transfer-controlled rate approach (Model 2) of mangosteen peel extraction using MAE with ethanol as solvent ($V = 400$ mL, $m = 40$ g, $N_p = 428$, $C_{AO} = 0.232$ g/cm^3, $R = 0.025$ cm)

t (min)	Antioxidant concentration (g/cm^3)					
	300 W		450 W		600 W	
	Data	Calculation	Data	Calculation	Data	Calculation
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.0008	0.0009	0.0011	0.0012	0.0013	0.0014
10	0.0014	0.0014	0.0018	0.0018	0.0020	0.0020
15	0.0019	0.0019	0.0023	0.0023	0.0025	0.0024
20	0.0023	0.0023	0.0027	0.0027	0.0030	0.0029
25	0.0026	0.0026	0.0032	0.0031	0.0034	0.0034
30	0.0029	0.0030	0.0035	0.0035	0.0039	0.0039
35	0.0033	0.0033	0.0040	0.0040	0.0043	0.0044
D_e (cm^2/s)		2.81×10^{-11}		3.42×10^{-11}		3.80×10^{-11}
K_c (cm/s)		6.36×10^{-08}		8.97×10^{-08}		1.05×10^{-07}
SSE		1.99×10^{-08}		2.02×10^{-08}		3.08×0^{-08}
Average error (%)		1.82		1.89		2.028

4. Calculating the value of C_A when $j = 0$.
5. Calculating the value of C_f.
6. Repeat steps 7–10 for $j = 1$ to $j = T$.
7. $C_{f(\text{calc})}$ is compared with $C_{f(\text{data})}$ until the minimum average error value and the smallest SSE are obtained.

The value of $C_{f(\text{data})}$ and $C_{f(\text{calc})}$ on the diffusion model in solids and the mass transfer from the surface to the controlling liquid (Model 2) were obtained from the calculations presented in Table 2.2 and Fig. 2.7. Model 2 is suitable for of mangosteen peel antioxidant extraction using MAE at all powers used.

Paryanto (2017) used this model and obtained diffusion coefficients of 2.20×10^{-11}, 3.40×10^{-11}, 3.95×10^{-11} cm^2/s for 300, 450, 600 W, and Henry's constant of 0.027. Recently, each material has diffusivity varies based on the physical properties and temperature (Huang et al. 2011). In addition, it can be seen that the higher microwave power, the higher the diffusion coefficients obtained. In mangosteen peel antioxidant extraction, the effect of temperature is represented by power, the higher the microwave power, the greater the heat energy produced and the faster the solute diffusion from solids to liquids will be (Hadi 2012). On the other hand, the power also affects the mass transfer coefficient, the greater the power, and the higher the mass transfer. This is because the greater the power, the faster the solute's motion.

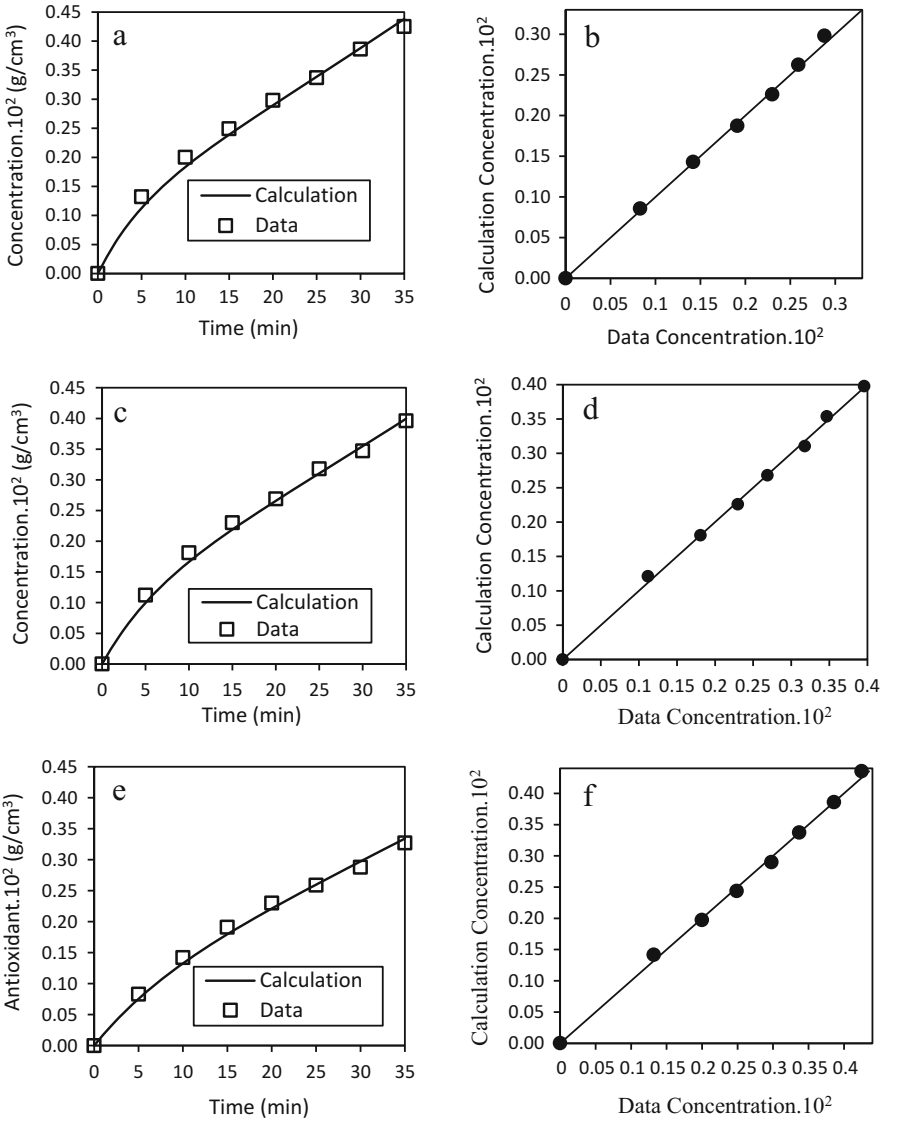


Fig. 2.7 The relationship between antioxidant concentration and extraction time with Model 2 approach on mangosteen peel extraction using MAE (V = 400 mL, m = 40 g, t = 35 menit, $N_p = 428.025$, $C_{AO} = 0.232$ g/cm^3, $R = 0.025$ cm; **a** and **b** = 300 W, **c** and **d** = 450 W, **e** and **f** = 600 W)

2.4.2.1 The Correlation Between Microwave Power (P) and Diffusion Coefficient (D_e) as Well as Microwave Power (P) and Mass Transfer Coefficient (K_c)

The correlations between microwave power and mass transfer coefficient, power, and diffusivity coefficient have not ever been analyzed. These correlations are done for scale-up calculations on an industrial scale. The equations used in these

Table 2.3 The calculation of the correlation between power (P) and diffusion coefficient (D_e) and power and mass transfer coefficient (K_c) on antioxidant extraction of mangosteen peel using MAE

P (Watt)	D_e (cm^2/s)	ln D_e	K_c (cm/s)	ln K_c
300	2.81×10^{-11}	−24.2949	6.36×10^{-08}	−16.5711
450	3.42×10^{-11}	−24.1000	8.97×10^{-08}	−16.2271
600	3.80×10^{-11}	−23.9934	1.05×10^{-07}	−16.0693

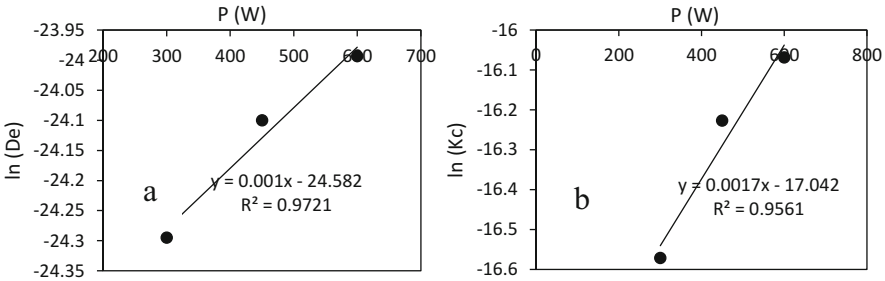


Fig. 2.8 The correlation between power (P) and D_e (**a**) and power (P) and (K_c) (**b**) on antioxidant extraction of mangosteen peel using MAE

correlations are empirical equations, such as Eqs. (2.26) and (2.27), where a, b, c, and d are constants. These constants can be calculated using linear regression and it becomes Eqs. (2.28) and (2.29), where a and c = exp. (intercept), b and d = − slope (Xin et al. 2009). The calculation results are presented in Table 2.3 and Fig. 2.8.

$$D_e = a \ \exp\ (-b.P) \tag{2.26}$$

$$K_c = c \ \exp\ (-d.P) \tag{2.27}$$

$$\text{In}\ D_e = \text{In}\ a - b.P \tag{2.28}$$

$$\ln\ (K_c) = \ \ln\ c - d.P \tag{2.29}$$

Based on Fig. 2.8, the correlation equations between P and D_e as well as P and K_c are depicted in Eqs. (2.30) and (2.31).

$$D_e = 2.109 \times 10^{-11}\ \exp\left(0.001\ P\right) \tag{2.30}$$

$$K_c = 3.970 \times 10^{-8}\ \exp\left(0.0017\ P\right) \tag{2.31}$$

2.4.3 *Mathematical Model for Mass Transfer-Controlled Rate of Antioxidant Extraction of Mangosteen Peel Using MAE (Model 3)*

Because the size of the mangosteen peel powder is too small, the solute diffusion from inside to solid's surface can be ignored. This mass transfer can be expressed by the homogeneous rate law. In addition, Krishnan and Rajan (2016) also did the same study on flavonoid extraction on *Terminalia bellerica* using MAE. The mass transfer of solute in the solid–liquid extraction can be expressed by the first- and second-order rate law, as described in Eqs. (2.29) and (2.30), with C_t = antioxidant concentration during the extraction (mg/mL), C_s = extraction capacity (mg/mL), t = time (min), and k_e = extraction rate constant (mL/mg/min) as well as the initial condition of $C_{t(t=0)} = 0$ and $C_{t(t=t)} = C_t$ (Krishnan and Rajan 2016).

$$-\frac{dC_t}{dt} = k_e(C_s - C_t) \tag{2.32}$$

$$-\frac{dC_t}{dt} = k_e(C_s - C_t)^2 \tag{2.33}$$

Eqs. (2.29) and (2.30) can be solved by integral method and its result is Eqs. (2.31) and (2.32) and then by using linearization method, these equations can be solved. The linear equations were expressed in Eqs. (2.33) and (2.34) and the parameters of k_e and C_s can be obtained (Fernando and Soysa 2015). The values of k_e and C_s are listed in Table 2.4 as well as the linear regression results are presented in Fig. 2.9.

$$C_t = C_s\left(1 - e^{-k.t}\right) \tag{2.34}$$

$$C = \frac{C_s^2 k_e t}{1 + C_s k_e t} \tag{2.35}$$

$$\log\ (C_s - C_t) = \log C_s - \frac{k}{2303}t \tag{2.36}$$

$$\frac{t}{C} = \frac{1}{k_e\ {C_s}^2} + \frac{t}{C_s} \tag{2.37}$$

Table 2.4 Extraction rate constant (k_e) and extraction capacity (C_s) of first-order rate law (Model 3) of mangosteen peel extraction using microwave-assisted extraction (operating condition: raw material weight of 20 g, raw material size of 500 μm, 70% ethanol volume as solvent of 260 mL)

Power (W)	Extraction capacity (C_s) (mg/L)	Extraction rate constant (k_e) (L/mg/min)
300	600	0.021
450	600	0.029
600	600	0.033

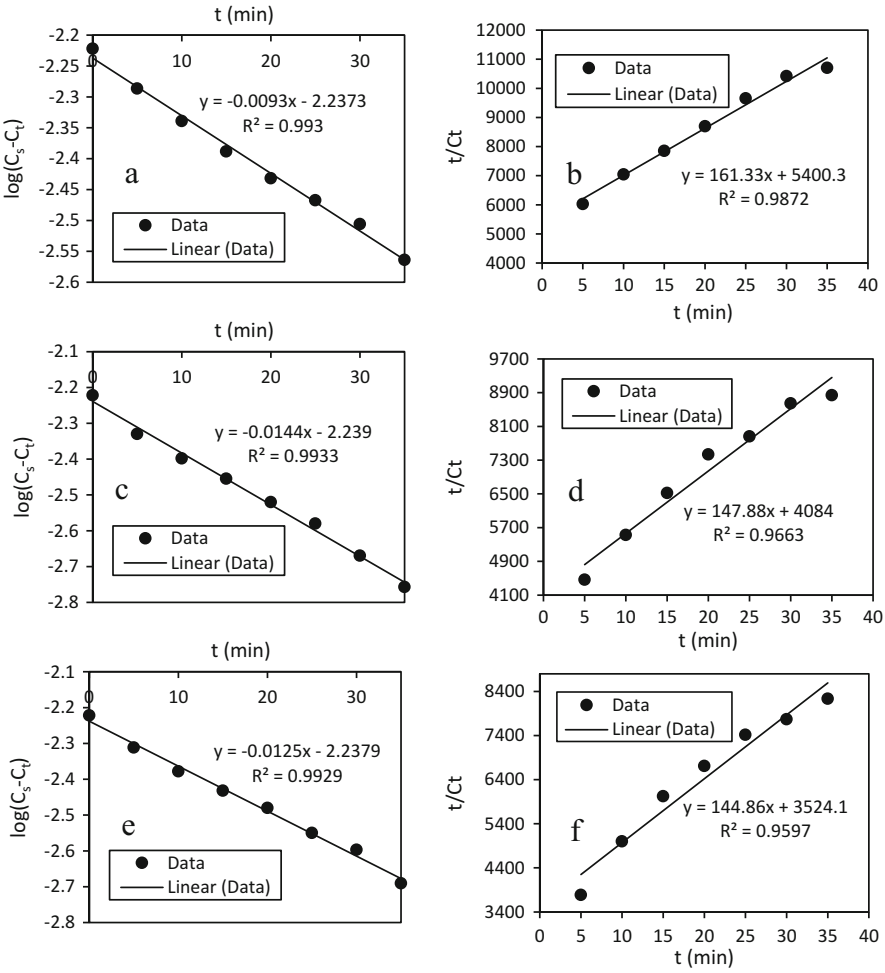


Fig. 2.9 Reaction rate law (Model 3) for mangosteen peel extraction using microwave-assisted extraction (**a**, **c**, **e** = first-order; **b**, **d**, **f** = second-order; operating condition: raw material weight of 20 g, raw material size of 500 μm, 70% ethanol volume as solvent of 260 mL)

2.5 Antioxidant Performance of Mangosteen Peel Extract on Biodiesel B20 Oxidation

Diesel fuel is very important for many aspects of daily life, especially transportation. Fossil fuels contribute 80% of the world's energy needs. Most industries use diesel engines for the production process, also in the transportation sector. This situation causes men a strong daily basis-dependence on fossil-based diesel fuel (Huang et al. 2011). To overcome this, alternative fuels, biodiesel, need to be developed. The source of biodiesel varies which generally comes from vegetable oils obtained in

nature. The properties of various fatty esters determine the properties of biodiesel fuel (Maisuthisakul et al. 2007). Biodiesel synthesis can also be done from *Chlorella* sp. oil (Widyastuti and Dewi 2015), which produces biodiesel with a density of 0.88 g/cm^3 (fulfilling one of the requirements of the Indonesian National Standard (SNI).

However, biodiesel contains unsaturated hydrocarbons which are unstable and easily oxidized to form fatty acids. Fatty acids can damage the quality of fuel which has an impact on the poor quality of fuel combustion in diesel engines. This problem can be avoided by adding antioxidants which can reduce NOx hydrocarbons, CO, particulates, polycyclic aromatic hydrocarbons, SO_2, and smoke in combustion engine exhaust emissions effectively. Antioxidants will suppress the oxidation of unsaturated fuels in their double bonds by ending the release of free radicals (Ramalingam et al. 2018). Free radicals are known as reactive and unstable molecules that contain one or more unpaired electrons in their outer orbitals. As an effort to achieve stability, free radicals will react with nearby atoms or monomers to obtain electron pairs. Meanwhile, antioxidants are compounds that can donate electrons (hydrogen atom givers) to free radicals which then can stop the chain reactions and convert free radicals into stable forms. Therefore, to reduce free radical activity, antioxidants are needed.

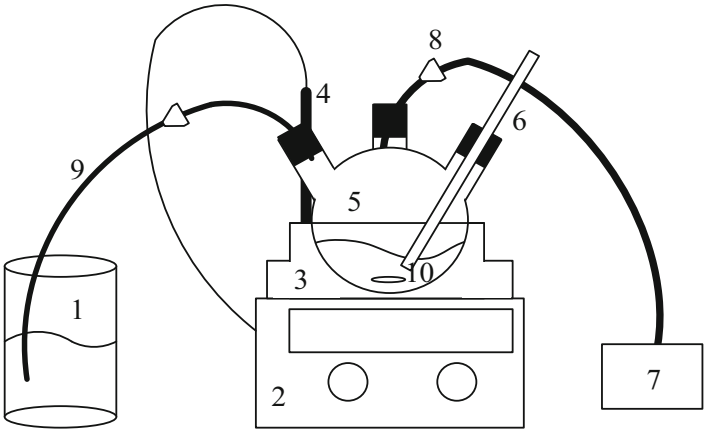
Theoretically, the oxidation reaction is divided into three stages; they are initiation, propagation, and termination. The first stage occurs when acid methyl esters (RH) release hydrogen atoms to form free radicals (R•), then it reacts with oxygen, or often called oxidized, forming peroxide radicals (ROO•), and/or hydroperoxide (ROOH). During the oxidation, peroxide radicals perpetually form new radicals (R•) to bind oxygen from the air and start chain reactions quickly (McCormick 2007).

Biodiesel oxidation occurs due to the reaction between oxygen and unsaturated fat methyl ester. Accordingly, diesel fuel is easily oxidized by oxygen, light, high temperature, and metals (Bouaid et al. 2009; Leung et al. 2006; Kivevele and Huan 2013; Park et al. 2008). This is certainly also experienced by biodiesel, because it is a mixture of biodiesel and diesel fuel in a certain ratio. In biodiesel, antioxidants function to capture free radicals formed during oxidation and stop chain reactions in fuel degradations (Spacino et al. 2016). Moreover, according to Coppo (Coppo et al. 2013) and Spacino (Spacino et al. 2016), natural antioxidants can inhibit biodiesel oxidation. Mangosteen peel extract can inhibit biodiesel oxidation with a concentration of 0.01% w/v, so the formation of free radicals can be inhibited by transferring hydrogen atoms into radical compounds or turning them into more stable forms. In the next discussion, mangosteen peel antioxidant extract used to inhibit biodiesel B20 oxidation will be explored using the oxidation kinetic. Meanwhile, the oxidation of mixture of biodiesel B20 and antioxidant obtained from mangosteen peel extraction was performed using air.

2.5.1 *Oxidation Experiment of Biodiesel B20 Using Mangosteen Peel Antioxidant Extract*

Experiments for studying biodiesel B20 oxidation using mangosteen peel extract can be carried out as follows. Before being used, biodiesel and the mixture of biodiesel and antioxidant's densities and kinematic viscosities were analyzed. The study was begun with mixing 95% biodiesel B20 and 5% antioxidant of mangosteen peel extract with a total volume of 100 mL into a three-neck volumetric flask. The mixture was then stirred at 600 rpm and heated to 100 °C. Then the air was flowed at a constant speed of 2.3 L/min. This oxidation was run for both biodiesel B20 and the mixture of biodiesel B20 and mangosteen peel extract antioxidants at various temperatures of 100, 110, and 120 °C for 0–70 min. The samples were taken every 10 min and their acid numbers were analyzed. The equipment used can be seen in Fig. 2.10. Biodiesel B20 was obtained from PT Pertamina, 96% ethanol from local chemical shop, Folin–Ciocalteu reagent from Chemistry Laboratory of Universitas Diponegoro, and Na_2CO_3 (Merck 106392), methanol (Merck 106009), NaOH (Merck 106462), and distilled water from Research Laboratory of Chemical Engineering Department of Universitas Negeri Semarang.

The densities and viscosities of biodiesel B20 and the mixture of biodiesel B20 and antioxidant of mangosteen peel extract can be seen in Table 2.5. Compared to the density of biodiesel, the density of the mixture of biodiesel and antioxidant was higher. The density value still meets the predetermined standard limit of 815–860 kg/m^3. This is in line with Fattah (2014) which stated that at 40 °C, the addition of antioxidants makes the density of biodiesel B20 higher. According to



1. Beaker glass, 2. Magnetic stirrer, 3. Heating mantle, 4. Thermocouple, 5. Three-neck volumetric flask, 6. Thermometer, 7. Air pump, 8. Check valve, 9. Hose, 10. Magnetic stirring bar

Fig. 2.10 Equipment of biodiesel oxidation with air

Table 2.5 The density and viscosity of biodiesel B20 and the mixture of biodiesel B20 and mangosteen peel extract antioxidant (operating conditions: temperature of 40 °C, stirrer rotation of 600 rpm, and air velocity of 2.3 L/min)

Sample	Density (kg/m^3)	Kinematic viscosity (mm^2/s)
B20	824	2.92
B20 + mangosteen peel extraction	844	2.90

Table 2.6 The conversions of biodiesel B20 and mixture of biodiesel B20 and mangosteen peel extract antioxidant during the oxidation process with air (operating conditions: antioxidant ratio of 5/95, stirrer rotation of 600 rpm, and air velocity of 2.3 L/min)

	Biodiesel B20			Mixture of biodiesel B20 and mangosteen peel extract		
Time (min)	100 °C	110 °C	120 °C	100 °C	110 °C	120 °C
0	0.000	0.000	0.000	0.000	0.000	0.000
10	0.043	0.105	0.173	0.018	0.043	0.089
20	0.106	0.179	0.298	0.061	0.100	0.139
30	0.167	0.289	0.457	0.082	0.123	0.177
40	0.267	0.394	0.516	0.101	0.169	0.211
50	0.311	0.463	0.667	0.139	0.170	0.282
60	0.418	0.540	0.730	0.172	0.230	0.376
70	0.466	0.667	0.806	0.198	0.243	0.413

Demirbas (2009) and Knothe (2007), in a fuel engine, a high viscosity will interfere with the fuel injection processes. The viscosity of the mixture of biodiesel B20 and mangosteen peel extract antioxidant was lower compared to biodiesel B20. The viscosity decreased but was still in the predetermined standard of 2–4.5 (mm^2/s). This is in accordance with a research conducted by Kivevele and Huan (2013), which stated that the addition of antioxidants could decrease the viscosity of biodiesel. Before being oxidized, the acid numbers of biodiesel B20 were 0.0881, 0.1058, and 0.1098 mg NaOH/mg at 100, 110, and 120 °C, respectively, while the acid numbers of the mixture of biodiesel B20 and mangosteen peel antioxidant were 0.2889, 0.3043, and 0.3077 mg NaOH/mg at 100, 110, and 120 °C, respectively. The addition of antioxidants increases acid numbers, but still within their limits—less than 0.5 mg KOH/mg (0.356 mg NaOH/mg) (Mahajan et al. 2006).

The effect of time and temperature on reaction conversion during the oxidation of biodiesel B20 and the mixture of biodiesel B20 and mangosteen peel extract antioxidant is presented in Table 2.6. At a fixed temperature, the conversion kept increasing during the oxidation process. At 100 °C, in 70 min of biodiesel B20 oxidation, the conversion increased until 0.466. In other words, it increased 0.67%/10 min. This shows that biodiesel B20 is easily oxidized. This is in line with Bouaid (2009), who stated that acid numbers, peroxide numbers, and viscosity increased with the increase in biodiesel storage time. However, in 70 min of the mixture of biodiesel B20 and mangosteen peel extract oxidation, the conversion increased until 0.198, or 0.28%/10 min. This shows that mangosteen peel extract antioxidants could slow down the oxidation for 26.32%. At 110 and 120 °C, the antioxidant extract of

mangosteen peel could inhibit the oxidation process of biodiesel B20 for 36.84 and 51.30%. The higher the temperature, the stronger the antioxidant effect to inhibit biodiesel oxidation process. This result is in line with a research conducted by Xin (2009) and Gregorio (2017).

The effect of temperature on biodiesel B20 oxidation can be investigated in 50 min. The conversions at 100, 110, and 120 °C were 0.311, 0.463, and 0.667, respectively. Every increase of 10 °C, the conversion increased by an average of 0.178 or 47.84%. Thus, the increase in the temperature gave a sensitive effect on the biodiesel B20 oxidation (Pereira et al. 2015). However, at the same oxidation time and temperatures, the conversions of the mixture of biodiesel B20 and mangosteen peel extract antioxidants were 0.139, 0.170, and 0.282, respectively. In other words, the conversion increase was only 14%. This further strengthens that antioxidants can inhibit the oxidation of biodiesel B20.

2.5.2 *The Oxidation Kinetics of Biodiesel B20 and the Mixture of Biodiesel B20 and Mangosteen Peel Extract Antioxidant*

Gregorio (2017) has conducted a study on the performance of natural antioxidants from pepper extract, coffee leaf, bacupari leaf, and sage to inhibit biodiesel B100 oxidation. Its performance was studied through an oxidation kinetics approach called pseudo-homogeneous first-order model. The effect of temperature on the oxidation kinetics was approached by the Arrhenius equation. Meanwhile, the performance of antioxidants obtained from mangosteen peel extraction on the oxidation kinetics will be evaluated based on the activation energy. The assumptions in the oxidation taken were: the reaction runs continuously with a constant volume (Zhou 2013); the air used is continuously flowed so that the reduction on oxygen concentration during the reaction can be ignored. The correlation between conversion and time in the first-order approach was derived into Eq. (2.38), with x_a = reaction conversion, t = time, and k = reaction rate constant. The conversion was calculated from the reduction of acid numbers during the reaction, as described in Eq. (2.39).

$$-\ln\ (1 - x_a) = k.t \tag{2.38}$$

$$x_a = \frac{AV_t - AV_0}{AV_0} \tag{2.39}$$

The rate reaction constant was influenced by temperature based on Arrhenius equation, as in Eq. (2.40) with A = frequency factor (1/min), E_a = activation energy (kJ/mol), R = universal gas constant, and T = temperature (K) (Levenspiel 1999). Parameters A and E_a were found out using curve-fitting method.

Table 2.7 Reaction rate constant (k) of biodiesel B20 and the mixture of biodiesel B20 and mangosteen peel extract antioxidant (operating conditions: antioxidant ratio of 5/95, stirrer rotation of 600 rpm, and air velocity of 2.3 L/min)

	k (1/min)	
T (°C)	Biodiesel B20	The mixture of biodiesel B20 and antioxidant
100	0.0056	0.0038
110	0.0076	0.0005
120	0.0104	0.0072

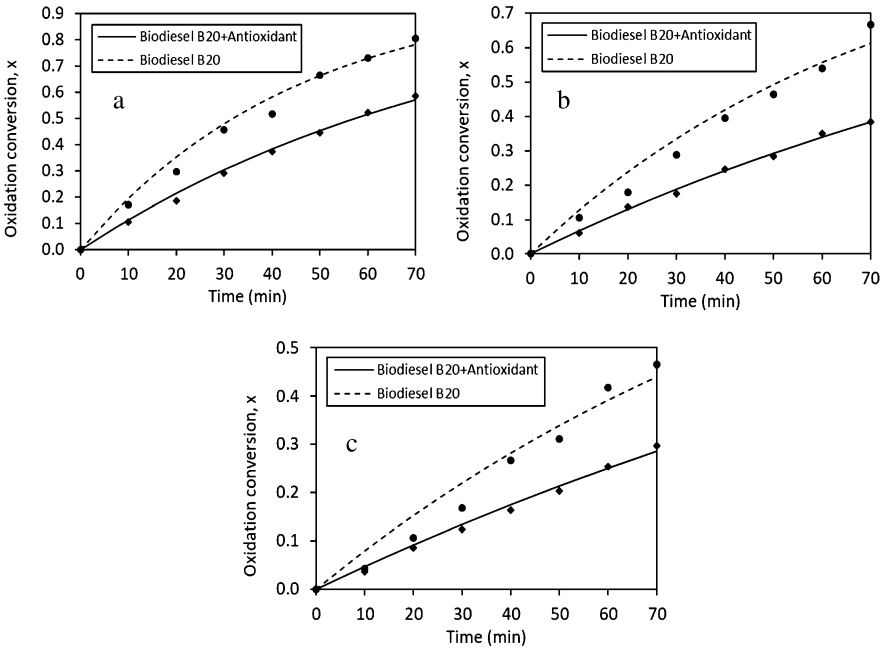


Fig. 2.11 The correlation between time (t) and conversion (x) in the mixture of biodiesel B20 and mangosteen peel extract oxidation (symbols = data; line = calculation; **a** = 100 °C, **b** = 110 °C, **c** = 120 °C, operating conditions: antioxidant ratio of 5/95, stirrer rotation of 600 rpm, and air velocity of 2.3 L/min)

$$\ln(k) = -\frac{E_a}{\mathrm{RT}} + \ln(A) \tag{2.40}$$

The conversion values obtained from biodiesel B20 oxidation were used to study the oxidation kinetics. The values of reaction rate constant (k) for biodiesel B20 and the mixture of biodiesel B20 and antioxidant oxidation, respectively, as well as are listed in Table 2.7. Fig. 2.11 shows the comparison results between the kinetics calculation using pseudo-homogeneous first-order model with experiment data. In order to check whether the values of reaction constant calculated from Table 2.7

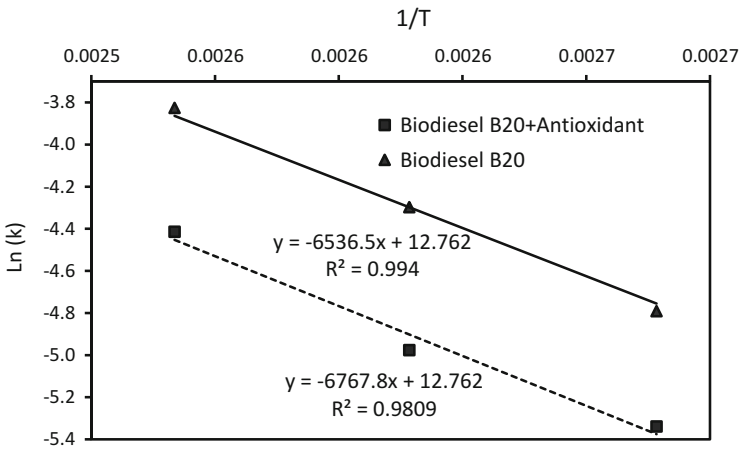


Fig. 2.12 Natural logarithm of the rate constant (ln (*k*)) versus 1/*T* for biodiesel B20 and the mixture of biodiesel B20 and mangosteen peel extract antioxidant (operating conditions: antioxidant ratio of 5/95, stirrer rotation of 600 rpm, and air velocity of 2.3 L/min)

satisfy the Arrhenius equation, the graph of ln (*k*) versus 1/*T* was made and shown in Fig. 2.12. The result shows that the data obtained from experiment agreed with Arrhenius law very well. The activation energies (E_a) for biodiesel B20 oxidation and the mixture of biodiesel B20 and mangosteen peel antioxidant calculated were 54.34 and 56.27 kJ/mol, respectively, as well as collision factors (*A*) of 348,711 and 348,711 1/min, respectively. The activation energy of the mixture of biodiesel B20 and mangosteen peel antioxidant was higher, so that the mixture of biodiesel B20 and mangosteen peel extract antioxidant is more difficult to oxidize. In a mixture of biodiesel and antioxidants of pepper extract, bacupari leaves, arabica coffee leaves, and sage leaves, when oxidized with oxygen, the activation energy was around 46.35–82.26 kJ/mol (Borsato et al. 2014; Gregorio et al. 2017).

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Chapter 3
Biotechnological Potential of Cottonseed, a By-Product of Cotton Production

E. Rojo-Gutiérrez, J. J. Buenrostro-Figueroa, L. X. López-Martínez, D. R. Sepúlveda, and R. Baeza-Jiménez

Abstract Cotton (*Gossypium hirsutum* L.) is an important fibre crop of global significance. It is grown and harvested in tropical and subtropical regions of more than 80 countries. The state of Chihuahua, in Mexico, is the leader in the production of cotton covering 70% of national production. According to statistics reported in 2016, 488,000 metric tons were obtained and utilized as follows: 93% for textile industry, 2.28% as cattle feed, 1.1% was re-harvested, and the other 3.56% was discharged, and in consequence an environmental impact occurs. That remaining cottonseed constitutes a potential agroindustry residue with biotechnological applications due to its chemical composition: fibre, proteins (as well as essential amino acids such as lysine, methionine, tryptophan, and other amino acids) carbohydrates, and lipids (it is important to highlight gossypol and the fatty acids profile). In this chapter, food and bioenergy applications of cottonseed in terms of bioactive compounds (phenolic content), bioactivity (antioxidant activity), and lipid content (production of biodiesel) are reviewed, as well as the chemical compounds responsible of such applications, different types of extraction methods and analytical protocols for their identification, purification, and quantification.

Keywords Bioactive compounds · Bioactivity · Biotechnological applications · Cottonseed

E. Rojo-Gutiérrez · J. J. Buenrostro-Figueroa · R. Baeza-Jiménez (✉)
Centro de Investigación en Alimentación y Desarrollo, Delicias, Chihuahua, Mexico
e-mail: jose.buenrostro@ciad.mx; ramiro.baeza@ciad.mx

L. X. López-Martínez
CONACYT-Centro de Investigación en Alimentación y Desarrollo, Hermosillo, Sonora, Mexico
e-mail: leticia.lopez@ciad.mx

D. R. Sepúlveda
Centro de Investigación en Alimentación y Desarrollo, Cuauhtémoc, Chihuahua, Mexico
e-mail: dsepulveda@ciad.mx

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3.1 Introduction

Cotton represents one of the most commercially important fibre crops due to its several industrial and agricultural applications (Egbuta et al. 2017; Mendoza et al. 2016; Wegier et al. 2016; McCarty et al. 2018). It has been utilized worldwide for more than 5000 years by different civilizations, being textile fibre the main use (Dugan 2019). About 50 different cotton plant species are found in nature, but only four of them have been domesticated: *Gossypium arboreum*, *Gossypium herbaceum*, *Gossypium hirsutum*, and *Gossypium barbadense* (Egbuta et al. 2017; http://www.cicr.org.in/pdf/cotton_varieties_hybrids.pdf). *G. hirsutum* constitutes approximately 90–97% of the world's cotton production due to its high yields and adaptability to different environmental conditions (Avci et al. 2013; Nix et al. 2017; Rocha-Munive et al. 2018).

Cotton is a semi-tropical or tropical climate crop; however, it is typical of warm areas. Cotton sowing is very sensitive: requires a proper conditionate land, deep soils, constant irrigation, and temperatures of 20–30 °C to grow (SIAP 2017). When cotton crops mature completely, they are processed for obtaining cotton bolls, which represents its main commercial significance (Hernandez 2016). However, important by-products can also be recovered from cotton processing chain, such as cottonseed, which is a value-added component for oil extraction and ruminant feeding due to its chemical composition (Egbuta et al. 2017; He et al. 2013; Bernard 2016; Okonkwo and Okafor 2016). Cottonseed also contains bioactive compounds, namely, antioxidants (flavonoids) or pigments (gossypol), which exhibit potential bioactivities (Nix et al. 2017; Tian et al. 2016).

In cotton ginning several components are present: sticks, stems, burrs, and mainly seeds. According to statistical reports (2018/19) of cotton production, 43.45 million metric tons of cottonseed were obtained, being the third oilseed produced in the world (https://www.statista.com/statistics/267271/worldwide-oilseed-production-since-2008/, 24 April 2019). Then, for an increased world cotton production, higher amounts of cotton residues are generated.

According to the aforementioned, cottonseed is suitable for food and bioenergy applications. Therefore, in the present chapter different types of extraction methods to recover the bioactive compounds present in cottonseed are reviewed as well as the analytical protocols for their identification, purification, and quantification for the diverse applications.

3.2 Cotton Production

World cotton production during 2018/19 was 25.89×10^6 metric tons; corresponding to an average yield of 780.5 kg/ha, for cultivated area of 33.18 million hectares. Compared to the period 2017/18, there was a decrease in world cotton production of 4.2%, because the main producers such as the United States, India,

Table 3.1 Major cotton producing countries during 2018/19

Position	Country	Production (1000 metric tons)
1	China	6042
2	India	5879
3	United States	4004
4	Brazil	2569
5	Pakistan	1676
6	Turkey	806
7	Uzbekistan	718
8	Australia	544
9	México	376
10	Greece	307
11	Mali	294
12	Benin	283
13	Argentina	250
14	Burkina	218
15	Turkmenistan	198

Source: Adapted from the Agricultural forum outlook (USDA 2019)

Pakistan, Central Asia, and Australia were affected by climatic shifts. On the other hand, a recent data (2019/20) forecast refers to an increase in global cotton production of 6.9%, equivalent to 27.54×10^6 metric tons (USDA 2019).

Table 3.1 shows the major cotton producing countries during 2018/19, and it can be seen that Mexico was the ninth cotton producing country, indicating an increase of 9.56% with respect to the period 2017/2018. A continuous annual increment (0.21%) is expected because cotton was included in the Mexican action plan "Planeación Agrícola Nacional 2017–2030", promoted by SAGARPA. According to data reported by SIAP in 2017 (SIAP 2017), 488,000 tons of cotton were produced in Mexico during 2016 and they were used as follows: 93% for textile industry, 2.28% was exported, 1.1% was re-harvested, and the residual 3.56% was considered waste. The latter can be suitable for potential biotechnological applications rather than being disposed of to the environment.

In Mexico, the northern states are leaders in cotton production. That area is arid and temperatures are warm, which is convenient for cotton cultivation. The main producing state is Chihuahua and it contributed with 708,332 tons in 2017. Other important producers are Baja California, Coahuila, Durango, Sonora, and Tamaulipas.

3.3 Chemical Composition of Cottonseed

Cottonseed chemical composition defines its quality and determines its different applications or uses as well as those of its by-products. Different intended or uncontrollable factors as growing location, variety, environmental conditions,

Table 3.2 Proximal composition of cottonseed

Parameter	Okonkwo and Okafor (2016) (% wt.)	USDA ARS (OECD 2015) (% wt.)	Commercial (OECD 2015) (% wt.)
Moisture	9.87	4.7	4.0–8.7
Crude protein	27.27	34.2[a]	21.8–28.2[a]
Crude fibre	22.94	21.4[a]	15.4–28.2[a]
Ash	4.55[a]	4.8[a]	3.8-4.9[a]
Lipid	27.83	36.3[a]	15.4-23.8[a]
Total carbohydrates	30.49	–	–

[a]Dry matter

genetic modifications, or agronomic approaches can affect cottonseed compositions (He et al. 2013; Okonkwo and Okafor 2016). Table 3.2 shows different proximal compositions of cottonseeds reported in studies or found in commercial seeds.

As it is depicted in Table 3.2, the moisture content is not elevated in cottonseed, which allows its preservation and reduces the growth of some microorganisms. On the other hand, protein, fibre, and lipid are the higher contents, which are important for feeding and can provide benefits to the digestive system. Lipids can also be extracted and further employed for the preparation of structured lipids or synthesis of biodiesel. Ash content is the inorganic matter where minerals are found. Cottonseeds are a rich source of magnesium (3.6 g kg^{-1} dry matter [dm]), manganese (16.0 mg kg^{-1} dm), copper (10.0 mg kg^{-1} dm), iron (70.0 mg kg^{-1} dm), phosphorous (5.9 g kg^{-1} dm), zinc (35.0 g kg^{-1} dm), potassium (12.0 g kg^{-1} dm), calcium (1.5 g kg^{-1} dm), and sodium (0.1 g kg^{-1} dm) (Heuzé et al. 2015).

3.3.1 Lipid Content

Vegetable fats and oils are mainly constituted by 98–99.5% of triacylglycerols (TAG), a trisubstituted glycerol (Nadeem et al. 2015). Common fatty acid profile is rich in unsaturated fatty acids, since they represent around 75%, while the remaining are saturated; consequently, showing an unsaturated/saturated ratio of 0.35. The unsaturated fraction is mainly conformed by oleic and linoleic residues, which represent 22 and 52% of the profile, respectively. Palmitic acid, on the other hand, is the major saturated fatty acid corresponding to a 24% content. In that matter, palmitic, oleic, and linoleic acids constitute more than 90% of the fatty acid composition of cottonseed (see Table 3.3). TAG content in cottonseed comprises mainly di-unsaturated (UUS, 43.4%) and tri-unsaturated (UUU 35.7%), and a low concentration of monounsaturated (USS, 12.5%) (Jahaniaval et al. 2000).

On the other hand, Table 3.4 shows the most common distributions of the saturated and unsaturated fatty acids in the different positions (*sn*-1, -2, and -3) of TAG backbone.

Table 3.3 Fatty acid profile of cottonseed

Fatty acid		Typical content (%)	Range (%)
C14:0	Myristic	0.66	–
C16:0	Palmitic	24.55	21.4–26.4
C16:1	Palmitoleic	0.66	–
C17:0	Margaric	0.19	–
C18:0	Stearic	0.21	2.1–3.3
C18:1 n9 cis	Oleic	19.05	14.7–21.7
C18:1 n9 t	Elaidic	2.10	–
C18:2	Linoleic	51.54	46.7–58.2
C18:3	Linolenic	0.19	0–1
C20:0	Arachidic	0.21	–
C20:1	Eicosenoic	0.12	–
C22:0	Behenic	0.17	–
Unknown compound	–	0.34	–
Total SFA	–	26.00	–
Total UFA	–	73.66	–
Total MUFA	–	21.93	–
Total PUFA	–	51.73	–
SFA/UFA	–	0.35	–

SFA Saturated fatty acids, *UFA* Unsaturated fatty acids, *MUFA* Monounsaturated fatty acids, *PUFA* Polyunsaturated fatty acids
Adapted from Mohdaly et al. (2017) and List (2017)

Table 3.4 Estimated composition of triacylglycerols (TAG) present in cottonseed oil

TAG profile	% wt.
PPoP	0.62
POP	3.67
POS	0.54
MLP	1.16
PLP	13.74
PLS	3.91
PPoL	1.93
POL	14.30
SOL	1.31
MLL	1.11
PLL	26.58
SLL	4.75
PoLL	1.30
OLL	10.43
LLL	12.88
Others	1.77

P Palmitic, *Po* Palmitoleic, *M* Myristic, *S* Stearic, *O* oleic, *L* Linoleic, *Ln* Linolenic, *A* Arachidic
Adapted from Ceriani and Meirelles (2004)

Table 3.5 Phytosterol profile in cottonseed oil

Sterol	Codex Alimentarius (1999) (%)	Yücel et al. (2017) (mg/kg)
β-Sitosterols	76.0–87.1	2411 ± 26
Y-Sitosterol	–	–
Campesterol	6.4–14.5	135 ± 4
Stigmasterol	2.1–6.8	27 ± 1
Cholesterol	0.7–2.3	–
Brassicasterol	0.1–0.3	–
Delta-5-avenasterol	1.8–7.3	–
Delta-7-stigmasterol	nd–1.4	–
Delta-7-avenasterol	0.8–3.3	–
Others	nd–1.5	–
Total (mg/kg)	2700–6400	2573

nd not detected

It is worth noting that there exists a trace percentage (0.5%) of two unique fatty acids that have some toxicity properties in the lipid fraction of cottonseed: malvalic and sterculic fatty acids. These residues are characterized by the presence of a cyclopropenoid subgroup in their chemical structure and are better known as cyclopropenoid fatty acids. Nevertheless, when the cottonseed lipids are extracted (oil), the conventional refining process reduces their contents ten times.

Other important lipid family found in cottonseed is phospholipids (PLs). The amount of PLs is very low and is typically measured in 0.7–0.9%, and they are included in the so-called "gums". PLs despite the fact that they generally benefit health properties lowering blood cholesterol, improve muscle performance, and resilience, among others, can affect the oil quality as well as the refined oil yield. PLs acts as an emulsifier, interfering in the neutralization, bleaching, and deodorization steps of the refining process, thus, enhancing oil losses, decreasing shelf-life, and promote that the oil turns dark (Ghazani and Marangoni 2016).

Phytosterols are also present in cottonseed oil (Table 3.5). These chemical compounds exhibit health-promoting effects like the decreasing of cholesterol, as well as antimicrobial, anti-inflammatory, and anticancerogenic properties. Nevertheless, phytosterols do not influence any physicochemical oil property. Between the phytosterols contained: β-sitosterol, campesterol, stigmasterol, and D5-avenesterol, typically β-sitosterol is the most abundant (Codex Alimentarius 1999); however, Mohdaly et al. (2017) reported Y-sitosterol as the most concentrated.

Cottonseed oil is also relevant for its tocopherols content, which reaches ca. 1000 ppm. There are four homologues (α-, β-, γ-, and δ-) and all of them are considered as derivatives of vitamin E, which have powerful antioxidant activities and several nutritional benefits. Moreover, they are a natural oxidative stabilizer of polyunsaturated fatty acids, prolonging the oil operational lifetime, and the shelf-life of foods that contain them. Only α- and γ-tocopherols are usually found in cottonseed oil (Table 3.6) and α-tocopherol is the most reactive of the homologues (Hernandez 2016; List 2017; Lepak et al. 2016).

Table 3.6 Tocopherol profile in cottonseed oil

Tocopherol	Codex Alimentarius (1999) (mg/kg)	Bockisch (1998) (%)	Yamamoto et al. (2018) (mg/kg)[a]
α-Tocopherol	136–674	60–80	360
β-Tocopherol	nd–29	nd	nd
γ-Tocopherol	138–746	20–40	310
δ-Tocopherol	nd–21	nd	nd
Total (mg/kg)	380–1200	900–1100	670

nd not detected
[a]Average values ($n = 3$)

3.3.2 Gossypol

All cotton species present a very particular compound distributed all over the plant, and it is known as gossypol, which is mainly concentrated in seeds (Gadelha et al. 2014). Its concentrations can vary depending on the cotton species and weather, but for *G. hirsutum* a range value of 0.5–1.89% of total gossypol has been reported (Scheffler 2016).

Gossypol is a phenolic terpene aldehyde with antioxidant and toxicity activities produced by pigment glands as defence mechanisms (pests and pathogens), and for the environmental adaptation of the plant (Tian et al. 2016; Scheffler 2016). The pigment glands also produce other 14 phenolic pigments like gossypurpurin (purple) and gossyfulvin (orange), but in depreciative concentrations compared to gossypol (yellow-green) (Gadelha et al. 2014; Cope 2018).

Gossypol consisted of a mixture of two enantiomers: (+)- and (−)-gossypol, which in the case of *G. hirsutum* are approximately present at 60% and 40%, respectively (Alexander et al. 2009). Moreover, most parts of (+)/(−) gossypol enantiomers are bound to proteins (mainly to their lysine and arginine residues), but low amounts remain as free gossypol (Gadelha et al. 2014). Despite both enantiomers can be equally effective against some hazardous factors as insects, (−)-gossypol exhibits higher bioactivity, thus, it is generally more toxic and more difficult to be removed (Alexander et al. 2009; Kakani et al. 2010).

The chemical formula and name of gossypol are $C_{30}H_{30}O_8$ and 1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-(2,2′-binaphthalene)-8,8′-dicarboxaldehyde, respectively. It contains a polyphenolic structure with three hydroxyl and one aldehyde groups bound in each naphthyl scaffold. The two enantiomers are a consequence of the restricted rotation around the internaphthyl bound (C2–C2′). The modification or removal of the functional groups (hydroxyls and aldehydes) through acid mediums, oxidation, ozonification, methylation, etc., produce a wide variety of gossypol derivatives, namely tautomeric forms, Schiff bases, among others. These derivatives exhibit different important biological activities like contraceptive, antimicrobial, antiviral, antiparasitic, antitumor, and antioxidant (Wang et al. 2009; Lu et al. 2017). Such bioactivities are further described in

Sect. 3.5.3. However, from food perspective, gossypol is the main limiting factor, since their intakes can produce liver damage (hepatotoxicity), poisoning, anaemia, disruption of reproduction (affects fertility and embryogenesis), and immunotoxicity (reduction of leukocytes) (Gadelha et al. 2014; Cope 2018).

Much effort has been devoted for the elimination of gossypol and to enable cottonseed-safe food uses. Some strategies developed to accomplish this, are the processing of glandless cottonseeds and ultra-low gossypol seeds. Glandless cottonseeds can be obtained by removing the glands by mechanical separation, addition of iron salts, or the use of high polar solvents (e.g. anhydrous acetone). The composition between glanded and glandless cottonseeds is similar (Hernandez 2016). The main benefit of glandless cotton is the absence of gossypol, but on the same hand, it also constitutes the main drawback because cotton plant loses its defence mechanisms and is exposed to plagues and diseases, limiting their cultivation to small scales (Tian et al. 2016).

3.3.3 *Flavonoids*

Flavonoids represent an important control for pest and herbivore, so they guarantee plant health and optimal functioning. They also play an important role in plant growth (Brown et al. 2001) and fibre development (Tan et al. 2013). They also have a potential antioxidant activity (Denev et al. 2013) and contribute to the colouring of the majority of plants flowers, leaves, seeds, among others, exhibiting by themselves a yellow colour (Egbuta et al. 2017). Flavonoids are conformed by several classes, namely, aurones, anthocyanins, biflavonoids, flavanols, flavones, flavonols, flavanones, and isoflavones. According to Nix et al. (2017), 52 flavonoids have been identified in *G. hirsutum*, where eleven are present in the seeds in the form of flavonols (Kaempferol, Kaempferol 3-diglucoside, Kaempferol-3-*O*-neohesperidoside, Isoquercitrin, Quercetin 3-diglucoside, Quercetin-3-*O*-neohesperidoside, Quercetin-3-*O*-robinoside, Rutin and Spiraeoside) and one flavanol.

3.3.4 *Cottonseed Proteins*

Cottonseeds, as it has been stated before, exhibit a high content of protein. Cottonseed proteins are highly degradable since approximately 70% of total proteins are soluble (albumin and globulin) (Heuzé et al. 2015), where vicilin and legumin families constitute the major components of the protein fraction. Different biological functions of the cottonseed proteins have been recognized which include storage, transcription/translation, synthesis, energy metabolism, antimicrobial activity, and embryogenesis. Moreover, such as oils, some proteins exhibit functional activities (He et al. 2018). Cottonseeds' protein also contains high concentrations of essential amino acids, where lysine and valine are the most abundant.

3.4 Extraction Methods

According to Sect. 3.3, the chemical composition of cottonseed is remarkable for the presence of compounds with important biological and biochemical functions. During so long, agroindustry residues were not considered and they were just disposed to the environment. Nowadays, with the development of novel analytical protocols for the identification, purification, and quantification of chemical compounds, the extraction of the different value-added components present in cottonseed is achievable. Such protocols are described as follows.

3.4.1 Oil–Cottonseed Meal

There exist several oil extraction methods and a single or a combination of them can be conducted. Before proceeding with the extraction, oil seeds need a preconditioning process to efficiently extract and remove impurities. The conditioning consists of the cleaning, delinting, dehulling, separation, flaking, and cooking of cottonseeds. The cottonseed meal is the defatted cake that remains after the kernels oil extraction.

3.4.1.1 Mechanical Extraction

Pure physical separation of the liquid (oil) and solid (cottonseed meal) phases, by the disruption of the lipid cells. Hydraulic or screw presses can be used for the extraction. Their advantages are: (1) chemicals are not applied and (2) low energy and equipment costs, compared to other methods such as solvent extraction. However, this method compromises oil yield, since 85% is generally the maximum yield achieved (Arișanuv and Rus 2017).

3.4.1.2 Solvent Extraction

Solvent extraction can be directly applied or after a pressing step (hydraulic or screw press), and it is a conventional extraction method for the oil industry. The solvent typically utilized for carrying out oil extraction is n-hexane, due to high yields reached and its commercial availability. Other solvents namely, acetone, aqueous ethanol, methylpentane, isohexane, heptane, petroleum ether, trichloroethane, chlorinated hydrocarbons, among others, can be also employed. Besides solvent, other operational parameters including temperature, solvent:oil ratio, particle size, and time, can also affect extraction yield. By means of this method, significant yield can be achieved (ca. 98%), but it requires large investment for equipment, energy demand, cost of solvents, and a further separation step. Saxena et al. (2011)

conducted a comparison between n-hexane and ethanol extraction. They found that both solvents at a temperature of 45 °C, at a ratio of 10:1 (solvent:seed) and a particle size of 0.6 mm, an oil yield of >99% was attained. However, ethanol is safer and eco-friendlier than *n*-hexane. Moreover, cottonseed meal from ethanol extraction contained 50% less gossypol than that of *n*-hexane.

3.4.1.3 Supercritical Fluid

Supercritical fluid is the most promising extraction method due to its simplicity, high efficiency, high oil quality, and short process time, compared to solvent methods; however, the expensive equipment and the impossibility of a continuous extraction are the main drawbacks for its sustainability. To perform the extraction, a supercritical fluid, such as CO_2, is used. Bhattacharjee et al. (2007) optimized a supercritical fluid extraction of cottonseed oil, by monitoring temperature, pressure, and time extraction effects using central composite rotate design and response surface methodology. Optimal conditions were 550 bar, and a temperature and extraction time between the ranges of 70–80 °C and 2–3 h, respectively. Temperature and pressure were the significant parameters for the efficiency of the extraction, and the maximum reported oil yield extraction was 17.26%. The oil yield increased proportionally to the pressure, but no further values were used due to operational limitations.

3.4.1.4 Microwave-Assisted Extraction

Attention has been paid to this novel method. By means of microwave-assisted extraction (MAE), a more efficient oil extraction can be obtained due to the oilseed cells rupture. Temperature, solvent:sample ratio, moisture, and properties of both solvent and sample are the variables to take into account to assay this method. Studies have agreed that the use of MAE enhance yields and require less solvent extraction. Other remarkable advantage is that MAE improves phenolics compounds extraction. Taghvaei et al. (2014) optimized a cottonseed MAE by increasing oil extraction under the optimal extraction times, solvent concentrations and moisture of samples using response surface methodology. Optimal conditions were as follows: 3.57 min of irradiation time, 14% of moisture content, and a ratio of 1:4 (sample: solvent). Following the previous conditions, they achieved an extraction efficiency of 32.6% and a phenolic content of 46 ppm.

3.4.2 Bioactive Compounds

Bioactive compounds (phenols, flavonoids, tocopherols) can be extracted by a wide variety of techniques, which also include the aforecited methods: solvent extraction, microwave- and ultrasound-assisted extraction, supercritical fluid extractions,

maceration, pressurized liquid extraction, and subcritical water extraction. Additionally, solvents used are as important as the extraction methods. Polar solvents are the conventional system for the recovery of these compounds. Since the nature of bioactive compounds varies depending on their chemical structures, their affinity for a specific polarity of a solvent varies as well.

Commonly used solvents are ethanol, methanol, acetone, hexane, ethyl acetate mixtures with water (Do et al. 2014). In the case of gossypol, different solvent systems are required, such as aqueous acetone, acetic acid, petroleum ether, anhydrous acetone, acetone–hexane, methylene chloride, and hexane–acetic acid mixtures (Hernandez 2016). Once the bioactive compounds have been extracted, a separation step must be conducted for their identification and quantification. The most used methods for that purpose include gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary liquid chromatography (CLC), thin-layer chromatography (TLC), among others. HPLC is the most sophisticated analytical protocol for the accurate characterization of these types of compounds, due to versatile detectors fitted to the apparatus: photodiode array, ultraviolet, fluorescence, and mass spectrometer.

3.5 Cottonseed By-Products Applications

The chemical composition of cottonseed allows the preparation of high-value products. Cottonseed oil (CSO) and cottonseed meal (CSM) are the main by-products of interest. On the other hand, remarkable bioactivities are exerted by the bioactive compounds also found in cottonseed.

3.5.1 Oil

Frying Oil Due to the amount of SFA and UFA content, high levels of antioxidants (e.g. tocopherols), and the absence of linolenic acid, refined CSO is distinguished as frying oil. SFA (palmitic acid) and tocopherols prevent oil oxidation; because of that, hydrogenation is not necessary, decreasing trans-fatty acids. The high content of linoleic acid (omega-6) favours the texture, mouthfeel, flavour of food, and promotes their shelf life. Arslan et al. (2016) developed different refined CSO and palm olein oil (POO) blend to obtain high-quality oil with improved stability during the frying process. By mixing the vegetable oils, the bioactive lipids, antioxidants (tocopherols and sterols) were increased, and the SFA/UFA ratio was balanced. More SFA was also increased with respect to UFA without compromising the sensorial and nutritional properties of foods, since SFA produces undesirable flavours. The oxidative stability was measured by the detection of polar and polymeric compounds; oxidation products. Their two different blends: A (50% CSO:50% POO) and B (40% CSO:60% POO) showed better quality properties, since the

polar and polymeric compounds for blend A and B increased only in 2.27% and 5.40%, respectively, while pure CSO exhibited an increase of 6.30%.

Biodiesel This is one of the most important and promising alternatives against the worldwide depletion of fossil fuel reserves. Crude CSO is a potential feedstock for biodiesel production, since its fatty acid composition confers the proper physicochemical properties required to meet the international quality standards. Biodiesel is obtained via transesterification of a vegetable oil/animal fat and a short-chain alcohol (usually methanol or ethanol) in the presence of a catalyst. Malhotra and Ali (2018) produced biodiesel from virgin CSO utilizing a novel solid catalyst (5-Na/ZnO/SBA-15). They could obtain more than 98% biodiesel yield after 4 h, for a methanol: oil ratio of 24:1, a catalyst concentration of 12% (wt.), and 65 °C. Jamshaid et al. (2018) optimized the biodiesel yield from CSO using the response surface methodology. The optimized variables were oil:alcohol molar ratio, catalyst concentration, temperature, and stirring speed. From their results, a yield of 98.3% was reached for a 6:1 methanol–oil ratio, 0.97% (w/w) of catalyst concentration, 63.8 °C, and 797 rpm. However, there is still a lot of work to do to attain a sustainable production of biodiesel in terms of catalyst (mainly the use of enzymes) and reaction systems.

Edible Oleogel Oleogels have proven to be a potential alternative to products with high lipid content and SFA, such as shortenings; hydrogenated vegetable oils that are solid at room temperature. Moreover, providing the same physicochemical and sensorial properties that are typically conferred by fats. Basically, this is the application consisted of the transformation of a liquid gel to a 3D network, obtaining a gel-like structuring of lipids. This process is conducted by adding a gelator molecule (plant waxes) to the oil of interest and its properly named oleogelation or organogelation (O'Sullivan et al. 2016). Pehlivanoglu et al. (2018) produced different oleogel blends with the aim of reduced fat content of cakes and their SFA as well as by replacing one of their main ingredients: shortenings. The formulation for the different blends included different percentage compositions of high oleic sunflower (HOS), CSO, and blend fat. All the formulations contained CSO due to the fact that it is constituted of >70% of UFA. Once the cake was prepared, their physicochemical and sensory properties were analyzed. Despite some quality properties were significantly affected (e.g. texture), the sensory scores showed that the cakes with oleogels were accepted, being the blend of 50% CSO:50% HOS the most acceptable. Moreover, they reported that all of the formulated oleogels exhibited a less SFA content.

Shortening CSO is a popular ingredient of many shortenings/margarines because of the high value of palmitic acid it presents. As the oil solidifies, it develops tiny fine crystals (β′-crystals) that forms a 3D-network (emulsion) and trap the oils. This confers smooth flavours, good texture and plasticity, good creaming properties, higher temperature stability, and an extended shelf life to foods that contain it. Shortenings are very important for food industry, namely bakery, pastry, chocolate, confectionery, among other industries. However, shortenings are typically hydrogenated to obtain specific textures and functionalities. Consequently, trans-

fatty acids are produced in the process, which have harmful effects on health. Interesterification of oils is an alternative that provides similar effects, and CSO has been used for their production. Imran and Nadeem (2015) used different canola oil (CO) and hydrogenated cottonseed oil (HCSO) mixtures for their interesterification. They concluded that the interesterified mixture of 50% CO:50% HCSO exhibited the best physicochemical and sensorial characteristics, and that all the mixtures had a lower trans-fatty acid concentration. Therefore, they stated that their mixtures could be used for the formulation of shortenings with a reduced health detriment.

Pesticide Studies have shown that fatty acids from vegetable oils present toxic activities and could be used for pest control (Bernklau et al. 2016; de Melo et al. 2018). Teodoro et al. (2017) utilized CSO as an insecticide against *Aceria guerreronis* (coconut plantation pest), in order to find alternatives to chemical control, since they are harmful to humans and the environment, besides the fact that pest resistance is a constant issue. Their results showed that CSO can be an effective biopesticide since it was highly lethal and repellent to *A. guerreronis*. Its bioactivity was mainly attributed to the linoleic and oleic acid contained in CSO.

Antibacterial Activity Flavonoids and phenols (tocopherols, sterols, gossypol) are multifunctional compounds that present antioxidant and antimicrobial activities. Vegetable oils are rich in these compounds and CSO is not the exception as it was described previously. In that matter, Xuan et al. (2018) evaluated the antimicrobial and antioxidant activities of commercial CSO and thirteen more, marketed in Japan. The determination of antioxidant activity (DPPH radical scavenging and β-Carotene bleaching method), total phenolic (TPC) and flavonoid (TFC) content and an antimicrobial activity test were conducted for the evaluation. CSO exhibited the second highest lipid peroxidation inhibition value. Middle TPC in comparison with the other oils were reported and the TFC were very similar among the oils. The identified flavonoid and phenolic compounds were benzoic acid, esculetin, and isoquercetin. Even when CSO did not show a high TPC, it exerted one of the highest antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli*.

3.5.2 Proteins

Feeding CSM is extensively used for beef production since they contain high nutrition value and up to around 40% of protein (Świątkiewicz et al. 2016). It is known that CSM can partially replace soybean meal (major protein source of farm animals) obtaining, in general, the same nutritional values. Broderick et al. (2013) evaluated the effects of partial replacement (50%) of soybean meal with cottonseeds (*G. hirsutum* and *G. barbadense*) and CSM (*G. barbadense*) on the production of lactating dairy cows. The results indicated that all the diets were comparable to soybean meal (100%) on the production and composition of milk. However, gossypol content is the limiting factor of true CSM potential as a protein source because

monogastric animals are much more susceptible and tolerate only small quantities. Świątkiewicz et al. (2016) concluded, based on their research and analysis of different studies, that a partial replacement between the range of 10–15% of soybean meal with CSM for poultry feeding is safe and even cheaper.

Such as CSM, cottonseed hulls (CSH) are destined for livestock feeding. Contrary to CSM, CSH presents lower protein content, but are an important roughage source, being quite useful for high-fibre diets. Moreover, they present a low cost, thus, decrease feeding costs. Eiras et al. (2016) analyzed the effect of a nutritional regimen based in CSH on the sensory attributes of young bull meat. They discovered that none of the three high-fibre diets (210, 270, or 330 g kg^{-1} cottonseed hull on dry matter) had an undesired effect in the sensorial properties (visual appraisal, flavour, tenderness) and all of them were accepted by the consumers.

Packaging Food packaging is critical for the protection of food quality and its safe distribution. However, in the last years, consumers have been increasingly looking for healthier and more sustainable products. In that matter, the developing of innovative packaging technologies was necessary to satisfy consumers' petitions. Active packaging goes beyond protecting food from external factors (dust, toxins, microorganisms, moisture) and facilitating the handling of food through the supply chain. In addition, active packaging can confer bioactive components such as antimicrobials and antioxidants to foods contained, or reduce the absorption of undesired components, improving the conditions and quality of packaged food (Yildirim et al. 2017). de Oliveira Filho et al. (2019) incorporated protein hydrolysates from CSM into alginate films, due to their high antioxidant properties. Besides the antioxidant properties, it showed antimicrobial and antifungal effects against *S. aureus*, *Colletotrichum gloeosporioides,* and *Rhizopus oligosporus*. Thus, their active film showed potential use as an active packaging. This strengthens the exploitation of unutilized cottonseed meal.

Wood Adhesive Industries of wood furniture are extremely important, and their demand rises as the world population increases. For the production of wood furniture, wood adhesives have become a key factor that determines the quality and production rate of the products. Most of the wood adhesives are synthetic adhesives derived from non-renewable products, and as they are mostly formaldehyde-base are toxic for people. An eco-friendly, renewable and non-toxic alternative are the vegetable protein adhesives. Pradyawong et al. (2018) used different blends of water washed CSM supplemented with cottonseed protein isolated or cottonseed protein residues after extraction. All the mixtures present protein values between 34.9% and 94.8%, and their physicochemical properties and adhesive strength were measured. Mixtures with a protein content of 65–70% exhibited comparable physicochemical properties and adhesive strength to their product with highest protein value, full cottonseed protein isolated (94.8%). Therefore, using mixes with water washed cottonseed meal can be more cost effective.

3.5.3 Gossypol

Gossypol is a multifunctional bioactive polyphenol that has a promising future in biotechnology applications. Nevertheless, more research is required to fully understand gossypol action mechanisms to potentiate its bioactive activities without compromising human and animal health.

Antibacterial, Antiviral, and Fungicidal Effects It has been proven the antibacterial effects against *S. aureus*, *E. coli*, *Saccharomyces cerevisiae*, among others. Due to the toxicity of gossypol in humans and animals, novel application methods for its safe use are in development. Studies have demonstrated that gossypol has been used safely in cutaneous gels. Based on these studies, Clément and Tang (2018) prepared two cutaneous formulations of gossypol and used them in ex vivo to analyze skin penetration. The results suggest that both formulations were safe since they were distributed in the outer layer of the skin. In that matter, they indicated the potential use of their gossypol formulas against skin bacteria including *Staphylococcus epidermidis*. However, for more reliable results, studies in vivo or clinical are needed to confirm the results.

Antiviral and fungicidal activities are other biological functions of gossypol. Li et al. (2015) obtained thirty-three gossypol Schiff derivatives, containing alkylimine, oxime or hydrazine moiety, and they were tested for their antimicrobial, antifungal, and antiviral activities. For the antiviral tests, all derivatives exhibited higher antiviral activities against *Tobacco mosaic virus*, where five of them exert an even higher inhibition than their controls; gossypol and ribavirin (commercial antiviral). On the same hand, high antibacterial activity in all derivative components was observed. In these assays, one particular component ([pyridin-3-yl] methanamine Schiff base) showed a complete inhibition (100%) of *Culex pipiens pallens.* The other included microbes in the study were *Mythimna separata*, *Helicoverta armigera*, and *Ostrinia nubilalis*. Lastly, in the fungicidal tests, *Physalospora piricola* was highly inhibited by all the compounds. Other fungi where the components showed good fungicidal activity were *Alternaria solani*, *Fusarium graminearum*, *Phytophthora capsici,* and *Cercospora arachidicola Hori*.

Contraceptive The contraceptive activity of gossypol has been studied in humans since the 1970s. It was first applied in men with the purpose of developing the first contraceptive method for men. Even though it was effective, serious side effects such as hypokalaemia and sterility finished the tests. Further studies showed that gossypol affects both male and female reproduction, inhibiting spermatogenesis, and affecting the oestrous cycle, pregnancy, and embryogenesis. In an effort to achieve a gossypol men's safe contraceptive, Wen et al. (2018) using a controlled drug release carrier (zero-order release), attained major progress. They could maintain the gossypol contraceptive activity using a 50-fold lower dose compared to conventional oral dose, without the hypokalaemia antifertility effects. For the complete restoration of fertility a recovery period is required.

Anticarcinogenic Gossypol has proven strong anticancer effects. Xilong et al. (2017) strongly inhibited the expression of two breast cancerous cells (MDM2 and VEGF) using gossypol. This resulted in cancer cell apoptosis and suppression of tumour angiogenesis. Besides confirming the anticarcinogenic effect of gossypol, a better understanding of its action mechanism was elucidated, promoting the development of novel anticarcinogenic drugs. Xie et al. (2017) synthesized a novel Schiff base drug constituted by gossypol and L-arginine. The drug presents excellent antitumor effects against lung cancer cells (A549) and human gastric cancer cells (SGC-7901).

3.6 Future Work

For our research centre, it is important to focus on the integral use of agroindustry wastes, in particular because our centre is located in the most important cotton producing zone. As it has already been mentioned, the main use of cotton is textiles and large amounts of cottonseed are disposed in the environment. Then, relevant biotechnological projects are being conducted in our centre for the extraction of oil and produce biodiesel by means of a novel supported biocatalyst and on the other hand, the recovery of bioactive compounds is being assayed for their bioactivities. It is worth noting that the projects are also evaluated for their technical and economic feasibility and sustainability.

3.7 Conclusions

In this chapter, the bioactivities and energy applications of the bioactive compounds present in cottonseed have been reviewed, as well as the different types of extraction methods and analytical protocols for their identification, purification, and quantification.

Even when several efforts have been reported, new and novel applications are needed and not only for cottonseed but also for other agroindustry wastes. The chemical composition of cottonseed is suitable for the recovery of the bioactive compounds present in order to achieve a wider characterization and the development of new applications and analytical methods for their extractions.

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Chapter 4
Bioprocessing with Cashew Apple and Its By-Products

Asha P. Antony, Swapna Kunhiraman, and Sabu Abdulhameed

Abstract Global industrialization has led to an enhanced production and use of enzymes and value-added products in various industrial sectors. As the demand for a cleaner and safer environment is inevitable in this twenty first century, better utilization of wastes for the production of value-added products has also been improved. Different bioprocesses are being used for the utilization of different agro-industrial residues for their transformation into useful products. The nature of substrate used is a major limiting factor in all fermentation processes. Cashew apple and its by-products are a new and promising substrate for bioprocessing as they are rich in carbohydrates, minerals, vitamins, amino acids, carotenoids, phenolics, organic acids, and antioxidants. Although 10–30 t/ha of cashew apples are accumulating globally, 90–94% have been discarded after harvesting the nut. Only 10% is commercially used for the preparation of wine, jam, juice, and ice cream and these products are hardly exported. Bioprocessing with cashew apple has wide variety of applications in different fields including bioethanol production, microbial production of enzymes, dextransucrase production as a preservative in food industry, production of biosurfactants, etc. Considering less cost, rich nutritional contents, and availability of cashew apple and its by-products, they can be exploited more as a promising substrate for the different fermentation processes.

Keywords Bioprocessing · Agro-industrial residues · Cashew apple · Value-added products · Enzymes

A. P. Antony · S. Kunhiraman · S. Abdulhameed (✉)
Department of Biotechnology and Microbiology, Kannur University, Kannur, Kerala, India

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4.1 Introduction

Global industrialization has made tremendous developments and rapid changes in different sectors. As a part of this, the generation of wastes also increased drastically. In the agro-industrial sector, the accumulation of agro residues or subproducts has increased and their improper disposal has made strong environmental impact. Hence there is an urgent need to solve this problem and this can be achieved by effective utilization of resources, efficient management of the residues, and also by implementing appropriate reuse strategies (de Araújo Sousa and Targino Pinto Correia 2010).

In the last few years, various environmental friendly and cost-effective methods have been developed both in industrial and academic levels aimed at exploiting the agro residues for the production of value-added products (Panesar et al. 2016). Microbial bioprocessing is the best alternative to deal with such agro-industrial residues as it produces specific high end finished products. Proper selection of microorganisms depending on the substrate and optimization of physicochemical parameters plays a key role in the production of value-added biological products (Panda et al. 2016).

Cashew apple (*Anacardium occidentale*) is a pseudo fruit rich in reducing sugars (fructose and glucose), vitamins, minerals, and some amino acid. According to the official data, 90% of cashew apples are been left underutilized after harvesting the cashew nut (Honorato et al. 2007). As a nutritionally rich substrate, it has immense potential to be processed to produce juice, syrup, jam, ice cream, candy, chutney, pickle, and other products. Apart from that it can be biotechnologically transformed using microorganisms to fermented products like wine, bioethanol, enzymes, mannitol, single-cell protein, biosurfactants, probiotic beverages, lactic acid, and oligosaccharides (Prommajak et al. 2014).

The current chapter summarizes the potential of cashew apple and its by-products as promising substrate for fermentation and production of different products like wine, enzymes, bioethanol, dextran, lactic acid, single-cell protein, biosurfactants, oligosaccharides, and other bioactive compounds.

4.2 Cashew Apple as a Promising Substrate for Bioprocessing

Cashew (*Anacardium occidentale*) is an important tropical plant that is highly resilient and has the ability to grow in poor soil condition with high productivity. The plant is a native from Brazil and its modified fruit called cashew apple is rich in bioactive compounds, reducing sugars such as fructose and glucose, vitamins, minerals, some amino acids, carotenoids, phenolics, organic acids, and antioxidants that have diverse biological applications (Silveira et al. 2012). Cashew is cultivated in 32 different countries around the world with West Africa, India, Nigeria, Vietnam,

Brazil, and Indonesia being the main producers. The major contributor is West Africa having 46% of total cashew production followed by India 23%, Vietnam 15%, East Africa 5%, Brazil 5%, Cambodia 3%, Indonesia 2%, and others 1% (Divya Priya and Pydi Setty 2019). It is the most important plantation crop that earns huge foreign exchange through export of cashew nut, kernel, and cashew nut shell liquid (CNSL). The major by-product is the peduncle or false fruit, the cashew apple, where 90% of it being left out in the fields after harvesting the real fruit, the cashew nut. For every single ton of cashew nut, 10–15 times more cashew apple is produced and been spoiled or wasted in the orchards under the trees (Attri 2009). Though nutritionally rich, it is not consumed when compared with other fruits due to the astringency, haze, sediment formation, short shelf life, and high perishability. Cashew apple has been found to cure many chronic diseases such as scurvy, uterine complaints, dropsy, diarrhea, rheumatism, cholera and also used to treat vomiting, worms, and syphilis (Thalasila et al. 2012).

Cashew apple pulp is a nutritionally rich agro-industrial residue and hence it is used for the production of several fermented and non-fermented products, viz juice, jam, jelly, syrup, pickle, wine, liquor, vinegar, bioethanol, and other value-added products such as dextran, lactic acid, mannitol, single-cell protein, oligosaccharides, biosurfactant, fiber, etc. (Divya Priya and Pydi Setty 2019; Attri 2009). Cashew apple juice is also an immense source of nutrients like minerals, salts, vitamin C, reducing sugars (glucose, fructose and Sucrose), flavor and aroma and amino acids. Cashew apple juice (CAJ) contains 4–10 times higher ascorbic acid content than orange and pineapple juice and also five times more iron, calcium, and vitamin B1 than other fruits such as banana, avocados, and citrus. It also contains phenolic compounds such as leucodelphinidin, cardol, tannins, anarcardic acid, and terpenoid such as carotenoids (Kaprasob et al. 2017; Gyedu-Akoto 2011). As the availability of substrate is high and very cost effective, cashew apple and its by-products such as juice, pulp, testa, etc. can be processed biotechnologically using microorganisms and can yield a variety of products as depicted in Fig. 4.1.

4.3 Types of By-Products from Bioprocessing of Cashew Apple

4.3.1 Bioethanol

Fossil fuels are the most exploited forms of energy today and it is expected to get exhausted in the next century. Most of the modern day fuels have lot of negative impacts on the environment like global warming, pollution etc., which led to the discovery of biofuels which are environmental friendly, renewable and sustainable energy fuels. Among these biofuels, bioethanol is considered to be the most used alternative fuel worldwide, as it significantly replaces the crude oil consumption, has got high oxygen content as well as octane number, thereby promoting a cleaner

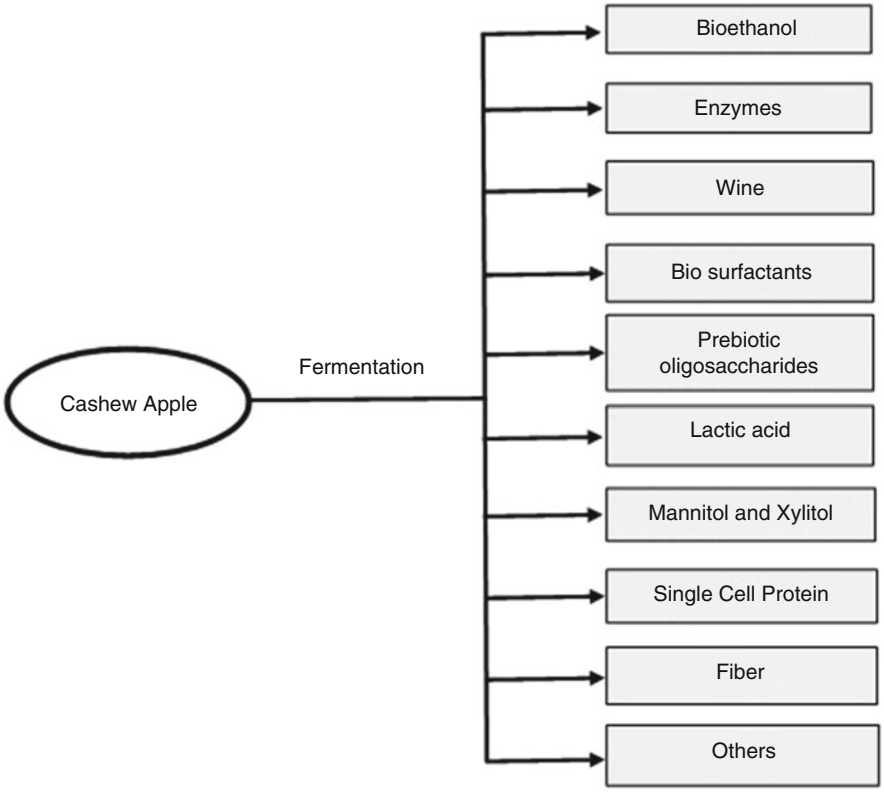

Fig. 4.1 Potential by-products from bioprocessing of cashew apple

environment for the future (Yusuf and Inambao 2019). Bioethanol is commonly known as ethyl alcohol or chemically C_2H_5OH or EtOH. Either it can be used directly as pure ethanol or can be blended with gasoline to produce "gasohol." Reports state that the world production of bioethanol has increased from 50 million m^3 in 2007 to over 100 million m^3 in 2012 (Kang et al. 2014). The USA is the world's largest producer of bioethanol accounting for nearly 58% of global output in 2014 and Brazil holds the second place accounting for nearly 25% (Mansouri et al. 2016).

4.3.1.1 Raw Materials

Bioethanol can be produced from raw materials like corn straw, barley, oats, rice straw, wheat straw, sorghum, sugarcane bagasse, cashew apple, etc. depending on their availability and regional conditions like climate, storage facility, and efficiency of transportation (Kim and Dale 2004). Lots of studies were being conducted on enhancing the bioethanol production using different types of yeasts such as *Saccharomyces cerevisiae* (RL-11), *Saccharomyces bayanus*, *Pichia stipites* (NRRL-Y-

7124), *Kluyveromyces fagilis* (Kf1) and other microorganisms employing microalgae (*Chlorococum* sp.) and various other substrates (Harun et al. 2010). However, yeasts, especially *Saccharomyces cerevisiae*, are commonly used in ethanol production as it has high ethanol productivity, high ethanol tolerance, stability over a wide range of pH and the ability to ferment different types of sugars (Mohd Azhar et al. 2017). Though it is commonly used, it does have some drawbacks also. The rise in temperature (35–45 °C) during sugar fermentation and ethanol concentration (over 20%) may adversely affect the growth rate and metabolism that in turn leads to lower ethanol production (Tofighi et al. 2014). Works have been conducted in which cashew apple bagasse (CAB) was used for the immobilization of *S. cerevisiae* during the production of bioethanol. The CAB was treated with a solution of 3% HCl and delignification was done using 2% NaOH prior to use (Pacheco et al. 2010).

Cashew apple approximately contains 30% fermentable sugars like fructose and glucose, which can be utilized for fermentation of ethanol. Its juice normally contains organic nutrients and minerals (Vitamin C, calcium, iron, phosphorus, sodium, and potassium) that makes it suitable for ethanol production by fermentation using microorganisms (Sasikumar and Viruthagiri 2008). It is already reported that bioethanol can be produced from cashew apple juice by using UV mutant strain of *S. cerevisiae* and the optimization of process conditions (substrate concentration, temperature, pH, and fermentation time) has also been carried out using response surface methodology (RSM) (Honorato et al. 2007).

4.3.1.2 Extraction of Cashew Apple Juice

Before extracting cashew apple juice (CAJ), they have to be properly washed and cut into halves. They are then compressed in a fruit processor, with a separate pulp collector to obtain the juice. The preparation steps involve pretreatment, centrifugation, and sterilization. Pretreatment can be carried out by adding 1% (w/v) gelatin powder to the raw CAJ and maintained at 4 °C for 24 h. This was then centrifuged at 3500 rpm for 20 min and added with 2.5 g/L of ammonium sulfate, followed by sterilization at 121 °C for 15 min (Deenanath et al. 2013).

4.3.1.3 Inoculum Preparation

Inoculum can be prepared using different kinds of media. Semisynthetic yeast nitrogen base (YNB) medium supplemented with the carbon source (1–6%) and yeast extract (0.05%), without shaking (standing cultures) or with shaking (100 rpm; limited aeration cultures), at different temperatures (30–45 °C) (Ryabova et al. 2003) and Yeast peptone dextrose (YPD) liquid broth containing 10 g yeast extract powder, 20 g peptone powder and 20 g D-glucose per liter of media. The culture conditions include volume (200 mL), temperature (30 °C), agitation (150 rpm), and duration (18 h). The yeast strain was activated by extracting the glycerol and

resuspending the pellet in 1 mL of YPD broth, and this suspension was introduced into 50 mL of YPD liquid broth (Deenanath et al. 2013).

Bioethanol production is a three-stage process comprising: (1) Hydrolysis in which starch is converted from biomass feed stocks like cereal grains, lignocellulose and macro algae into fermentable monosaccharide sugars, (2) Fermentation where these monosaccharide sugars are converted into alcohol with the aid of microorganism in a closed aerobic or anaerobic atmosphere, and (3) Distillation which is a thermochemical separating process where ethanol gets concentrated to 95% (Deenanath et al. 2013). Its production can be divided into four generations based on the raw materials and the technologies used. First-generation bioethanol is produced only from the food part of the crops (sugar-based raw materials) and it uses conventional technologies to convert them into bioethanol, while the second generation aims at full valorization of plants, especially uses lignocellulosic raw materials and employs advanced technologies. Third generation exploits the potential of seaweeds and uses more advanced technologies and fourth generation makes use of the conversion of plankton vegetal (micro seaweed) to bioethanol using ultra-high processing technologies (Virginie et al. 2018).

4.3.1.4 Process in Bioethanol Production

Pretreatment

Pretreatment enhances the breakdown of complex sugar molecules to make it more susceptible for hydrolysis (Nath et al. 2011). Common methods in practice are physical pretreatment which uses mechanical milling to ground the substrate, chemical pretreatment includes ozonolysis, acid hydrolysis, and alkaline hydrolysis, whereas biological pretreatment employs different kinds of fungal species and physicochemical pretreatment can be achieved by ammonia fiber explosion and steam (Mohd Azhar et al. 2017; Alvira et al. 2010). NaOH and $Ca(OH)_2$ are used for alkaline hydrolysis which results in the removal of all lignin, part of hemicellulose and enhance the reactivity of cellulose in further hydrolysis steps (Hamelinck et al. 2005).

Hydrolysis

The aim of hydrolysis is to break down the complex sugars into simple sugars using acid or enzymes. Fermentation medium was incubated with dilute sulfuric acid (0.5–1.5% H_2SO_4) at 100–150 °C and the sugar monomers which are produced during this step were recovered in the liquid fraction after filtration. Dilute hydrochloric acid and nitric acids were also used, but use of dilute sulfuric acid achieves reasonably high sugar yields from hemicellulose. The residual solids were further subjected to enzymatic hydrolysis which uses enzymes like cellulases. The major groups of enzymes involved in the hydrolysis of cellulose are 1,4-β-D-glucanglucanohydrolase (EC 3.2.1.3), 1,4-β-D-glucancellobiohydrolyase (EC 3.2.1.91), and β-D-glucosidase (EC 3.2.1.21) (Hamelinck et al. 2005; Joshi et al. 2011).

Fermentation

During the process of fermentation both pentose and hexose sugars are fermented to ethanol under either anaerobic or aerobic conditions (Yusuf and Inambao 2019). *S. cerevisiae* is used for ethanol production from hexoses while *P. stipitis* and *Candida shehatae* are capable of fermenting both hexose (glucose) and pentose (xylose) sugars to ethanol (Parekh and Wayman 1986). Separate Hydrolysis and Fermentation (SHF), Simultaneous Saccharification and Fermentation (SSF) and Simultaneous Saccharification and Co-fermentation (SSCF) are the commonly used processes in bioethanol production (Saavedra-Molina et al. 2018). Fermentation can be carried out in batch (substrate addition at the beginning, without addition or removal of the medium), fed-batch (continuous addition of substrate) and repeated batch or continuous mode (Hadiyanto et al. 2013).

Distillation

The product resulting from fermentation is called "beer" which is a mixture of ethanol, cell mass, and water. Distillation is carried out to purify ethanol. It involves passing the vapor stream and the liquid stream in opposite directions. Usually about 95% ethanol can be recovered through distillation (Lynd 1996).

4.3.2 Enzymes

4.3.2.1 Tannase

Tannin acyl hydrolase (EC 3.1.1.20), also known as tannase, is an enzyme that catalyzes the hydrolysis of ester (galloyl ester of an alcohol moiety) and depside (galloyl ester of gallic acid) bonds of hydrolysable tannins to release glucose and gallic acid (Aguilar et al. 2001). It is an inducible enzyme with a wide variety of industrial applications. The major product of tannic acid degradation is gallic acid which is readily utilized as substrate by oxidative breakdown to simple aliphatic acids that enter into citric acid cycle (Bhat et al. 1998). Tannase is used in the manufacture of gallic acid, a precursor of antibacterial drug trimethoprim. Gallic acid is the substrate for the synthesis of propyl gallate which is an antioxidant in fats and oils, as well as in beverages industry. Tannase is used as a clarifying agent in the industrial processing of fruit juices and coffee-flavored soft drinks. It has great importance in the manufacture of instant tea. The commercial production of tannase is through microorganisms using submerged and solid state fermentation (Aguilar et al. 2007). Microorganisms most studied for tannase production include bacteria, fungi, and yeast. Filamentous fungi includes species of *Aspergillus, penicillium, Fusarium, Chryphonectria, Rhizopus, Fusarium, Cheatomium, Mucor, Neurospora, Trichoderma, Ascochyta, Helicostylum Cunnighamella, Syncephalastrum* etc. Among bacteria, species of *Bacillus, Klebsiella, Corynebacterium, Pseudomonas, Selenomonas ruminantium, Streptococcus*, and *Achromobacter* are reported as good tannase producers. Very few yeast species are reported as tannase producers

including *Candida, Mycotorula japonica, Pichia* sp., and *Debaryomyces hansenii* (Bhat et al. 1998; Lekha and Lonsane 1997; Bajpai and Patil 1996).

At the industrial level, mostly tannase production is achieved using *Aspergillus* sp. under solid state growth (Beena 2010). An inducer-tannin is used in all microbial production of tannases. Cashew apple has quite an appreciable amount of tannin content and hence it can be used as a suitable substrate for tannase production. N. Lokeswari has studied the tannin content of bark of eight commonly available plants by paper chromatography and quantified using colorimetric method and found that maximum amount of tannin was found in the extract of the husk of cashew. The results show that the extract of *A. occidentale* was best for the induction of tannase production using *Aspergillus oryzae* in submerged fermentation (Lokeswari 2010). Optimization of process parameters for maximal enzyme productivity in solid state fermentation using cashew apple baggase as substrate was reported by different researchers (Rodrigues et al. 2007, 2008; Porto et al. 2016; Tatiana et al. 2016). Viswanath et al. investigated the tannase productivity of *A. niger* CEPC 11 (MTCC 5898) on an abundantly available waste by-product cashew testa under solid state fermentation and response surface methodology was effectively applied for the optimization of factors for the production of tannase. They reported that the optimum response of the Box–Behnken method enhanced the tannase production resulting in a 3.02-fold increase from 97.32 to 301.70 U/g of dry sample of tannase (Viswanath et al. 2016).

4.3.2.2 Pectinase

Pectinases or pectinolytic enzymes are the complex and diverse group of enzymes that hydrolyze pectic substances, which are glycosidic macromolecules with high molecular weight containing galacturonic acid as basic monomer. Generally pectinases can be divided into three groups (Jayani et al. 2005):

(a) Protopectinases: degrade insoluble protopectin and convert them to highly polymerized soluble pectin.
(b) Esterases: act on the ester linkage to remove methoxy esters and help in the de-esterification of pectin.
(c) Depolymerases: hydrolyse glycosidic bonds between galacturonic acid moieties α-(1-4 linkage) and play a major role in the pectin breakdown.

Pectinases are widely used in food industry especially for fruit juice clarification, extraction of vegetable oils, refinement of vegetable fibers, curing of coffee and cocoa, and in the manufacture of pectin-free starch. Pectinases from microbial source pose a share of 25% in the global sales of food enzymes. There is an immediate need to discover new pectinase producing microbial strains and optimize their enzyme production conditions in both submerged and solid state fermentations in order to meet this increasing demand (Demir and Tari 2016).

In a study, polygalacturonase production was performed under solid state fermentation process in a tray bioreactor, using cashew apple dry bagasse and *A. niger*

CCT0916 for checking the influence of temperature and substrate thickness and found that both the parameters have positive effect on polygalacturonase activity (Alcântara and Flávio 2012). Works have also verified the influence of other parameters like concentration of spores and ammonium sulfate, moisture content and temperature on polygalacturonase activity of *A. niger* CCT0916 and found highest activity of 33.27 U/g with 10^6 spores per gram and 1.5% (w/w) of ammonium sulfate at 35 °C after 29 h of incubation (Alcântara and Da Silva 2011).

Industrially important enzymes are normally produced using microorganisms especially from filamentous fungi, under solid state fermentation. The reason is that, growing conditions in solid state fermentation approximate the natural habitat of filamentous fungi and are able to grow well on solid substrate and excrete large quantities of enzymes (Silva et al. 2005).

4.3.3 Wine

Wine is an alcoholic beverage produced by fermenting different kinds of fruit juices. Traditionally fermentation was carried out using wild yeast, but nowadays genetically modified strains of *S. cerevisiae* are used. The quality of wine produced depends on the parameters like sugar level, acid content, color, and aroma (Singh and Kaur 2009). A typical wine contains ethyl alcohol, acids, sugar, tannins, higher alcohols aldehydes, esters, anthocyanins, vitamins, amino acids, minerals, minor constituents like flavoring compounds etc. Wines made from fruits are often named after the fruits. As wine is a fruit-based fermented and undistilled product, it contains most of the nutrients present in the original fruit juice. The nutritive value of wine increases as the amino acids and other nutrients from the yeast are released into the wine during fermentation. Fruit wines contain 8–11% alcohol and 2–3% sugar with energy value ranging between 70 and 90 kcal per 100 mL. White wine (yellow, gold, or straw colored, depending on whether it includes the skin of the grape or just the juice), red wine (made from red grapes, which are actually closer to black in color), and pink wines (light pink in color, made from a mixture of "black" and "white" grapes) are the different types of wines commonly used around the world (Swami et al. 2014). Cashew apples are also used for the preparation of wine. The steps for the production of wine using cashew apple are discussed below. Figure 4.2 displays the different steps involved in wine production.

4.3.3.1 Preparation of the Cashew Apple Juice

Fresh fully ripened undamaged cashew should have to be selected. The nuts were removed from the fruits and washed thoroughly with distilled water. Then the cashew apples are soaked in 5% salt solution to remove the tannin content in them, so as to get rid of the astringent aroma of the product (Swain et al. 2005). Fruits are autoclaved at 15 lbs pressure for 15 min and crushed in a grinder to extract

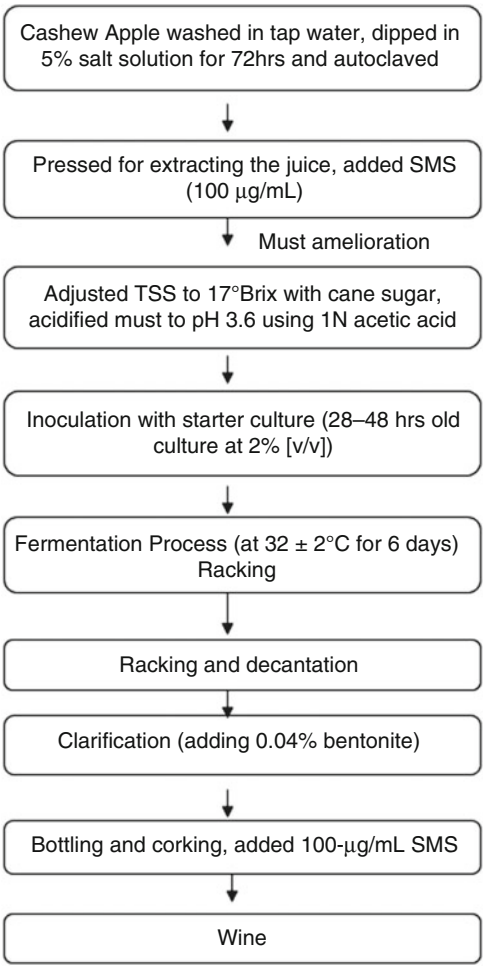

Fig. 4.2 Flowchart for making cashew wine; *SMS* sodium metabisulfite, *TSS* total soluble sugars (Swain et al. 2005)

the juice. To avoid the growth of undesirable microorganisms, the juice was treated with sodium metabisulfite (0.45–100 µg/mL) (Akinwale 2009). Along with that, 1% citric acid and 0.69 g/L of ammonium sulfate were also added as a nutrient source for yeast (Akinwale 2009). The carbon supplement for the starter culture was provided by the addition of invert sugar until the sugar content attains 17° Brix (Swain et al. 2005). The pH of the media was maintained at 3.6 using 1 N acetic acid. The resultant liquid was called the must into which the starter culture should be inoculated (Awe et al. 2013).

4.3.3.2 Preparation of the Starter Culture

The most commonly used starter culture is *S. cerevisiae*, i.e., the commercially available baker's yeast (Osho 2014). Varieties of strains can be used for the wine production using cashew apples as substrate (Awe et al. 2013). The starter culture can be prepared by the inoculation of the yeast in a small quantity of the prepared must-seed culture (1–5 g of yeast per 1000 mL of the must) or the previously prepared grape wine can be used as the starter culture (Swain et al. 2005).

4.3.3.3 Fermentation Process

Fermentation can be carried out at room temperature (28–32 °C). It was then inoculated with the starter culture or the seed culture. It takes 6 days to 6 weeks for the fermentation to get completed. The fermentation can either be strictly anaerobic or anaerobic cum aerobic (Awe et al. 2013). In anaerobic fermentation, periodic aeration cannot be provided. In the latter one, the inoculated must is periodically aerated by stirring at room temperature. The aerobic fermentation is carried out for 6 days and then can be subjected to anaerobic fermentation. Anaerobic air trapped jar can be used for the anaerobic fermentation. It takes 2–6 weeks to get completed. In order to supply CO_2 gas, Camden tablet can be added to the filtrate which can keep the wine away from the risk of contamination. After this the wine was stored to flocculate the yeast which was then subjected to racking (Awe et al. 2013).

4.3.3.4 Racking

During racking, the total sugar content in the wine reaches 2–3° Brix which will remove any sediment in the wine (Swain et al. 2005). Racking can be done by siphoning the fermented supernatant into another large clean vessel. It not only facilitates the clearing of wine, it also prevents the wine from undesirable flavor (Akinwale 2009).

4.3.3.5 Clarification and Ageing

Clarification was done by the addition of bentonite, pectinase, PVP, and gelatin (Lowor et al. 2016). The vessel was tightly closed to avoid air contamination and was kept for about 6 weeks at a temperature of 10–17 °C (Awe et al. 2013).

4.3.4 Biosurfactant

Microorganisms synthesize a wide range of structurally diverse Surface Active Compounds (SAC) and are generally known as biosurfactants. They are composed of hydrophilic component (amino acid, peptide cations or anions, mono-, di- or polysaccharides) and a hydrophobic component (unsaturated or saturated hydrocarbon chains or fatty acids). Because of these structures they got the ability to reduce the surface tension at the air/water interfaces as well as at the oil/water interfaces and to form micelles. Lipopeptides, glycolipids (rhamnolipids, trehalolipids, sophorolipids, and mannosylerythritol lipids [MELs]) and proteins are some of the examples for biosurfactants. Nowadays they gain more attention than their synthetic counterparts, as they are relatively nontoxic, biodegradable, thermostable, and have tolerance in wide range of pH (Banat et al. 2010). Biosurfactants possess important biological activities, including antibiotic, antifungal, insecticidal, antiviral, immunomodulator, and antitumoral activities. The applications of biosurfactants span in a wide variety of industries that produce household and industrial cleaners, personal care products, food processing industries and in the production of plastics, paints and coatings, textiles, pulp and paper, and agricultural products, cosmetics, pharmaceuticals, emulsifiers, wetting agents, and in the synthesis of fine chemicals (Arutchelvi and Doble 2011).

Rhamnolipids produced from *Pseudomonas aeruginosa*, trehalolipids from a number of different microorganisms, such as *Mycobacterium*, *Nocardia* and *Corynebacterium* and trehalose dimycolates from *Rhodococcus erythropolis* are available in the market as fungicide for agricultural purposes or as an additive to enhance bioremediation activities (Rapp et al. 2009). Sophorolipids are produced mainly by yeasts, such as *Candida bombicola*, *Centrolene petrophilum*, *Candida apicola*, and *Rhodotorula bogoriensis*, while MELs are produced by *Pseudozyma yeasts*, *Pseudozyma aphidis*, *Pseudozyma antarctica*, and *Pseudozyma rugulosa* (Arutchelvi and Doble 2011; Konishi et al. 2007).

4.3.4.1 Applications of Biosurfactants

Environmental Applications

Used in the remediation of inorganic compounds such as heavy metals both at sea and land. They accelerate the biodegradation of hydrophobic hydrocarbons in oil contaminated beach, soils and soil slurried in bioreactors (Dyke et al. 1993).

In agriculture, surfactant compounds can be used to enhance the pesticide and agrochemical solubility, acting as emulsifiers, spreaders, and dispersing agents and also they can be used for eliminating the plant pathogen and for enhancing the bioavailability of nutrients for beneficial plant-associated microbes (Sachdev and Cameotra 2013). Studies on pesticide biodegradation reported that trifluralin, atrazine, and coumaphos can be degraded by rhamnolipid mixture (Rh-mix) and Triton X-100 (TX-100) (Mata-Sandoval et al. 2001).

Antimicrobial Activity
Several biosurfactants have shown antimicrobial action against bacteria, fungi, algae, and viruses. The lipopeptide iturin from *B. subtilis* showed potent antifungal activity. Inactivation of enveloped virus such as herpes and retrovirus was observed with 80 μM of surfactin. Rhamnolipids inhibited the growth of harmful bloom algae species *Heterosigma akashivo* and *Protocentrum dentatum* at concentration ranging from 0.4 to 10.0 mg/L. A rhamnolipid mixture obtained from *P. aeruginosa* AT10 showed inhibitory activity against many microbes like *Escherichia coli*, *Micrococcus luteus*, *Alcaligenes faecalis* (32 μg/mL), *Serratia marcescens*, *Mycobacterium phlei* (16 μg/mL), and *Staphylococcus epidermidis* (8 μg/mL) and have antifungal properties against *Aspergillus niger* (16 μg/mL), *Chaetomium globosum*, *Penicillium crysogenum*, *Aureobasidium pullulans* (32 μg/mL), and the phytopathogenic *Botrytis cinerea* and *Rhizoctonia solani* (18 μg/mL). Sophorolipids and rhamnolipids were found to be effective against plant and seed pathogenic fungi (Nitschke and Costa 2007).

Food Applications
Biosurfactants are used to control the agglomeration of fat globules, stabilize aerated systems, improve texture and shelf life of starch containing products, modify rheological properties of wheat dough and improve consistency and texture of fat-based products.

Industrial Application
Lipopeptides, such as surfactin, lichenysin and emulsan have proved very effective in enhancing oil recovery. Because of the de-emulsifying properties of biosurfactants, they are used to break emulsions which form at various steps in oil extraction and processing, thus allowing a better recovery of the product (Banat et al. 2010). MELs in cosmetics can increase the stratum corneum water content in the skin and also can repair the damaged hair (Paulino et al. 2016).

4.3.4.2 Production of Biosurfactants

Commonly, Cooper medium, a glucose-based mineral salt medium, is used for the microbial production of Surfactin. It is proved that substituting the medium components NH_4Cl and EDTA with $(NH_4)_2SO_4$ and citrate, as well as changing the glucose concentration from 40 to 8 g/L could enhance the surfactin productivity for the strain *B. subtilis* DSM 10T (Willenbacher et al. 2015). It is reported that *P. aeruginosa* ATCC 10145, when grown on CAJ supplemented with peptone can be used for the production of rhamnolipids. For preparing the media, CAJ was centrifuged at 3500 rpm for 20 min and filtered using a 25 μm filter paper, and then diluted with water (1% [v/v]). 5.0 g/L of peptone was added to the diluted CAJ. pH was adjusted to 7.0 and it was again sterilized by filtering through a 0.45 μm Millipore membrane. Three loops of culture (overnight bacterial culture) were inoculated into 50 mL of Nutritive broth (NB) in a 250-mL Erlenmeyer flask and incubated in a rotary shaker at 30 °C and 150 rpm for 18–24 h. Optical density (600 nm) of bacterial suspension

was adjusted to 0.1. About 6 mL of inoculum (2%) was introduced into 500 mL Erlenmeyer flask containing 300 mL of CAJP medium, and incubated at 30 °C, 150 rpm in a rotary shaker (Rocha et al. 2007).

Studies conducted on biosurfactant production by *Acinetobacter calcoaceticus* RAG-1 using CAJ medium has reported that it is a viable substrate for biosurfactant production, as it can replace the traditional carbon sources (Rocha et al. 2006). *B. subtilis* LAMI008 was proven to be grown and produced biosurfactant in the different cultivation mineral media supplemented with yeast extract and with clarified cashew apple juice as carbon sources (Ponte Rocha et al. 2009).

4.3.5 Prebiotic Oligosaccharide

Prebiotic is defined as food ingredient that is not digested in the stomach but selectively digested by probiotic bacteria like *Bifidobacteria* and *Lactobacillus* in the intestine (Fernando et al. 2011). Functional foods have the potential to improve health benefit by providing nutrients and help to decrease gastrointestinal disorders. Prebiotic oligosaccharides are the new functional ingredients of foods including milk drinks and functional symbiotic to improve its quality (Manosroi et al. 2014).

Dextransucrase (EC 2.4.1.5) is a glycosyl transferase enzyme involved in the synthesis of dextran from sucrose. If the medium contain any other carbohydrate other than sucrose, the enzymatic pathway was shifted from dextran synthesis to oligosaccharide synthesis. Glycosyl moiety is transferred from sucrose to an accepter molecule that can be mono-, di-, oligosaccharides and also the products of this enzyme. Fructose is formed as a by-product during this transfer and hence it can be used to monitor the process. Mostly dextransucrase is produced by Lactic Acid Bacteria (LAB) (Demuth et al. 2000).

Chagas et al. (2007) reported that the use of cashew apple juice as substrate is an interesting alternative to grow *Leuconostoc mesenteroides* to produce dextransucrase. The enzyme activity was at least 3.5 times higher than synthetic medium. Even when the substrate was used without yeast extract or phosphate addition, high enzyme activities were obtained.

The report by Kaprasob et al. (2018) demonstrated a new biotechnological option for enhancing B-group vitamins and fructo-oligosaccharides of cashew apple through the fermentation by selected LAB. The five strains of probiotic bacteria studied were *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Bifidobacterium longum.* The fermented cashew apple juice with *Lb. acidophilus* and *Lb. plantarum* had higher oligosaccharide (fructo-oligosaccharides [FOS] and raffinose family oligosaccharides [RFO]) contents as prebiotic. Vergara et al. (2010) evaluated the prebiotic effect of cashew apple juice fermented with *L. mesenteroides* B-512F and *L. mesenteroides* B-742. It was tested using probiotic *Lactobacillus johnsonii* NRRL B-2178. In vitro growth of *L. johnsonii* in fermented cashew apple juice was about threefold higher than non-fermented juice.

4.3.6 Lactic Acid

Since ancient times, lactic acid has been utilized in fermentation and preservation of human foodstuffs. Lactic acid has a broad range of applications in food, textile, pharmaceutical, cosmetic, and chemical industries. As it is classified as GRAS (Generally Recognized As Safe) and permitted to be used as a food additive by the US FDA (Food and Drug Administration), it is widely utilized in every segment of food industry (Datta et al. 1995).

Lactic acid is mainly produced by two methods: (1) chemical method and (2) fermentative method. One of the major drawbacks of chemical synthesis is that, it results in racemic mixtures while an optically pure product is obtained through fermentative processes. Lactic acid is the main end product of carbohydrate fermentation by LAB and an approximately 90% of lactic acid is produced through fermentation (Hofvendahl and Hahn-Hägerdal 2000).

Silveria et al. (2012) investigated the use of cashew apple juice as a low-cost substrate for *Lactobacillus casei* B-442 cultivation and lactic acid production. The influence of different parameters such as the concentration of reducing sugars, ammonium sulfate concentration (as nitrogen source), pH, and temperature was evaluated regarding biomass formation, lactic acid production, and productivity.

A comprehensive mathematical model has been developed by Guilherme et al. (2012) for the production of lactic acid from cashew apple juice by *L. casei* B-442 in batch reactor. The result of the study performed by Sivagurunathan et al. (2018) also implies that cashew apple juice can be effectively utilized as a substrate for lactic acid production. Parameter optimization was also performed employing cashew apple juice from yellow variety and five best isolates. The authors reported that the maximum production of lactic acid was observed at pH 6.5 at an optimum temperature 38 °C and after 3 days of incubation and the addition of ammonium sulfate has improved the yield of lactic acid. Among the five different isolates studied, *L. casei* (LAB-I) produced a maximum of 24.4 $\pm$ 0.52 g/L of lactic acid.

4.3.7 Mannitol and Xylitol

Mannitol is the most abundant polyol or sugar alcohol in nature that can be produced by bacteria, yeasts, fungi, algae, lichens, and many plants. It is a valuable nutritive non-metabolizable sweetener, nontoxic and in many organisms, it is used as carbon and energy source. Mannitol is partially metabolized by humans and hence it does not induce hyperglycemia and can be used in food formulations for diabetics. It has been used in functional foods as food additive, texturing agent, sweet builder in "sugar free" chewing gum and also in pharmaceutical preparations (Wisselink et al. 2002; Song and Vieille 2009).

Xylose is one of the major sugars derived from the hydrolysis of lignocellulosic biomass, along with other sugars such as mannose, galactose, arabinose, and rhamnose. D-Xylose is the second most abundant sugar in nature, and it can be converted

to xylitol that has wide application in food and pharmaceutical industries. Yeasts are considered as the best xylitol producers (de Arruda et al. 2011).

In a study, the authors have reported the use of cashew apple juice supplemented with ammonium sulfate as substrate for mannitol production by two *Leuconostoc* strains. The replacement of yeast extract with ammonium sulfate has enhanced the yield of *L. mesenteroides* B-512F and was observed to be the best mannitol producer. A good mannitol yield of 95% and productivity of 1.6 g/Lh was obtained at pH 5.0 (Fontes et al. 2013). In a previous study, they have already reported the use of cashew apple as best substrate for mannitol production. They reported that the best results were obtained applying only cashew apple juice as substrate, containing 50 g/L of total reducing sugar (28 g/L of fructose), yielding 18 g/L of mannitol with 67% of fructose conversion into mannitol and productivity of 1.8 g/Lh (Fontes et al. 2009).

T. L. de Albuquerque et al. (2014) evaluated the cashew apple bagasse hydrolysate as a potential medium for biotechnological production of xylitol and they also claimed that detoxification treatments can be employed to reduce potential toxic compounds present in the medium without significant loss of sugars. *Kluyveromyces marxianus* CCA510 was used for the production and found that it was able to produce xylitol using cashew apple bagasse hydrolysate, with the highest yield of 0.36 g/L and maximum concentration of 12.73 g/L. The authors reported that when urea was added in the medium highest xylitol yield was observed showing that urea as nitrogen source improved the xylitol production.

Another study by Valderez et al. (2014) evaluated the effect of dilute acid pretreatment on cashew apple bagasse for ethanol and xylitol production. The organisms used in the study were *S. cerevisiae* and *K. marxianus*. The authors claimed that dilute acid hydrolysate of cashew apple bagasse is a promising substrate for microbial xylitol production by *K. marxianus* CCA510, since higher concentrations of bioproducts were achieved by this microorganism when compared to *S. cerevisiae.*

4.3.8 Single-Cell Protein

As the population growth is increasing in an alarming rate, the demand for the food gains the attention. The growing concern over the food shortage resulted in the investigation for an alternative food of choice. Underdeveloped countries like Algeria, Botswana, Nigeria, Madagascar and developing countries like India are facing nutrition deficiency and food scarcity problems. Single-Cell Proteins (SCP) are dried cells of bacteria, algae, yeast, and fungi, which are rich in proteins and thus can be used as protein supplement (Anupama and Ravindra 2000). *Spirulina* are unicellular and filamentous blue green algae, used as feed for fish, shrimp, and poultry. In ancient days people in Africa and Mexico used to harvest *Spirulina* from the waters and consumed it as food after drying. They grow well in a pH range of 9–11 and thus the chance of contamination by other microbes can be minimized (Usharani et al. 2012). Orange peel residue, sweet orange residue, sugarcane residue, paper mill waste, rice husk, wheat straw residue, cassava waste, sugar beet pulp,

coconut waste, grape waste, mango waste, and cashew apple juice are some of the substrates which have been used for the production of various types of SCPs (Suman et al. 2015).

The important factors which need to be taken into consideration while choosing the substrate for SCP production are the nature of the sugars as well as the total amount of fermentable sugars present in the substrate (Layokun et al. 1986). The result of analysis conducted on the cashew apple juice showed that it contains a high level of sugar (8.19%) concentration which makes it a suitable substrate for SCP production (Osho 1995).

4.3.9 Fiber

Dietary fiber is often referred to as the edible parts of plant material in the diet which is resistant to enzymatic digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. It includes cellulose, oligosaccharides, noncellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilages and a non-carbohydrate component lignin (Dhingra et al. 2012). Studies proved that the cashew apple residue powder contains 87% dietary fiber. Thus it can be incorporated with food items. Figure 4.3 represents the steps involved in the processing of cashew apple dietary fiber (Nam et al. 2014).

The production of hamburgers with partial substitution of beef with cashew apple residue powder found to have improved the nutritional quality of the product as well as it was found to have high dietary fiber content (value was between 0 and 7.66%) but low fat content (35% lipid reduction) (Pinho et al. 2012). Cashew apple residues from fruit juice industry as dehydrated fruit powders can be used for wheat flour substitution for cookies formulations. pH, fiber, and protein contents were significantly affected by fruit powder substitution levels during the biscuit-type cookies process (Emmanuelle et al. 2016).

4.3.10 Others

Other bioactive compounds including antibiotics, pigments, antioxidants, hypercholestrolemic agents, antihypertensive agents, antitumor agents, and bioactive peptides are some of the bioactive compounds produced during fermentation.

4.3.10.1 Antioxidants

Anacardic acids (AAs) are alkyl phenols that display antioxidant, anti-inflammatory, anticancer, antimicrobial activity. They are mainly present in the cashew nuts shell liquid (CNSL), which is a by-product obtained during the processing of cashew nuts

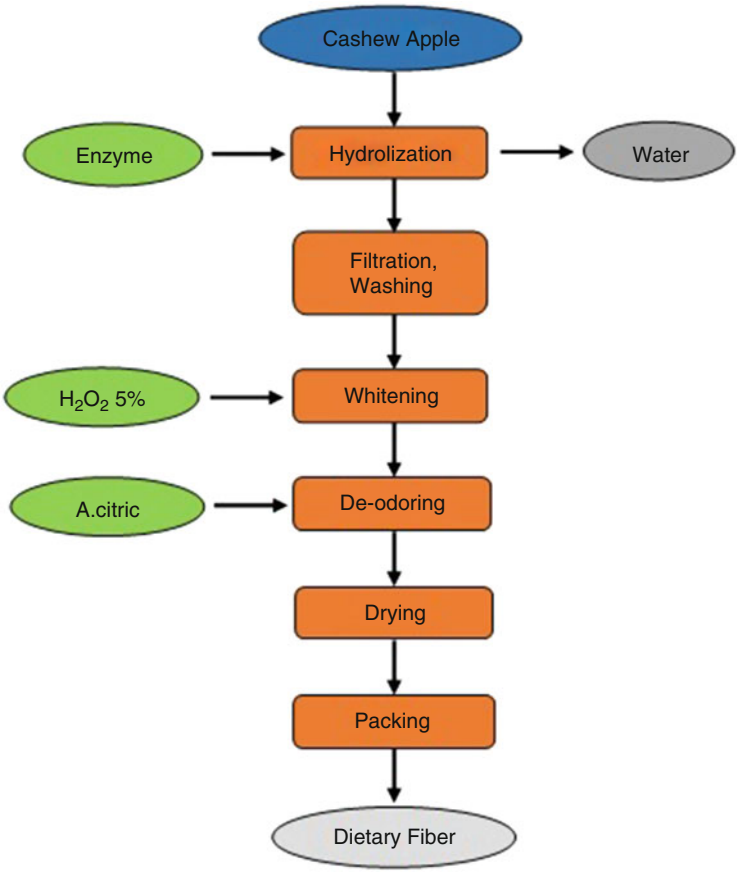

Fig. 4.3 Flowchart for processing cashew apple dietary fiber (adapted from Nam et al. 2014)

(Medeiros-Linard et al. 2018). Bioprocessing of CAJ from cashew by-products with beneficial lactic acid bacteria (LAB), *Lactobacillus plantarum* and *L. casei* is an effective approach for obtaining type 2 diabetes relevant antioxidant nutraceuticals (Kaprasob et al. 2019). The anacardic acids isolated from the cashew apple juice and cashew nut shell oil displayed potent antibacterial activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* strains (Muroi and Kubo 1996).

4.4 Conclusions

Bioprocessing using cashew apple is a highly economic, efficient, and key strategy for the production of wide variety of products. As 90% of the cashew apple is being wasted after harvesting the cashew nut, it is necessary and demanding to avoid such accumulation of agro wastes in the fields for a safer and cleaner environment. Different bioprocesses are being used for the utilization of different agro-industrial residues for their transformation into useful products. Cashew apple and its by-products offer a potential source for bioethanol, wine, and enzyme production as they are rich in lignocellulosic material, pectin, and tannin. The production of biosurfactants using underutilized agro residues, such as cashew apple, is a promising alternative against chemically synthesized surfactants as they are safe and economic. However, better utilization and exploitation of such agro residues are necessary and further studies and validations are required in the purification and commercialization of these products.

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Chapter 5
Agro-processing Residues for the Production of Fungal Bio-control Agents

Mousumi Das M and Sabu Abdulhameed

Abstract Environmentally sustainable and cost-effective alternatives for the control of plant pathogens and plant pests are required in the current scenario because of the development of agrochemical resistance in targeted pathogens, as well as the negative impacts of chemical pesticides on public health and the environment. Therefore, biological control agents (BCAs), such as fungi, are an attractive input to replace hazardous pesticides and offer great potential for field application. Agro-industrial bioprocessing generates large quantities of waste, such as straw, pulp, leaves, husk, and bagasse, which poses serious environmental problems worldwide. Effective use, added value, and bioconversion of these materials via bio treatment for the production of fungal BCA have received considerable attention in recent years. Many researchers have succeeded in using cheap agro-residues for the mass production of fungal biological control agents, mainly using SSF technology. The fermented substrate can be used directly for field application and the difficulties with downstream processing and product formulation are excluded. Among the microorganisms present in the rhizosphere, *Beauveria bassiana*, *Verticillium lecanii*, *Metarhizium anisopliae*, *Paecilomyces* spp., *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma asperellum*, *Trichoderma virens*, etc. are the most commonly used fungal biological control agents. Coffee husk is reported to be an excellent agricultural residue for the growth of *Trichoderma* spp. and *Paecilomyces* spp. Many aspects of the use of agricultural by-products in solid-state fermentation remain to be exploited for the production of bioactive compounds, green chemicals, biopesticides, etc., as well as for the development of more economically feasible processes.

Keywords Bio-control agent · Agro-processing residue · Trichoderma · Solid-state fermentation · Coffee husk

M. Das M · S. Abdulhameed (✉)
Department of Biotechnology and Microbiology, Kannur University, Kannur, Kerala, India

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5.1 Introduction

In the recent years, eco-friendly pest management strategies for sustainable crop production have gained considerable importance as a potential alternative to agro-chemicals for controlling plant pathogens. This has eliminated the hazards of conventional chemical pesticides on the environment and nontarget organisms and also reduced the production cost. This approach is being adapted around the world and is highly compatible with sustainable agriculture and effective against array of soil-borne phytopathogens (Heydari and Pessarakli 2010). Therefore, bio-control agents (BCAs), such as fungi, are an attractive input to replace chemical pesticides and offer great scope for field application, but the development of a viable bioprocess for its commercial production is not an easy task. Fungal BCAs are diverse group of naturally occurring organisms that are perceived as less harmful to the environment. Among the microorganisms present in the rhizosphere, *Trichoderma harzianum, Trichoderma viride, Trichoderma asperellum, Trichoderma virens*, *Beauveria bassiana, Nomuraea* sp., *Verticillium lecanii, Metarhizium anisopliae, Paecilomyces* spp. are some of the important fungal BCAs active against a variety of different insect pests and plant pathogens. Field applications of BCAs are mainly accomplished by means of fungal conidiospores, which must be virulent and viable for long periods of storage. Solid-state fermentation (SSF) offers many advantages for large-scale and cost-effective production of conidiospores. The use of different types of agro-industrial waste (straw, pulp, leaves, husk, bagasse, and winery wastes) solved this problem as substrates for large-scale production of BCAs. These agro-residues are produced globally in large amounts every year and left unutilized or untreated, which can cause serious environment and economic complications. In recent decades, efforts to use agro-industrial residues, particularly those from the tropics, have intensified. There is an increasing public concern for more efficient use of these agro-industrial residues such as coffee husks/pulps, wheat bran, cassava bagasse, sugarcane bagasse, sugar beet pulp, cakes and straw, in bioprocesses as carbon/nutrient source to produce microbial biomass which has been recognized as a bio-transforming agent for the agro-industrial waste materials (Pandey and Soccol 2000; Pandey et al. 2000a, b, c; Pandey 1992). Bioconversion of such agro-residues to value-added fine chemicals, through microbial fermentation, is the natural way to recover renewable bio-resources. Each microbial bioprocess is associated with biomass production, be it enzyme production, metabolite production, or bioconversion of waste materials. Nutritional quality, toxicity, and components of agro-residues determine the suitability of microorganism for utilization of these waste materials as substrate (Taherzadeh et al. 2003). Accordingly microbial biomass production is inevitable when value addition is achieved through microbiological ways. However, this chapter discusses relevant studies on the production of fungal BCAs using various agro-processing residues as well as the technology of SSF.

5.2 Bio-Control Agents: An Eco-Friendly Approach for Plant Disease Management

Biological control of pests, plant pathogens, and diseases has received considerable attention in the current scenario and it reduces the risk associated with the use of chemical pesticides in the crop field as well as resistance to the agrochemicals of the target organisms. It also helps in maintaining and balancing the plant species along with their natural enemies. They pose less threat to the environment and to human health. Biological control of plant diseases involves the use of biotic or abiotic agents acting through one or more mechanisms to reduce the damage caused by insect pest population and plant pathogens directly or indirectly by activating the host defense systems to reduce the intensity of the disease. Biotic agents include fungi, bacteria, oomycetes, protozoa, and viruses that suppress the development of crop diseases caused by microbial pathogens and plant pests in various crops, although each separate active ingredient is relatively specific for its target (Usta 2013). Abiotic agents such as solar energy, heat, ultraviolet rays, organic amendments, organic and inorganic compounds and naturally occurring plant and animal substances also have the ability to limit development of pathogens by direct and indirect effects. Biological control can result from different types of interactions between organisms such as predation, competition for nutrients and space, antibiosis, mycoparasitism/hyperparasitism, cell wall degradation by lytic enzymes, physical or chemical interference, hydrogen cyanide, induced systemic resistance (ISR), and plant growth promotion (Sharma et al. 2013; Pal et al. 2006). Nowadays, there is a growing commercial demand for fungal and bacterial BCAs to control phytopathogenic agents. Compared with bacterial BCAs, fungal BCAs show a great deal of progress, mainly because of their high degree of host specificity, high reproductive capacity, persistence, dispersal efficiency and their ease of cultivation and their maintenance in production which can ensure their survival longer without a host (Sandhu et al. 2012).

5.2.1 Filamentous Fungi as Bio-Control Agents

Fungal BCAs offer an alternative to chemical pesticides with increased target specificity and ecological safety, so that they are used either uniquely or in combination with other pest management programs. The most commonly used BCAs are living organisms, which are pathogenic for the pest. These include biofungicides (*Trichoderma*), bioherbicides (*Phytopthora*) and bioinsecticides (*B. bassiana*, *M. anisopliae*), and bionematicide (*Paecilomyces*) (Gupta and Dikshit 2010). The potential benefits to agriculture and public health programs through the use of BCAs are remarkable. Thus, special attention has been paid on fungal BCAs, as they have the ability to grow on cheap agro-processing residues, higher spectrum of disease management, and higher biomass production.

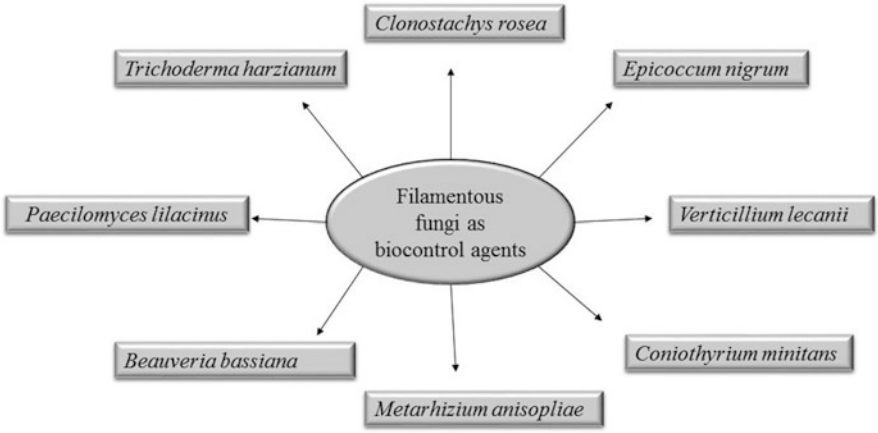

Fig. 5.1 Important fungal BCAs

Fungal BCAs are used to control different kind of parasites and pests. For example, filamentous fungi that control certain weeds, and other fungi kill specific insects (Gupta and Dikshit 2010; Brand et al. 2010). An entomopathogenic fungus is a filamentous fungus that can act as a parasite of insects and kills or infects them. Since they are considered as natural and environmentally friendly agents of mortality, there is worldwide interest in the use and manipulation of fungal BCAs for the control of various insects and pests in agriculture. It causes deadly infections and is host-specific with a very low risk of attacking nontarget organisms or beneficial insects. These fungi invade their hosts by penetrating through the host exoskeleton or cuticle, entering the hemolymph, producing toxins and developing with the nutrients in the hemocele to prevent the immune responses of insects (Usta 2013; Yashaswini and Sudarsanam 2017). Important fungal bio-control agents are mentioned in Fig. 5.1.

Among the wide variety of fungal species, *Trichoderma* have gained remarkable relevance due to their great bio-control potential among other entomopathogenic fungi. *Trichoderma* spp. is one of the most investigated fungi, able to antagonize and control a wide range of plant pathogens, pests, and nematodes, and contributing as high as 50% of BCAs fungi (De La Cruz Quiroz et al. 2015). Remaining 50% of BCAs fungi was contributed by *B. bassiana*, *M. anisopliae*, *V. lecanii*, *P. lilacinus*, etc., as they showed great effectiveness in laboratories and field studies. Several filamentous fungi can be applied in the field as conidia or sporulating mycelium for effective biological control of plant pathogens. The filamentous fungi that are used in plant disease control are shown in Table 5.1. Therefore, it is necessary to mass produce target-specific fungal antagonists to maximize its field application.

Table 5.1 Fungal BCAs used for the biological control of phytopathogens, pests, and nematodes (Sandhu et al. 2012; Brand et al. 2010; De La Cruz Quiroz et al. 2015; Arzumanov et al. 2005; Alavo 2015; Whipps and Gerlagh 1992)

Filamentous fungi	Plant pathogens/pests/nematodes
T. harzianum	*A. alternata, F. oxysporum, P. capsici, R. solani, P. expansum, B. cinerea, M. piriformis, A. flavus*, and *F. moniliforme*
B. bassiana	*Lygus lineolaris, Cosmopolites sordidus, Panonychus citri, Tetranichus cinnabarinus, Xylosandrus germanus*
T. viride	*Serratia* spp.
M. anisopliae	*Fusarium oxysporum, Cladosporium herbarum*, and *Curvularia clavata*
V. lecanii	*Puccinia graminis var. tritici, Puccinia striiformis, Uromyces appendiculatus, Phaeoisariopsis personata*, and *Puccinia arachidis*
C. minitans	*Sclerotinia sclerotrorum* and *Sclerotinia trifoliorum*
P. lilacinus	*Meloidogyne incognita*
Nomuraea sp.	*Spodoptera litura, Trichoplusia ni, Heliothis zea, Plathypena scabra, Bombyx mori, Pseudoplusia includens,* and *Anticarsia gemmatalis*
T. virens	*Stearia viridis* and *Amaranthus retroflexus*

5.3 Production of Fungal Bio-Control Agents Under SSF

Research on fungal BCAs has expanded constantly during the last decades, confirming real interest in their bio-control potential. The most important aspect to be considered while selecting a BCA for commercial application is the availability of a cost-effective production and stabilization technology for manufacturing an effective formulation. The production of fungal spores by SSF has arisen as a sustainable production method, which is slowly becoming preferred over submerged fermentation (SmF) considering the advantages of using of agro-industrial wastes as substrates. Solid substrates such as agro-processing wastes are found to be the better choice for growing sporulating fungi. In addition, SSF produces BCAs of better quality and micro-propagules with higher conidia content than SmF (Ooijkaas 2000; Sala et al. 2019).

SSF is generally defined as the growth of microorganisms on solid substrates with low moisture content, such as bran, bagasse, paper pulp, husk, usually of agricultural origin (Pandey 1992). These agricultural by-products are nutrient-rich waste, which can be easily recycled as substrates. In addition to provide nutrients such as carbon and nitrogen, the solid substrate also plays the role of the physical structure that supports the growth of microorganisms (Subramaniyam and Vimala 2012; Mishra et al. 2016).

SSF processes are usually cost effective, and they require reduced labor. In many cases, the fermented substrate can be used directly for field application, and thus most technical difficulties associated with downstream processing and product formulation are excluded. BCAs are mainly applied as spores and SSF offers many advantages such as higher virulence and UV tolerance of aerial spores, higher

spore stability, spore resistance to drying, and higher spore germination rates for longer storage periods (Sala et al. 2019). The under-explored field of mass production of BCA from waste biomass using fermentation processes should be technically refined to become the most cost-effective method among other strategies for converting biomass from waste into value-added products.

Fungal biomass production and their potential antagonistic activity against plant pathogens vary with the nutrient composition of the medium (Schisler et al. 2007). Therefore, the support chosen for biomass production should favor the growth of microorganisms and its cost should be economically feasible, so that the SSF technology remains economically viable. The substrate commonly used in fermentation process are cereal grains (rice, wheat, barley, and corn), legume seeds, wheat bran, rice husk, rice bran, lignocellulosic materials such as straws, sawdust, wood chips and a wide range of plant and animal materials (Sadh et al. 2018). Table 5.2 summarizes various fungal BCAs grown on agro-processing residues with products obtained or its functional application by fermentation.

Advances in biotechnology innovations, mainly in the field of bioprocess technology, many new avenues have been opened up for their effective use in fungal BCA production. Many researchers utilized different agro-residues for the mass production of filamentous fungi and optimized various process parameters for their large-scale production.

5.3.1 Agro-Processing Residues as a Substrate/Support on SSF

Agricultural development is usually accompanied by agro-residues generated from the irrational application of intensive farming methods and the abuse of hazardous chemicals used in cultivation, significantly affecting rural environments in particular and the global environmental in general (de la Cruz Quiroz et al. 2019). In 1990, global production of wheat straw was estimated at 709.2 million metric tons, compared to 673.3 million tons for rice straw (Belewu and Babalola 2009). Similarly, a large quantity of rice husks and wheat husks are also produced each year in the rice–wheat threshing process (Sharma et al. 2014). Most of this waste is used as animal feed or burned as an alternative to disposal, which generates uncontrolled emissions while wasting a potential energy source. Husks are usually burned out, producing ashes, fumes, and toxic organic gases. The incineration of rice husks produces rice husk ash (RHA), considered a global environmental problem because of its role in air and water pollution (Sharma et al. 2014). Coffee husks and coffee pulp, defective coffee beans and spent coffee grounds are another agro-industrial residues generated during its processing. The adequate disposal of these residues poses a major environmental problem to be solved. They are known to contain sugars, lipids, proteins, tannins, and polyphenols (Fan et al. 2003). Therefore, in addition to the effective waste management, the developing of value-added products

Table 5.2 Fungal BCAs produced through SSF in different agro-processing residues

Substrate for SSF	Microorganism used in SSF	Fermentation product/function	References
Rice, rice bran, rice husk	*Verticillium lecanii*	Fungal spores (Biological control)	Feng et al. (2002a, b)
Coffee husk	*Trichoderma* spp.	Fungal spores (Biological control)	Mamo and Alemu (2012)
Bagasse	*Trichoderma harzianum*	Fungal spores (Biological control)	Roussos et al. (1991)
Wheat bran	*Coniothyrium minitans*	Biological control	Chen et al. (2005)
Rice bran, sugarcane bagasse, wheat straw, chick pea husk, rice straw	*Trichoderma viride* *Trichoderma harzianum*	Biological control	Gangadharan and Jeyarajan (1990)
Refused potatoes and sugar-cane bagasse, coffee husk	*Beauveria bassiana*	Biopesticide	Santa et al. (2005)
Coffee fruit skin, poultry manure	*Trichoderma* spp.	Fungal spores (Bio-control agents)	Sawant (1996)
Corn bran	*Trichoderma harzianum*	Biological control	Cavalcante et al. (2008)
Wheat bran and rice straw	*Beauveria bassiana*	Biopesticide	Kang et al. (2005)
Wheat bran and sugar cane bagasse	*Beauveria bassiana*	Biopesticide	Nuñez-Gaona et al. (2010)
Sweet potato	*Isaria javanica*	Biological control	Kim et al. (2014)
Wheat bran	*Paecilomyces fumosoroseus*	Biological control	Asaff et al. (2006)
Sugarcane pressmud	*Paecilomyces lilacinus* *Paecilomyces farinosus*	Biological control	Robl et al. (2009)
Goddard sand/barley bran mixture	*Verticillium chlamydosporium*	Nematophagous	Bourne and Kerry (1998)
Rice/sugar cane bagasse	*Metarhizium anisopliae* (Metschn.) Sorokīn	Entomopathogenic	Arzumanov et al. (2005)
Cassava bagasse and coffee husk	*Paecilomyces lilacinus*	Bionematicide	Brand et al. (2004)
Cassava by-product	*Trichoderma harzianum*	Biological control	John and Jeeva (2014)

also plays a crucial role. The use of these agro-processing residues as raw materials can help to reduce the production cost while setting an eco-friendly alternative for their disposal. Therefore, bioconversion of agro-residues with microorganisms through fermentation process resulted in the production of microbial biomass and a range of significant beneficial compounds. Microbial biomass production thus has economic benefit as well as environmental benefit.

5.3.2 Principal Agro-Residues Used in Bio-Control Agent Production by SSF

The SSF is the best option to produce spores of BCAs, because the spores have a long shelf life, compared with the spores obtained in liquid cultures. Currently, these researches are aimed at the high biomass production of the BCAs using different kind of substrate including coffee husk, sugarcane bagasse/molasses, wheat bran, beer waste, cassava bagasse/peels, oil cakes, fruit and vegetable wastes.

5.3.2.1 Coffee Husk/Coffee Pulp: A Promising Agro-Processing Residue for Biomass Production

Coffee (*Coffea* spp.) is one of the most important agricultural commodities in the world. *C. arabica* and *C. robusta* are the two main varieties of the genus cultivated worldwide for commercial production. Brazil is the largest coffee producer in the world. One million tons of coffee is produced each year in more than 50 countries and the waste generated between harvesting, processing, and consumption represents much more than 50% of processed coffee (Singhania et al. 2008). Solid coffee processing wastes such as coffee husks and coffee pulp, defective coffee beans and spent coffee grounds contain tannins, caffeine, and polyphenols, which are generally considered as anti-nutritional factors, making direct use of coffee by-products less suitable for animal feed applications. Inappropriate disposal of these solid residues can cause several environmental problems. In this regard, several studies have been undertaken and are being developed in terms of alternative uses for such solid residues (Franca and Oliveira 2009). The detoxification of these residues by physical and chemical means can lead to a significant reduction in caffeine and tannin levels. However, this could become quite expensive, which would make it economically unfeasible (Shiono et al. 2017). But then, it has been confirmed that fungal strains are detoxifiers of this day-to-day waste. *Penicillium* sp., *Aspergillus* sp., *P. roqueforti*, and *Stemphylum* sp. are powerful detoxifiers; they degrade caffeine and tannins (Asano et al. 2009; Hakil et al. 1999; Kurtzman and Schwimmer 1971). Pretreated coffee by-products could therefore be used for forage applications.

Coffee husk is a potential agricultural residue for the biomass production of *Trichoderma* spp. (Sawant 1996). *Trichoderma* spp. plays a major role as a

bio-control because of their ability to improve crop fields through multiple roles, such as biopesticide and growth promotion (Kumar et al. 2012). In order to improve the marketability of *Trichoderma* as BCA, feasible commercial production processes are of utmost importance. Coffee husk-based *Trichoderma* formulation reduces the disease incidence of black pepper caused by *Phytophthora* sp. (Sawant 1996). Similarly, a bio-compost produced using coffee husk was used for the production of nematophagous fungi, *P. lilacinus*, which infects the *Meloidogyne incognita* coffee nematode (Brand et al. 2010). In addition to the economic production, the extended shelf life (up to 18 months) of BCAs multiplied on the coffee husk makes it more attractive in the field of bioprocess technology (Kumar et al. 2017).

5.3.2.2 Sugarcane Bagasse/Molasses

Sugarcane bagasse is a fibrous residue of cane stems left over after the crushing and extraction of juice from sugarcane (*Saccharum officinarum*). Sugarcane bagasse is generated in large quantities during the processing of cane sugar and consists mainly of 50% cellulose and 25% of each hemicellulose and lignin (Elumalai and Thangavelu 2010). Bagasse offers many advantages over other crop residues, such as rice straw and wheat straw, because of its low ash content which makes them suitable in bioconversion processes using microbial cultures. These residues have a wide range of applications in bioprocesses, mainly the production of protein-enriched livestock feeds and enzymes, biofuel production (ethanol), and biopesticide production (Singhania et al. 2008).

A large number of microorganisms including bacteria, yeasts, and fungi have been used for biomass production using bagasse. However, filamentous fungi, particularly basidiomycetes, are the preferred choice for enzyme production and protein enrichment and have been used most extensively. Some of them are *A. niger*, *N. sitophila*, *T. viride*, *T. ressei*, and *Xanthomonas* sp. (Singhania et al. 2008).

Several studies have been carried out using sugarcane by-products as substrate for fungal biomass production. According to Dalla Santa et al. (2004), the mixture of 60% potatoes and 40% sugarcane bagasse was found to be the best substrate/solid support for the production of *B. bassiana* spores. *B. bassiana* is a potential myco-insecticide against a wide range of pathogenic pests and insects. These fungal spores produced during SSF have biological control activity against the caterpillar larvae of mate plants, such as *Theolsia camina* and *Hylesia* spp. The spores were highly virulent, resulting in mortality of *T. camina* and *Hylesia* spp. larvae after 7 days of incubation with conidia suspension containing 10^8 conidia/mL. In this bioprocess, the sugarcane bagasse was used as a support for fungal growth and the refused potatoes were used as a source of carbon for fermentation because of its sugars, proteins, mineral salts, and vitamins.

Sugarcane bagasse was also used for the mass production of other deuteromycetes fungi. Latifian et al. (2013) reported the mass production of entomopathogenic fungi *M. anisopliae* using extracts of sugarcane molasses and rice, a two-phase liquid–solid method developed to control date palm pest. In the case of *V. lecanii*, an

entomopathogen with high potential for biological pest control, solid-state fermentation with sugarcane bagasse as a carrier-absorbing liquid medium has been used to produce *V. lecanii* spores. This study reveals that sugarcane bagasse with high porosity and water absorption can provide an ideal environment and inert carrier for solid-state fermentation of the fungus for spore production (Shi et al. 2009).

5.3.2.3 Wheat Bran

Wheat bran is another residue of the food industry and finds its applications in food and feed (Singh Nee Nigam and Pandey 2009; Hossain et al. 2018). These residues constituted a rich source of fiber, mineral, vitamins, and phenolic compounds. The industrial importance of wheat bran has increased in recent decades and several treatments, mainly fermentation, milling, heating, extraction and extrusion, have been used to improve their applicability. Wheat bran is used as a solid substrate for the growth of many BCAs, such as *Trichoderma harzanium* sp., *Trichoderma viride* sp., *Trichoderma koningii* sp., and *Trichoderma polysporum* sp. etc. (Cavalcante et al. 2008). Cavalcante et al. (2008) described a study where low-cost substrates such as rice, wheat bran, corn bran, and SSF were used to produce *Trichoderma* spores, which is antagonistic to several soil-borne phytopathogens. There are reports of sporulation of *Coniothyrium minitans* under SSF using wheat bran as a substrate. *C. minitans* is a natural antagonist capable of infecting sclerotia of many phytopathogenic fungi such as *Sclerotinia minor*, *Sclerotinia sclerotiorum* etc. (De Vrije et al. 2001). Chen et al. (2005) have studied the effect of addition of different carbon and nitrogen sources in wheat bran for the sporulation of *C. minitans*. The results of their study have showed a constant increase in *C. minitans* spore production, which allowed for maximum spore production of 9.94×10^9 spores/g IDM, with an increase in the C/N ratio of the substrate.

Mishra et al. (2016) described a study in which agro-processing residues such as wheat bran, rice husks, tea leaf wastes, pigeon pea and urad etc., were used as a production medium for spore production by *Beauveria bassiana* HQ917687. Their study mentioned that many of these agro-residues, mainly rice husk, wheat bran, and tea leaf waste, have supported *B. bassiana* sporulation demonstrating its potential for use as the substrate of choice for mass production of this fungus. Among the substrates used, the rice husk (4.3×10^8 spores/g) and wheat bran (2.1×10^8 spores/g) were found to be the most suitable substrate for *B. bassiana* spore production. In addition, these nutrient-supplemented substrates displayed comparatively higher spore production. Rice husks and wheat bran with the C/N ratios of 22.7 and 19.5, respectively, have been found to be excellent carriers for improved spore production. In the case of wheat bran, spore production has been improved through nutrient supplement (5.4×10^8 spore/g). The results of this study should pave the way for the commercialization of the *B. bassiana* biopesticide.

5.3.2.4 Beer Waste

Another agro-food residue, beer waste, generated after brewing process has potential applications in food, feed, and industrial biotechnology. The brewing process generates solid wastes such as spent grain, hot rub, residual yeast, and diatomaceous earth (Thiago et al. 2014). These brewery wastes were rich in fibers, carbohydrates, proteins, vitamins, and phenolic compounds (Mussatto et al. 2012; Robertson et al. 2010). To obtain products with high added value, these agro-residues are subjected to industrial bioprocess (Singhania et al. 2008). Beer waste provides excellent support and a source of nutrients for the production of fungal biomass. The bioconversion of this waste can enhance their potential for use as fertilizer. Gopalakrishnan et al. (2003) reported that brewery waste modified spent malt as a substrate for the mass production of *Trichoderma*. *Trichoderma* sp. is an effective biological control agent against many plant diseases of economic importance. The authors used spent malt as a source of carbon and brewer's yeast as a source of nitrogen (cheap brewery waste) for the SSF of *T. harzianum*. They have also multiplied three different species of *Trichoderma* including *T. harzianum*, *T. viride*, and *T. virens* to determine the efficacy of these isolates to utilize spent malt as a carbon source. Their studies concluded that the 3 g/100 g spent malt is the optimum combination for the growth of *T. harzianum* in SSF. *T. harzianum* of the three species tested produced the highest viable propagules (88.1×10^6), followed by *T. virens* (75.8×10^6) after 12 days of incubation. The cost of producing *Trichoderma* on these substrates is much lower. As a result, several commercial industries use this brewery waste for large-scale production. Since these substrates are available in abundance in the brewing industry, their use for mass production of *Trichoderma* will not only contribute to proper disposal, but also to the plant disease control program.

5.3.2.5 Cassava Bagasse/Peels

Cassava bagasse/peel is yet another agricultural by-product, left after industrial processing of cassava tubers for obtaining starch with mass balance. Cassava (*Manihot esculenta* Crantz) is the sixth largest food crop in the world and is the staple food for more than 700 million people in many countries. It is evident that processing 250–300 tons of cassava tubers yields about 1.6 tons of solid peels and about 280 tons of high moisture bagasse (85%). These wastes are usually disposed off in the environment without any treatment. Their disposal is a serious concern for the environment. Although they are fibrous residues rich in starch (carbohydrates), their direct use, like animal feed, is much lower because of the low protein content. However, these agro-industrial residues are used in the production of value-added products such as organic acids, ethanol, flavorings, fungi, etc. (Pandey et al. 2000c).

Several microorganisms, such as yeasts and fungi use starch as a substrate for growth and activity, have generally been preferred for bioconversion processes using cassava bagasse because of its high starch content. However, filamentous fungi have

been the most widely used. Brand et al. (2004) reported a study in which cassava bagasse and coffee husks were used as substrates for the production of an entomopathogenic fungi, *P. lilacinus*. *P. lilacinus* is an effective and proven BCA in field application for root knot nematode, *Meloidogyne incognita*. The authors used different proportions of cassava bagasse and coffee husks to obtain different C/N ratios and compared spore production in the two substrates. They also studied whether the virulence of the fungi was related to the nature of the substrate. The feasibility of using the strain *P. lilacinus* for spore production in SSF using cassava bagasse and coffee husk as substrate was evaluated. When coffee husk was used alone, the final spore count was 2.6×10^9 spores/g of dry substrate, and for cassava bagasse was 7.6×10^9 spores/g of dry substrate. Thus, they concluded that cassava bagasse as a substrate was much better than the coffee husk or mixed substrate. The results demonstrated that *P. lilacinus* spore produced under SSF have potential bio-control activity against the *M. incognita* nematode.

Another study by John and Jeeva (2014) described the use of cassava by-product as a carrier material for spore production of *T. harzianum*, a potential BCA against *Sclerotium rolfsii* causing collar rot in elephant foot yam. Their study revealed that cassava-based carriers prolong the shelf life and antagonistic potential of *T. harzianum* by more than a year compared to commonly used substrate such as saw dust and talc.

5.3.2.6 Oil Cakes

Oil cake is a coarse residue obtained from oil processing industry, rich in organic nitrogen, proteins and minerals and valuable for poultry and other animal feed. It can be broken and sold or be ground into oil flour. Some seed cakes such as castor beans and tung nuts are poisonous and are used as fertilizer rather than animal feed. Several plant seeds including soybeans, sesame seeds, peanuts, flax seeds, rapeseed, cotton-seed, coconut, palm kernel, olives and sunflower seeds have been used to extract vegetable oils and have left large quantities of solid residues such as oil cakes during its processing (Singhania et al. 2008; Ramachandran et al. 2007). Because of their high content of oil and other valuable substances and their low costs, oil cakes are potential substrates for bioprocesses chemicals. Their application in bioprocesses also offers advantages in bioremediation and biological detoxification of hazardous chemical compounds. The cakes are of two types, edible and nonedible, in which edible cakes have a high nutritional value (Ramachandran et al. 2007). Cakes have also been reported for use in production of biopesticides.

Petlamul and Prasertsan (2014) reported a study in which decanter cake was used for the spore production of entomopathogenic fungus, *Beauveria bassiana* BNBCRC as a part of biological control of various plant pests. The decanter cake is a solid waste generated during manufacture of palm oil, representing approximately 3.60% by weight of fresh fruit clusters constituting the raw material for the milling (Petlamul and Prasertsan 2014). It can be used as a soil amendment or as a fertilizer in oil palm plantations or as a livestock feed and could be an alternative

substrate for mass production of *B. bassiana* as a component of integrated pest management (Singh et al. 2010). This by-product would provide a source of carbon to the basal fermentation medium. The authors optimized the fermentation medium with the response surface methodology (RSM) method and indicated that the predicted spore yield values of 4.72×10^8 g^{-1} were close to the true value with a spore production of 4.62×10^8 spores/g.

Another study by Arora et al. (2017) focused on the use of nonedible cake waste (Jatropha, Karana, Neem and Mahua) as a substrate for the growth of *Paecilomyces variotii* and production of a bioactive compound, dipicolinic acid (DPA) (Arora et al. 2017). Their studies showed that *P. variotii* is a potential BCA with antagonistic activity against two phytopathogenic fungi, *Fusarium oxysporum* and *Verticillium dahilae* causing wilt in tomato plants. Tomato plants applied with DPA and *P. variotii* fungal extract were tolerant of severe attack by the pathogens *Fusarium* and *Verticillium*. Thus, inexpensive materials, such as nonedible cake, are a suitable substrate for the production of *P. variotii* and a large amount of bioactive components obtainable by optimization of the medium components (Badr et al. 2016).

Shah et al. (2008) reported that the neem seed cake improved the efficacy of the entomopathogenic fungus, *Metarhizium anisopliae*, for the control of black vine weevil (BVW) and *Otiorhynuchs sulcatus* (Coleoptera: *Curculionidae*) in outdoor plants, Euonymus, thus proposing a strategy without chemical pesticides to fight against the pests present in the environments (Shah et al. 2008).

5.3.2.7 Fruit and Vegetable Wastes

Fruit and vegetable waste is generated in large quantities around the world. This waste is commonly used as livestock feed and its use as a substrate in bioprocesses has a positive impact on food security, the economy, and the climate (Md Salim 2019). These wastes constitute pulp, seeds, peels, rind and pomace, and are good sources of economically useful bioactive compounds, mainly carotenoids, polyphenols, dietary fiber, vitamins, enzymes, and oils (Sagar et al. 2018). The utilization of these wastes in bioprocess technology for the production of essential bioactive components is an important step toward sustainable development.

Fruit wastes are rich in carbohydrates, starch, cellulose, soluble sugars, minerals and organic acids, vitamins, and minerals. Many researchers have used this waste for the growth of industrially important microbes. Different crop residues such as apple pomace, grape marc, pineapple waste, fruit blends, mausambi waste, banana waste, sugar beet pod, and kiwi fruit peel were studied for their potential use in fermentation process (Singhania et al. 2008).

Emerson and Mikunthan (2015) found that the BCA, *Trichoderma*, was able to grow on a palmyrah jaggery solution (30×10^7 spores/mL) and that palmyrah fruit pulp extracts produced maximal proliferation of mycelium and spores. Another work done by Sobita and Anamika (2011) reported that vegetable and fruit wastes, crop residue, farm yard manure (FYM), and poultry manure were suitable for mass production of fungal antagonist, *Trichoderma viride* and *Trichoderma harzianum*.

5.4 Lab Scale In Vitro Mass Production of *Trichoderma harzianum*

There are several studies that report the production of BCAs under SSF. Currently, the SSF is a commonly used system because the raw materials such as coffee husk, sugarcane bagasse, wheat bran and beer waste, among others are cheaper. The optimization of temperature, pH, moisture content, inoculum size, and incubation time are some factors that cause difficulty in the rigorous control of the fermentation process. *Trichoderma harzianum* have been used as antagonists in the biological control of several soil-borne plant pathogens, such as *F. oxysporum*, *R. solani* and *P. capsici* and *X. campestris* (Mousumi Das et al. 2019; Anwar and Iqbal 2017). A recent investigation from our laboratory on the production of a novel *Trichoderma harzianum* CH1 using solid agro-residues such as coffee husk, beer waste, wheat bran and sugarcane bagasse etc. has showed substantial positive results for biomass production along with bioconversion of waste materials. Fungal biomass was estimated in terms of n-Acetyl D-glucosamine which is the most abundant and stable component of cell wall. The present study focuses on improving the biomass production, propagule stability, infectivity, and shelf life of BCAs. The growth of *Trichoderma* sp. on these agro-residues has been shown in Figs. 5.2 and 5.3.

In this study, different process parameters have been optimized for higher biomass production. *T. harzianum* showed an optimal incubation time for maximum biomass production within 5–8 days when the coffee husk was used as a substrate. However, when wheat bran was used as a substrate, the maximum biomass was reached after 5–6 days of incubation, whereas when using beer waste as a solid substrate, the maximum biomass yield was reached in the third day after inoculation. In the case of sugarcane bagasse, the maximum biomass yielded with 8–10 days of incubation (Fig. 5.4) (Unpublished).

From the study it was clear that *Trichoderma* are able to grow on a wide variety of agriculture by-products and this can be useful to farmers to cultivate these fungi very easily. In terms of shelf life, our study revealed that higher biomass production was found in coffee husk and coffee husk-based *Trichoderma* formulation remained

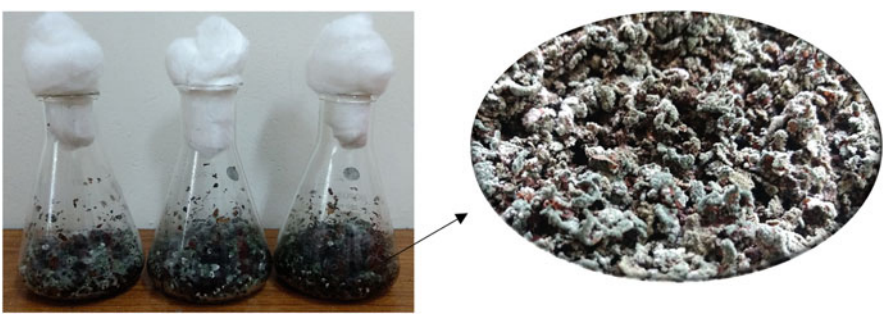

Fig. 5.2 Growth of *Trichoderma harzianum* on coffee husk

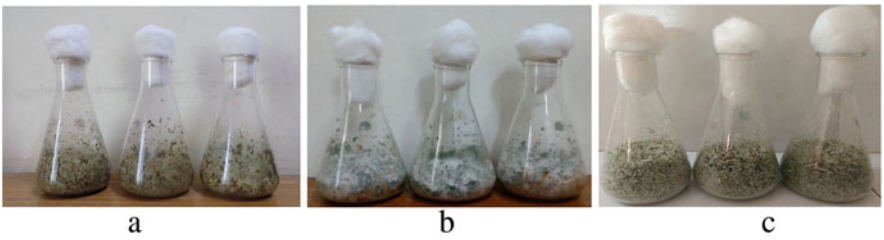

Fig. 5.3 Fermentation of *T. harzianum* in (**a**) beer waste, (**b**) wheat bran, (**c**) sugarcane bagasse

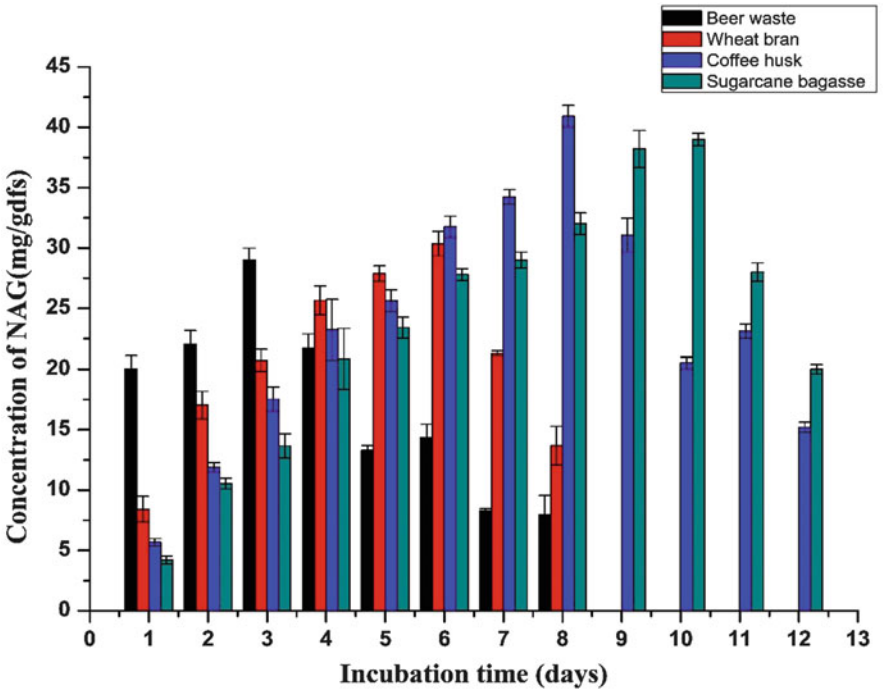

Fig. 5.4 Production of *T. harzianum* biomass at different incubation periods in beer waste, wheat bran, coffee husk, and sugarcane bagasse. Error bar represents standard deviation ($n = 3$)

viable for 12–15 months at 4–15 °C. The best SSF conditions with coffee husk and *T. harzianum* are as follows: substrate (30 g), initial pH (4.4), inoculum size (2×10^7 spores/g), temperature (30 °C), moisture content (65%), and incubation time (8 days). Thus biomass production of these BCAs not only helps in plant disease management but also facilitate agro-industrial waste disposal and production of value-added products through bioprocess.

5.5 Conclusion

Microorganisms (bacteria, actinomycetes, fungi) dominate the nature in terms of economical and environmental-friendly services. Their large diversity makes them suitable for various industrial microbial processes. These include biomass production, enzyme production, fermented food and feed production, microbial cell and metabolite production, and bioconversion of wastes materials. Microbial BCAs, particularly fungi-based BCAs, are a promising new alternative with features such as high biodegradability and specificity, low probability of pest resistance development, low or no known health risks and suitability for incorporation into integrated pest management programs. However, the development of a suitable mass production procedure for its commercial production is not an easy task. Therefore, using agricultural waste as a substrate or carrier for the growth and sporulation of microorganisms under SSF can produce larger amounts of spores at a reduced cost. In addition, this process reduces environmental pollution without wastewater. At the end of the SSF process, the product is completely dry and the fungal spores produced in SSF are cost-effective and show good stability and viability for field applications on a commercial scale.

This chapter summarizes the potential use of various agro-residues such as coffee husk/pulp, wheat bran, cassava and sugarcane bagasse, beer waste and fruit and juice wastes, for biomass production of fungal BCAs. Apart from this, isolation and identification of antibiotics from bio-control agents and their application in crop fields may prove to be advantageous. In spite of the potential fungal BCAs discussed in this chapter, additional studies reporting field level application, viability, and efficacy after long-term storage are necessary. Further research should focus on the improvement of shelf life and efficacy of fungi-based formulations after storage, because it will provide useful information about the field performance of formulation and application methodologies. Furthermore, the influence of growth, sporulation, and virulence in different soil should be investigated.

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Chapter 6
Production of Activated Carbon from Agro-industrial Wastes and Its Potential Use for Removal of Heavy Metal in Textile Industrial Wastewater

Ajeng Y. D. Lestari and Achmad Chafidz

Abstract Water pollution due to textile industrial wastewater discharge has become one of serious issues especially in developing countries, including Indonesia. The wastewater has a dangerous impact on the surrounding environment and living things including animals and human, since it contains some heavy metals, which have a tendency to accumulate in nature and do not decompose by nature. Accumulation of these heavy metals in the human body until certain level could cause some diseases. Therefore, this wastewater has to be treated first before safely discharged into the environment. One of the most promising methods to remove these heavy metals from the wastewater is by adsorption process. In the recent years, there have been a trend to utilize biomass or agro-industrial wastes based adsorbent due to their availability (abundant in nature), minimal effort, and biodegradability. The use of agro-industrial wastes to produce biomass-based activated carbon for removal of heavy metals in textile industrial wastewater could become one of the best promising alternatives to solve wastewater problem from textile industry as well as waste from agro-industry. This chapter focuses on the preparation, physical characterization, and adsorption properties of several bio-based activated carbon made of agro-industrial wastes.

Keywords Agro-industrial wastes · Bio-based activated carbon · Adsorption · Heavy metals · Textile industrial wastewater

A. Y. D. Lestari · A. Chafidz (✉)
Department of Chemical Engineering, Universitas Islam Indonesia, Yogyakarta, Indonesia
e-mail: achmad.chafidz@uii.ac.id

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6.1 Introduction

6.1.1 Textile Industrial Wastewater Problem

Textile industrial wastewater discharge has become a serious issue related to water pollution in most of developing countries, including Indonesia. This wastewater mostly contains heavy metals, which can endanger the living organisms as well as the ecosystem. Accumulation of these heavy metals in the human body can cause some diseases such as cancer, brain disorders, heart failure, digestive problems, and even death. Therefore, this wastewater should be treated first before disposed into the environment. Technologies for the removal of heavy metals from industrial wastewater have developed continuously. Over decades, various remediation strategies have been developed for heavy metals particle expulsion, for example, chemical precipitation, ion exchange, and membrane filtration. These strategies have some detriments, including raised vitality prerequisites, inadequate metal expulsion, age of poisonous muck, maintenance requirement, and costly equipment. One of the most promising methods to remove these heavy metals is adsorption process. The adsorption methods that have been reported in previous research studies commonly used inorganic-based adsorbents including activated alumina, activated silica, zeolites which would have an impact in the environment when not used anymore. Therefore, the selection of material to be used as an adsorbent gradually changes to biomass or solid organic waste which is much better in terms of availability, cost, and biodegradability (Kallel et al. 2016).

6.1.2 Potential of Indonesia's Agro-Industrial Waste

As an agro-industrial country, Indonesia has many agro-industrial wastes such as oil palm empty fruit bunch, sugarcane bagasse, rice husk, and corn cob. These wastes are usually left, buried underground, or even burned which could cause air pollution. Therefore, the use of these agro-industrial wastes to produce activated carbon for removal of heavy metals in textile industrial wastewater could become the best possible alternatives to solve the wastes from agro-industry and textile industry simultaneously. The following are some of agro-industrial wastes that have been investigated by the authors to produce activated carbon for removal of heavy metals.

*6.1.3 Elephant Foot Yam (***Amorhophallus campanulatus***)*

Elephant foot yam is a tuber that lives in the wilderness of East Java Province, Sumatra Island, and Kalimantan Island, Indonesia (see Fig. 6.1). The taste of the

Fig. 6.1 Elephant foot yam tuber (Aruna 2008)

tuber is not good when consumed, which makes this tuber usually used as animal feed. The composition of the tuber is presented in Table 6.1.

6.1.4 Petai

Petai is a native plant to the Southeast Asia countries such as Indonesia (Hasim et al. 2015). It has been realized that the stinky beans and cases comprise cancer prevention agents, nutrients, oils, and phenolic compounds. In Indonesian customary culture, rank beans and the skin are utilized as a bothersome drug. Petai also contains dynamic natural mixture, such as flavonoids, saponins, and tannins. It is worth to note that the petai residues, like the pods, have not been optimally utilized because it contains cellulose, and thus it can be utilized as raw materials for an adsorbent. Figure 6.2 shows the photograph of petai.

6.1.5 Rice Husk

Rice has been a fundamental food commodity for more than 100 nations and expended as a basic nourishment by the majority of individuals on this planet. In 2014, the total area of rice development in the entire world was around 162.7 million ha, and around 741.5 million tons of rice were produced. More than 90% of this is produced in Asia, whereas China, India, and Indonesia represented 27.85%, 21.20%, and 9.55% of the total yield, respectively (FAOSTAT 2014). The kernel of rice consists of endosperms, husks, wheat, and germs, where endosperms contribute 70%, rice husks 21%, rice grain 8%, and rice seeds 1%, from the total seed weight. During

Table 6.1 Composition of elephant foot yam tuber (*Amorhophallus campanulatus*) (Opara 2003)

Composition	Percentage
Moisture	77.8
Sugars	0.14
Protein	2.24
Ash	1.36
Starch	16.6
Dietary fiber	1.45
Lipids	0.06

Fig. 6.2 Photograph of petai

the rice processing process, a lot of rice husk are resulted as a by-product. At present, a large portion of the rice husks produced are not used or consumed due to the properties of the rice husk, e.g., poor nutrition, hard surfaces, high silicon level, low thickness, and in this manner it is hard to be decayed by microorganisms. Presently, in Indonesia, the rice husks were treated in basic manner, e.g., for burning to deliver warmth or steam, or left unused in open stockpiling, or landfilling. The issue is that these treatments could cause environmental issues such as smoke, dust, and further nursery impacts (Kuan et al. 2012; Soltani et al. 2015). The rice husk consists of low dimensions of unrefined protein and fat which are in the scope of 2.0–2.8% and 0.3–0.8%, separately. Additionally, it also contains rough fiber, in the range of 34.5–45.9% (Champagne et al. 2004), cellulose (28.6–41.5%), hemicellulose (14.0–28.6%), and lignin (20.4–33.7%) (Quispe et al. 2017). Figure 6.3 shows the photograph of rice husks.

6.1.6 Corncob

Corncob is also an agro-industrial waste that is commonly used as an adsorbent, a mixture of concrete materials, raw materials for making ethanol, raw materials for making acetic acid, etc. Corncobs are abundant in the world as corn production in the world reaches 1120 million metric tons in 2018 (USDA 2019). Untreated corncob

Fig. 6.3 Rice husk ash (taken from https://www.alibaba.com/product-detail/Dao-Ke-Powder-black-rice-husk_60753967034.html)

contains 40.16% cellulose, 42.25% hemicellulose, and 10.78% lignin (Li et al. 2014). Figure 6.4 shows the photograph of corncobs.

6.1.7 Empty Coconut Palm Oil Fruit Bunch

Oil palm is one of the main agriculture commodities produced in Indonesia, which makes Indonesia as one of the major producers of palm oil in the world. Mostly, the palm oil is used to produce household cooking oil, or converted into biodiesel. Empty fruit bunch (EFB) of oil palm can be considered as waste since it does not have additional values and has not been fully utilized. Nevertheless, the EFB has potential to be used as raw material to produce adsorbent, since it has high surface area, good porosity and chemical stability, and very low cost since it is abundantly available as waste from the palm oil industry. Table 6.2 shows the properties of dry basis oil palm biomass.

Fig. 6.4 Corncob (taken from https://www.exoticblanks.com/corn-cob-pen-blanks-stabilized-small-sized-7mm.html)

Table 6.2 Properties of dry basis oil palm biomass (Chan et al. 2015)

Biomass	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Extractive and ash (%)
Raw EFB oil palm	26.8	26.7	18.7	27.8
Palm mesocarp fiber	22.3	23.1	30.5	24.1
Palm kernel shell	22.5	24.6	33.4	19.2

6.2 Preparation of Bio-Based Activated Carbon Made from Agricultural Waste

In general, there are two main steps to prepare bio-based activated carbon made from lignocellulosic biomass. The first step is carbonization of lignocellulosic biomass at temperature of about 800 °C (or lower) with the absence of oxygen (Cagnon et al. 2009; Sun and Jiang 2010; Daud et al. 2003; Lua et al. 2006; Tsai et al. 1998). The second step is activation process of the prepared carbon to develop surface area and pore volume. The activation process includes physical and chemical activation.

6.2.1 Carbonization

Carbonization procedure is the initial step of activated carbon preparation. The goal of this stage is to build carbon content in the biomass raw material by wiping out non-carbon parts by means of thermal decomposition. The underlying porosity of the roast could still be controlled in this progression (though low) before further controlled in the following activation process. The carbonization procedure factors must be prepared cautiously since this procedure will influence the properties of the carbon produced (Daud et al. 2000).

In this process, the temperature influences the carbonization procedure fundamentally, followed by the heating rate, nitrogen flow rate, and residence time (Ioannidou and Zabaniotou 2007; Lua et al. 2006). Normally, higher carbonization temperature, for example 600–700 °C diminished char yield while expanding the gases and fluid discharge rate (Ioannidou and Zabaniotou 2007). High temperatures can likewise

expand the ash and carbon content while diminishing the dimension of unpredictable segments (Ioannidou and Zabaniotou 2007; Lua et al. 2006; Putun et al. 2005). Consequently, high carbonization temperatures can deliver great nature of char yet in addition diminishing the yield. It is known that at high temperature, the biomass deteriorated and furthermore the char buildup experiences optional disintegration (Ioannidou and Zabaniotou 2007).

Higher temperatures during carbonization procedure will build the ash level and fixed carbon content because of diminishing unstable parts, which produces ash with better quality. To get low volatility and high yield of char, low heating pace (for example 10–15 °C/min) must be utilized. The delivered char with high fixed carbon substance is significant for synthesizing activated carbon. Low heating rate can increase the lack of hydration and adjustment of the polymeric substances (Suhas et al. 2007; Ioannidou and Zabaniotou 2007).

Moreover, the microporosity of the char was reported not influenced by the synthesis of the precursor and the carbonization heating rate (Suhas et al. 2007). The carbonization parameter which significantly contributes in the starting pore structure of char is the release of volatile matters from the char/carbon. Accordingly the carbonization parameters should have been considered before the initiation procedure. It is known that the inner pore structure of the char can significantly influence the pore attributes of the activated carbon produced.

6.2.2 Activation

Activation procedure is the second step to produce activated carbon. The reason for the activation procedure is to build the pore volume, the measurement of the pores, and porosity of the initial carbon. This procedure should be possible by means of three strategies, which are physical, chemical, and physiochemical (combination of physical and chemical) activation. Steam or CO_2 are normally utilized for physical activation, while for chemical activation, different chemical substances are used (Gupta and Suhas 2009; Crini 2006).

During the main period of activation process, chaotic carbon is removed, which leads to the progression of microporous structure. In the last period of the reaction, existing pores are expanded or large size pores are molded when dividers between the pores are completely seared off (Ahmed 2016).

This leads to the growing transitional pores and macroporosity, while the volume of micropores decreases. Hence, the level of carbon material consumed or the degree of activation is a considerable factor during the synthesis of activated carbon. During activation, the temperature is set between 800 and 1000 °C to develop porosity and surface domain of lignocellulosic carbon (Nor et al. 2013).

For physical activation, using steam is more effective than using CO_2 in order to obtain activated carbon with higher surface area. The smaller molecule size of water is highly recommended to promote scattering inside the carbon's porous structure. Steam initiation is said to be a couple of times faster than CO_2 at a comparable degree of progress (Ahmed 2017).

In the other hand, different chemical compounds, e.g., phosphoric acid, sodium hydroxide, potassium hydroxide, and zinc chloride have been utilized for chemical activation of activated carbons which build up the porosity based on dehydration and degradation. In general, chemical activation happens at lower temperature than physical initiation. This improves the advancement of pore in carbon structure because of the impact of chemical activator. One of the most significant points of interest of chemical activation over physical activation is the lower treatment temperature and shorter treatment time. Also, the activated carbon produced by the chemical activation has larger surface territory and better controlled microporosity in smaller ranges. Additionally, the carbon yield of chemical activation is higher than that of physical ones (Nor et al. 2013).

6.3 Properties of Adsorbents

This section will discuss several properties of adsorbents prepared and investigated by the authors.

6.3.1 Morphology of Adsorbents

Figure 6.5 exhibits the SEM micrographs with 5000× magnification of KB (i.e., dried raw elephant foot yam skin) and 500× amplification of KM (i.e., modified elephant foot yam skin). The figure shows that KM has bigger pore than KB due to the difference of amplification. The pore measurement of KB was 412.6 nm, while the pore diameter of KM was approximately 64.69 μm. The modification the elephant foot yam skin has affected the morphology of the adsorbents. The KM adsorbent was expected to have more superior adsorption performance than the KB adsorbent (Lestari et al. 2018a).

Additionally, Fig. 6.6 shows the SEM micrographs with 1000× magnification of Raw Starch of Elephant Foot Yam (PB), Porous Elephant Foot Yam Starch (PT), and Modified Porous Elephant Foot Yam Starch (PTM). Figure PB represents the pure foot yam starch which was shown by circle with few pores. There were some granules presented, which was believed to be contamination of starch as reported by Zhu (2015), whereas, Figure PT represents the porous foot yam starch, which was demonstrated by circles which were smoother and had a greater number of pores than Figure PB. Additionally, Figure PTM exhibits the modified porous foot yam starches. They had more amorph, permeable and clean structure. Different procedures have affected the morphology and adsorption capacity of adsorbents. Based on the morphological study results, the PTM had more bargaining as adsorbent for adsorption process (Lestari and Dewi 2018).

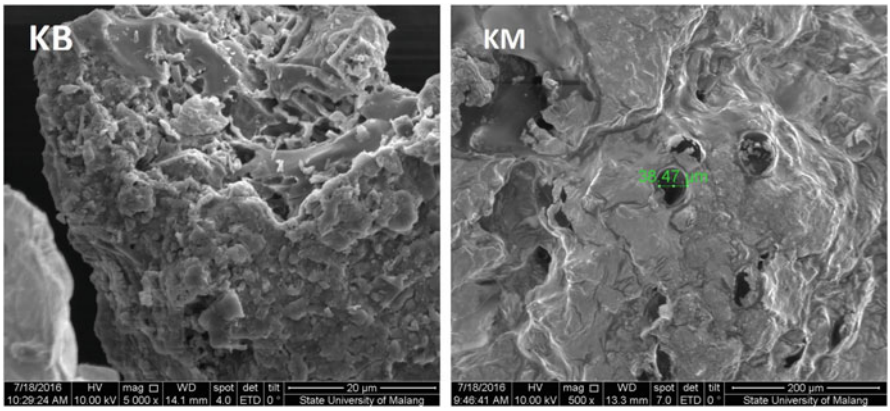

Fig. 6.5 SEM of raw (left) and modified (right) elephant foot yam based activated carbon (Lestari and Dewi 2018)

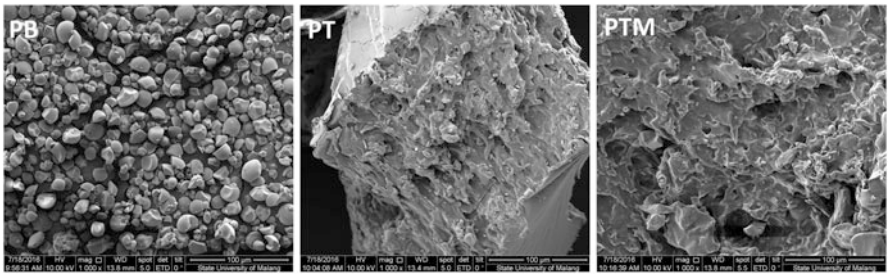

Fig. 6.6 SEM of raw starch of elephant foot yam (PB, left), porous elephant foot yam starch (PT, center), and modified porous elephant foot yam starch (PTM, right) (Lestari et al. 2018a)

6.3.2 *Chemical Bonding in Various Agricultural Waste-Based Adsorbents*

Figure 6.7 shows FTIR spectra of the chemical bonding that occurred in the adsorbent made of elephant foot yam skin. The black line represented native elephant foot yam skin spectrum and the light blue line represented modified elephant foot yam skin spectrum. The FTIR spectrum showed the –OH bonds on wavelength around 3.550 cm^{-1}, the C=O bonds on wavelength around 1.600 cm^{-1}, the C-O bonds on wavelength 950 cm^{-1} (Lestari et al. 2018a).

Figure 6.8 exhibits FTIR spectra of the chemical bonding that occurred in the adsorbent made of elephant foot yam starch. The black line represented modified porous elephant foot yam starch (PTM) spectrum, the dark blue line represented porous elephant foot yam starch (PT) spectrum and the light blue line represented native elephant foot yam (PB) spectrum. The FTIR analysis result demonstrated the presence of hydroxyl bonds on wavelength around 3400 cm^{-1}, the C=O bonds on

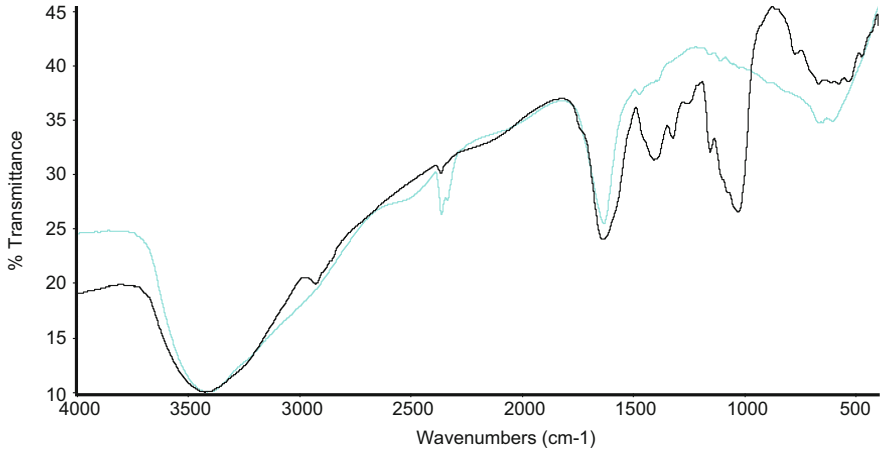

Fig. 6.7 FTIR spectrums of native and modified *Amorphophalus campanulatus* skin (Lestari et al. 2018a)

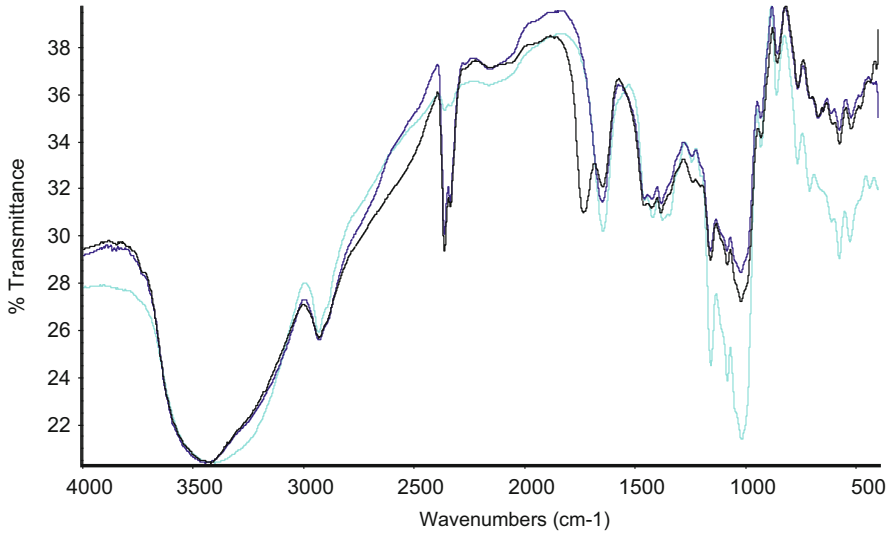

Fig. 6.8 FTIR spectrums of native and modified elephant foot yam starch (Lestari and Dewi 2018)

wavelength around 1800 cm^{-1}, the C-O bonds on wavelength 1000 cm^{-1}. The main focus was the hydroxyl bonds. The hydroxyl bonds of PB sample was noticed to be a little wide to the right. After the alteration, both of PB and PTM's hydroxyl bonds tends to be progressively balanced. It was believed that the procedure of alteration with citrus extract may modify the hydroxyl bonds (Lestari and Dewi 2018).

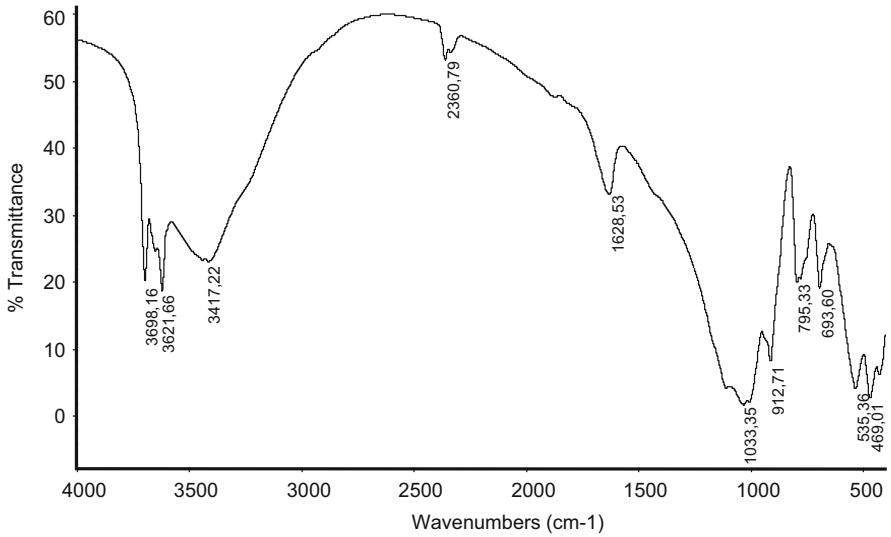

Fig. 6.9 FTIR spectrums of modified water hyacinth leaves (Lestari et al. 2018b)

Figure 6.9 shows the FTIR spectrum for modified water hyacinth leaves. There is an NH group strain vibration at wave number 3698.16 cm^{-1}. Along wave number 3621.66 cm^{-1} indicates the existence of stretch vibration of OH group. Wave number 3417.22 cm^{-1} indicates the stretching of OH groups which are hydrated by adsorbed water molecules. This is reinforced by the adsorption band at 2360.79 cm^{-1} which shows the buckling vibration of C≡C. The adsorption band that extends at numbers 1033.35 cm^{-1} shows the characteristics of C-O amines, besides that the vibration of C-H is also observed at wave number 795.33 cm^{-1}. Adsorption bands at wave numbers 1033.35 cm^{-1} to 795.33 cm^{-1} indicate the presence of asymmetric stretching vibrations from Si-O. The existence of Si-O groups is identified by the appearance of sharp adsorption bands and high intensity at wave numbers of 1033.85 cm^{-1}. The high intensity at the adsorption peak of 1033.85 cm^{-1} shows the high content of montmorillonite in bentonite. The bending vibration of the hydroxyl group of Al-Al-OH appears in the adsorption band 911.90 cm^{-1}, while the bending vibrations of Si-O-Al and Si-O-Si are shown in wave numbers 535.02 cm^{-1} and 468.73 cm^{-1} (Lestari et al. 2018b).

Figure 6.10 shows FTIR spectra of the chemical bonding that occurred in petai hull and corncob. The dark purple line represented native petai hull-based adsorbent, the light blue line represented modified petai hull-based adsorbent, whereas the light green line represented native corncob-based adsorbent, and the black line represented modified corncob-based adsorbent. The FTIR analysis results show that the modified treatment with nitric acid (HNO_3) on corncobs and petai hull has changed the structure and quality of chemical bond in pure compound. The modification process on the biosorbent also affects the difference in the number of peaks of each biosorbents due to the different types of content and the treatment applied to each biosorbents. The broad adsorption bands in the range of 3500–3200 cm^{-1} can be

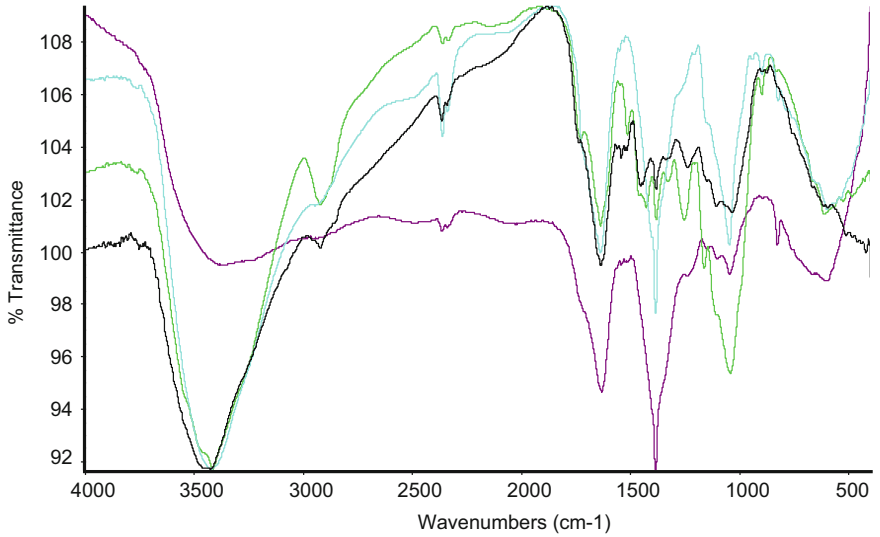

Fig. 6.10 FTIR spectrums of native and modified petai hull and corncob adsorbents (Putri et al. 2018)

attributed to the presence of hydroxyl (–OH) functional group. The functional groups present in cellulose are carboxyl and hydroxyl groups. The adsorption peak at 1041.44 cm^{-1} can be assigned to moderate-sharp intensity C–N (amine) bond and a sharp intensity alcohol C–O bond. The C–O group of alcohols can stretch the cellulose in the biosorbent. This shows that the chemical bonds of the four biosorbents can be used as Pb(II) metal ion biosorbent (Putri et al. 2018).

6.3.3 Adsorption Performance of Adsorbents

6.3.3.1 Adsorption Capacity of Pb Ion with Corncob and Petai Hull Adsorbent

The influence of initial lead concentration on the adsorption capacity of several natural based adsorbents, e.g., B (with ratio of corncob-based activated carbon/petai hull-based activated carbon 1:3); C (with ratio of corncob-based activated carbon/petai hull-based activated carbon 1:1); D (with ratio of corncob-based activated carbon/petai hull-based activated carbon 3:1); and H (with ratio of corncob-modified activated carbon/petai hull-modified activated carbon 1:1) are shown in Fig. 3.7. Based on Fig. 6.11, it can be seen that sample B reached the largest adsorption capacity at 500 ppm concentration of 0.617 mg/g. Sample C reached its maximum capacity at 500 ppm concentration which is equal to 1.289 mg/g. Sample D had the maximum adsorption capacity at 2.005 mg/g at 500 ppm. Maximum adsorption of

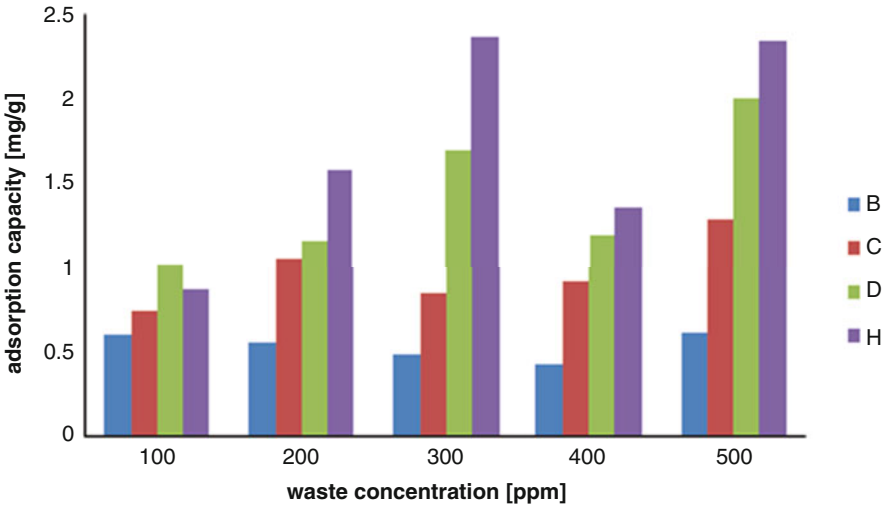

Fig. 6.11 Lead adsorption capacity on various adsorbents from *Amorphophallus campanulatus* (Lestari et al. 2018b)

sample H occurred at a concentration of 300 ppm with an adsorption capacity of 2.368 mg/g. As noticed from the results, for all the samples, the maximum adsorption capacity occurred at the highest concentration, i.e. 500 ppm. In exception for sample H, in which the maximum adsorption capacity occurred at concentration of 300 ppm. In general, it can be concluded that, adsorption capacity increased with increasing concentration of the adsorbate. Additionally, based on these results, the sequence of adsorbents due to their maximum capacity to adsorb Pb(II) metal ions is the following: H > D > C > B (Lestari et al. 2018b).

6.3.3.2 Adsorption Capacity of Pb Ion with Water Hyacinth

The Pb ion adsorption process using synthesized adsorbents occurred in variety of initial Pb waste concentrations (i.e., 100, 200, 300, 400, 500 ppm). The filtrate was analyzed by atomic absorption spectrophotometer (AAS) method. Table 6.3 shows the nomenclature of the synthesized adsorbents, while Fig. 6.12 shows the lead adsorption capacity in various synthesized adsorbents. According to Fig. 6.12, it can be seen that the largest adsorption capacity of Pb is on adsorbent H. The ability of water hyacinth to absorb metal due to water hyacinth has leaves that contain fiber so that it serves as a tool to absorb metal compounds, so that the dissolved metal toxicity decreases. The data showed that the optimal result occurred at sample H with maximum adsorption capacity of 80.28%, in which the remaining content of Pb was 78.9 ppm from 300 ppm of initial concentration.

Table 6.3 Nomenclature of the synthesized adsorbents

Sample	Bentonite mass (g)	Water hyacinth leaves mass (g)	Chitosan (g)	Sample	Bentonite mass (g)	Water hyacinth leaves mass (g)	Chitosan (g)
A	1.00	0.00	1.00	E	0.00	1.00	1.00
B	0.75	0.25	1.00	F	0.50	0.50	0.50
C	0.50	0.50	1.00	G	1.00	0.00	0.00
D	0.25	0.75	1.00	H	0.00	1.00	0.00

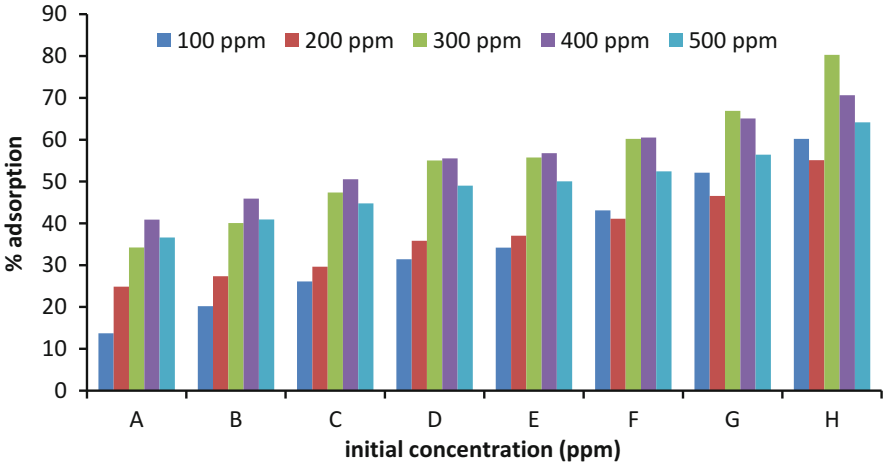

Fig. 6.12 Lead adsorption capacity in various adsorbents

6.3.3.3 Adsorption Capacity of Pb Ion with Petai Hull and Corncob for Lead Removal

Figure 6.13 shows the adsorption capacity of B (1 corncob:3 petai hull), C (1 corncob:1 petai hull), D (3 corncob:1 petai hull), and H (1 modified corncob:1 modified petai hull). It shows that the maximum capacity of Pb(II) metal ions adsorbed from the biosorbents B, C, and D, respectively, with capacity of 0.8525 mg/g; 1.0813 mg/g; 1.4175 mg/g with 30 min stirring time. From the result of maximum capacity of each biosorbent without modification it can be seen that D biosorbent adsorbed metal Pb(II) better than biosorbents B and C. In the biosorbent of corncobs and petai hull modified with nitric acid, the maximum adsorption metal ion Pb(II) capacity was 2230 mg/g at concentration of 400 ppm. The order of biosorbents to their ability in adsorbing metal ions Pb(II) from the result of its maximum capacity is H > D > C > B (Putri et al. 2018).

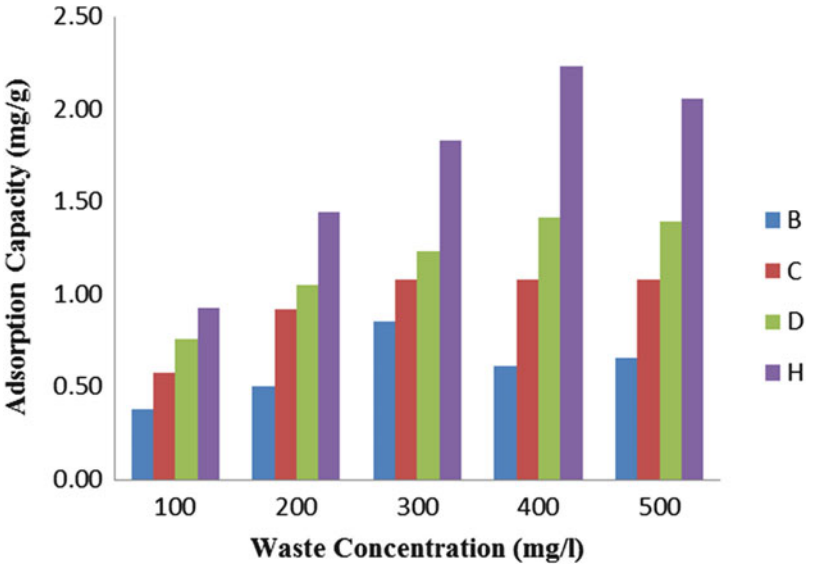

Fig. 6.13 Adsorption capacity of B (1 corncob:3 petai hull), C (1 corncob:1 petai hull), D (3 corncob:1 petai hull), and H (1 modified corncob:1 modified petai hull) (Putri et al. 2018)

6.3.4 Isotherm Adsorption

6.3.4.1 Lead Isotherm Adsorption into Corncob and Petai Hull Adsorbents

Determination of the lead adsorption isotherms on petai hull and corncob can be seen from the R^2 value. Table 6.4 shows that the Dubinin model showed very good correlation with experimental data, indicated by the high coefficient of determination.

6.3.4.2 Magnesium and Calcium Isotherm Adsorption of Elephant Foot Yam Skin

Table 6.5 shows that the calcium adsorption onto KB (i.e., native elephant foot yam skin) and KM (i.e., modified elephant foot yam skin) are suitable with Dubinin isotherm, whereas Table 6.6 shows the details of magnesium adsorption isotherm parameters onto KB and KM adsorbents that are also suitable with Dubinin isotherm.

Table 6.4 Pb isotherm adsorption parameter on petai hull and corncob adsorbent (Lestari et al. 2018b)

	Langmuir			Freundlich		
	K_L	q_o	R^2	K_F	n	R^2
B	0.104	0.494	0.2141	0.782	−13.908	0.1639
C	0.053	1.086	0.4888	0.437	6.588	0.4197
D	0.100	1.523	0.4540	0.608	5.956	0.4595
H	0.020	2.422	0.6915	0.446	3.720	0.3854
	Temkin			Dubinin–Radushkevich		
	A_t	B	R^2	K_{ad}	q_s	R^2
B	0.000	−0.036	0.1536	8.455	313.156	0.9523
C	4.790	0.145	0.3849	5.648	268.513	0.9298
D	3.330	0.234	0.4158	1.547	210.882	0.9553
H	0.754	0.382	0.3218	3.223	194.144	0.8434

Table 6.5 Calcium isotherm parameters KB (native elephant foot yam skin) and KM (modified elephant foot yam skin) (Lestari et al. 2018a)

	Langmuir			Freundlich		
	K_L	q_o	R^2	K_F	n	R^2
KB	0.044	0.009	0.131	2.418	0.789	0.101
KM	0.207	0.249	0.601	0.946	3.448	0.968
	Temkin			Dubinin–Radushkevich		
	A_t	B	R^2	K_{ad}	q_s	R^2
KB	0.005	0.080	0.153	1.674	120.542	0.994
KM	0.001	0.105	0.676	0.902	77.246	0.988

Table 6.6 Magnesium isotherm parameters on KB (native elephant foot yam skin) and KM (modified elephant foot yam skin) (Lestari et al. 2018a)

	Langmuir			Freundlich		
	K_L	q_o	R^2	K_F	n	R^2
KB	0.006	0.117	0.843	0.040	0.820	0.667
KM	0.254	0.451	0.161	0.946	3.448	0.586
	Temkin			Dubinin–Radushkevich		
	A_t	B	R^2	K_{ad}	q_s	R^2
KB	0.055	0.102	0.647	2.512	196.960	0.962
KM	0.005	0.007	0.003	1.448	134.424	0.926

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Chapter 7
Utilization of Glycerol from Biodiesel Industry By-product into Several Higher Value Products

Zahrul Mufrodi, Erna Astuti, Arief Budiman, Supranto, Sutijan, Agus Prasetya, and Rochmadi

Abstract Since the 1980s the energy demand has been increasing steadily, including diesel fuel. On the other hand the oil reserve in the world was increasingly limited because of being the product that could not be renewed. Therefore, effort was carried out to look for the alternative fuel that could be renewed and environment friendly. The alternative energy from new renewable energy is a solution to reduce the dependence of fossil energy. The renewable energy consists of the energy of water, wind, biomass or biofuels, solar energy, ocean energy, and geothermal energy. One of the biofuels is biodiesel. Biodiesel is diesel fuel which is made from vegetable oil by transesterification. The abundance of glycerol will result in declining sales value of glycerol as a by-product of the biodiesel plant. It should be anticipated to improve the usefulness of glycerol both in terms of quantity and its variants. The increasing usefulness of glycerol will result in the higher price of glycerol that will increase the profitability of biodiesel plants. Among the usefulness of glycerol investigated is as an ingredient in pharmaceutical products, polyether, emulsifiers, fabric softener, stabilizers, preservatives in bread, ice cream, cosmetic ingredients, a propellant binder, and others. This chapter explains the utilization of glycerol to produce triacetin as bioadditive and polyglycidyl nitrate (PGN) as a propellant binder. Triacetin is used to increase octane number of fuel and improve the biodiesel's performance. Propellant binder consists of two kinds of non-energetic polymers and polymer energetic. The most energetic polymer is PGN. The focus of this chapter is to determine each step of reactions, operating conditions of process and the results of products.

Keywords Glycerol · Utilization · By-product · Triacetin · Polyglycidyl nitrate

Z. Mufrodi (✉) · E. Astuti
Department of Chemical Engineering, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
e-mail: zahrul.mufrodi@che.uad.ac.id

A. Budiman · Supranto · Sutijan · A. Prasetya · Rochmadi
Department of Chemical Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia

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7.1 Introduction

Biodiesel is an alternative energy for fossil fuels. In the process of making biodiesel, a by-product in the form of 10% glycerol will be produced from the results of the biodiesel obtained. The abundance of biodiesel products with the establishment of many biodiesel industries will result in a decrease in the value of glycerol. This needs to be anticipated by increasing the benefits of glycerol. With the increasing usage of glycerol, the glycerol selling price will increase. This has led to an increase in the benefits of the biodiesel plant. Some of the uses of glycerol that have been studied are as ingredients for pharmaceutical products, polyether, emulsifiers, softening agents, stabilizers, preservatives in bread, ice cream, cosmetic ingredients, and others (Bonet et al. 2009; Galan et al. 2009). The use of glycerol in industry and its percentage can be seen in Fig. 7.1 (Bonet et al. 2009).

Glycerol is also used for the manufacture of fragrance esters and synthetic resins, medicines, cosmetics, toothpastes and in the food industry (Kirk and Othmer 1980). The higher selling value of glycerol has an impact on increasing profits at the biodiesel plant. The biodiesel industry produces two products, namely biodiesel (methyl ester) and mixed glycerol. The mixture of both can be separated by gravity because of differences in density (biodiesel of 7.35 lbs/gal and glycerol of 10 lbs/gal). Every 45.3 kg of biodiesel produced is 4.53 kg of crude glycerol or 10% of the amount of biodiesel produced. The purity of crude glycerol is between 60 and 80%. Glycerol impurities are dew, ash, soap, etc. (Ayoub and Abdullah 2012). Purification of crude glycerol can be done until the purity reaches of 99.9% (Kiss and Ignat 2012). The physical and chemical properties of dirty glycerol, glycerol from the research, technical glycerol and glycerol 87% can be seen in Table 7.1.

Villamagna and Hall use crude glycerol, modified crude glycerol and pure glycerol, as one component of explosives (Villamagna and Hall 2008). Various processes for synthesizing glycerol into other products that are more valuable are proposed by Pagliaro and Rossi (2008). Sailah conducted a preliminary study of the glycerol polymerization in the production of polyglycerol from the by-product of the biodiesel industry (Sailah 2007). Pardi carried out optimization of the monooleate glycerol from glycerol (Pardi 2005). Haryanto et al. polymerized glycerol with adipic acid (Haryanto and Budiman 2005). The results can be used for aircraft putty, paint, and coating (Sandler 1994). Hydrogenolysis of glycerol to form propylene glycol at low pressure is carried out by catalysts of nickel, palladium, platinum, copper, and copper chromite (Dasari et al. 2005). Marris et al. carried out hydrogenolysis of glycerol with a carbon bimetal catalyst supported by PtRu and AuRu produced ethylene glycol and propylene glycol (Marris et al. 2007). Hydrogenolysis of glycerol with Pt/WO3/ZrO2 catalyst produces 1,3 propanadiol (Kurosaka et al. 2008). Esterification of glycerol with acetic acid will produce triacetin which is useful as a bioadditive for fuel oil (Galan et al. 2009; Melero

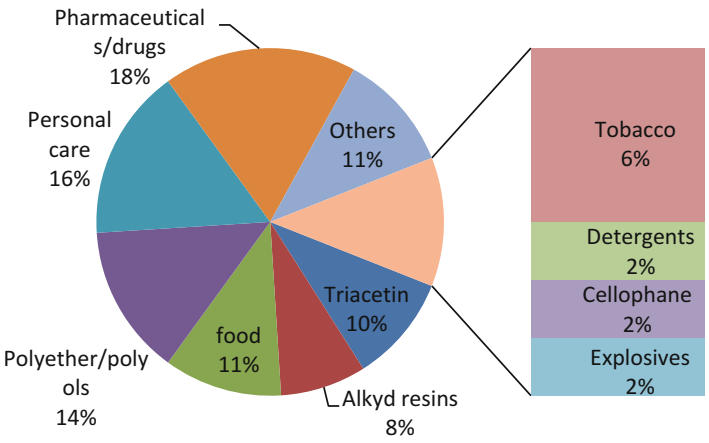


Fig. 7.1 The market for glycerol (volume and industrial use)

Table 7.1 Physical and chemical properties of glycerol

Properties	Crude glycerol	Glycerol from research	Technical grade of glycerol	Glycerol of 87%
Glycerol content, %	>30	>90	>90	87
Water content, %	0.3	1 s/d 3	n/a	>10
Soap content, %	22.55	7–13	0	0
Color	Brown	R = 2.4; Y = 5; B = 3	R = 0; Y = 0.2; B = 0	R = 0; Y = 0.2; B = 0
Free fatty acids	1.73	1.4	0.9	0.9
pH	10	7	5	5
Density, g/cm^3	n/a	1.2	n/a	1.15

et al. 2007). Ayoub and Abdullah provide information on the comparison of the physical properties of crude glycerol from the biodiesel industry, pure glycerol and commercial glycerol as shown in Table 7.2 (Ayoub and Abdullah 2012).

Table 7.2 shows crude glycerol obtained from the biodiesel industry containing impurities such as dew, ash, soap, and chloride. Soap is produced from the reaction of free fatty acid and base. Glycerol as by-product from the biodiesel industry needs to be purified before being used as raw material for other industries.

Table 7.2 Parameters of glycerol

Parameter	Crude glycerol	Pure glycerol	Commercial glycerol
Glycerol content (%)	60–80	99.1–99.8	99.2–99.98
Moisture content (%)	1.5–6.5	0.11–0.8	0.14–0.29
Ash (%)	1.5–2.5	0.054	<0.002
Soap (%)	3.0–5.0	0.56	n/a
Acidity (pH)	0.7–1.3	0.10–0.16	0.04–0.07
Chloride (ppm)	Nd	1.0	0.6–9.5
Color	Dark	34–45	1.8–10.3

Table 7.3 Cold properties improvement when mixing triacetin with biodiesel

Parameter	Biodiesel	Biodiesel + triacetin 1%	Biodiesel + triacetin 5%	Biodiesel + triacetin 10%
Density (g cm^{-3})	0.859	0.862	0.869	0.881
Melting point of the mixture (°C)	−7	−16	−16	−17
Viscosity at −10 °C	–	–	931.2	453.9

7.2 Triacetin

7.2.1 Physical Properties

Triacetin is also known as 1,2,3 triacetypropene, glycerin triacetate or triacetyl glycerol (TAG). Triacetin has physical properties: molecular weight of 218.21 g/mol, specific gravity of 1.2 (water = 1), boiling point of 259 °C, melting point of 4.1 °C, flash point at close cup of 149 °C and at open cup of 153 °C. Delgado stated that triacetin can be used to improve the performance of biodiesel, by mixing 10% of triacetin into biodiesel, resulting in an increase in the density of biodiesel, decreasing freezing from −7 to −17 °C and decreasing viscosity to more than 50% (Table 7.3) (Delgado 2002).

In addition to being used to improve the performance of biodiesel (as seen in Table 7.3), triacetin can also be used as a bioadditive to increase the octane number in gasoline and reduce the cetane number in diesel oil, as shown in Table 7.4.

The cetane number for a 10% triacetin mixture can reach 45. In Table 7.4 it can be seen that triacetin can be used to increase the octane number in gasoline and reduce the cetane number in diesel oil. The 10% of triacetin can also be used as a mixture of biodiesel to improve engine performance in all aspects (Rao and Rao 2011). In addition, triacetin can also be used as a pharmaceutical and cosmetic material (Reddy et al. 2010).

Table 7.4 Cold properties improvement when mixing triacetin with biodiesel (Delgado 2002)

Motor Octane Number (MON)		Research Octane Number (RON)		Cetane Number (CN)	
Gasoline	Mix of 10% of triacetin	Gasoline	Mix of 10% of triacetin	Diesel	Mix of 10% of triacetin
85.3	88.0	97.0	98.3	50.5	45.0

7.2.2 Raw Material

Production of triacetin can be done in various processes. From studies that have been done, production of triacetin can be done using glycerol and acetic anhydrous or using glycerol and acetone. Then it was also done using glycerol, acetic acid, and anhydrous acetate. Most triacetin production uses glycerol and acetic acid. At first, triacetin was made from glycerol and acetic anhydride. The reaction was carried out at temperatures of 120–135 °C in a batch reactor without using a catalyst within 7 h (Trevoy and Tegg 1963). The other research with the same material was also carried out by Silva et al. (2010). The study used various solid catalysts such as H-Beta, K-10, Amberlist-15, and Phosphate Niobium. Subsequently triacetin was synthesized by using glycerol and acetic acid with or without catalyst.

7.2.3 Type of Catalyst

Research into the production of triacetin by batch processes have been carried out, especially by using solid catalysts. Yang and Lu made triacetin from glycerol and acetic acid using a catalyst and got the best results at 450 °C (Yang and Lu 1996). Zhang and Yuan tried to make triacetin with phosphotungstic acid catalyst with a catalyst and reactant ratio of 3.8%, a temperature of 135–155 °C and a reaction time of 7 h to get a triacetin yield of 84.6% (Zang and Yuan 2001). Meanwhile using the aminosulfonic acid catalyst, Hou et al. got yield of as much as 90% (Hou et al. 1998). Another study conducted by Liu et al. using p-toluensulfonic acid obtained triacetin with yield of 92% (Liu et al. 2007).

The effect of the use of various solid catalysts for the production of triacetin was carried out by Gonsalves et al. The catalysts used were Amberlist-15, K10, Niobic acid, HZMS-5, and HUSY. The highest glycerol conversion of 97% was obtained when the catalyst used was Amberlist-15. From the various types of catalysts that were tried, the best result was achieved when using Amberlist-15 and K10 with triacetin selectivity of only 13% and 5% (Gonsalves et al. 2008).

Experiments using different catalysts have also been carried out. The use of the tungstophosphoric acid (TPA) catalysts has been studied by Balaraju et al. In this study it was stated that glycerol conversion and selectivity depend on the acidity of the catalyst, which was related to the amount of niobic acid from the landfill used. In

Table 7.5 Homogeneous catalytic activity using different conventional acids (Mufrodi et al. 2018a)

No.	Catalyst	Glycerol conversion, %
1	H_3PO_4	57
2	HCl	49
3	HNO_3	61
4	H_2SO_4	96
5	Without catalyst	22

this study the optimum conditions at the reaction time were 4 h, the reaction temperature was 120 °C, the ratio of acetic acid:glycerol of 1:5 and the catalyst weight of 25% TPA/Nb_2O_5 of 200 mg (Balaraju et al. 2010).

The use of acetic acid with dodecamolybdophosphoric acid (PW) supported on activated carbon as catalyst for the glycerol esterification reaction has been investigated by Ferreira et al. (Mufrodi et al. 2012). By using PW2_AC catalyst after the reaction has been running for 3 h, the largest conversion of glycerol was 86% with selectivity of monoacetin of 25%, diacetin of 63%, and triacetin of 11%.

Research using homogeneous catalyst using H_3PO_4, HCl, HNO_3, and H_2SO_4 (Mufrodi et al. 2012, 2018a, b) catalysts has been carried out by Khayoon and Hameed (2011). The study was carried out under the temperature of 120 °C, molar ratio of glycerol/acetic acid of 1:8, and reaction time of 5 h. The conversion of glycerol obtained is shown in Table 7.5.

In Table 7.5, it can be seen that compared to other homogeneous catalysts, the sulfuric acid catalyst is a catalyst that can provide the greatest conversion, which is four times compared to the reaction without using a catalyst. Glycerol conversion obtained was 96%. Sulfuric acid catalyst was also used in a batch reactor equipped with stirrer and reverse cooler with optimum results achieved at the mole ratio of acetic/glycerol of 7:1, temperature of 120 °C, and reaction time of 5 min with conversion of 67.6323% (Windriyanto and Satriadi 2012).

7.2.4 Operating Conditions

The operating conditions in a reaction greatly affect the expected results. The operating conditions include reaction time, reaction temperature, comparison of glycerol and acetic acid reactants used, mechanism or method, type of catalyst used and others. The results of the study described the effect of reaction time on conversion and percent of product distribution both monoacetin, diacetin, and triacetin can be seen in Table 7.6.

Table 7.6 illustrates that the use of different catalysts have different effects on the results of monoacetin, diacetin, and triacetin. The longest reaction time (3 h) results in a greater percentage of product distribution than reaction time of 1 h. There are several uses of catalysts that produce 100% conversion namely p-TSA, SAS, and SSBA catalysts for reaction times of 1 and 3 h, use of Amberlist-15 catalyst for 3 h reaction time. The increase in the average yield of diacetin and triacetin as a result of

Table 7.6 Glycerol conversion and product distribution over different solid acid catalysts (Testa et al. 2013)

	Conversion, %		Distribution product, %					
			Monoacetin		Diacetin		Triacetin	
Catalyst	1 h	3 h	1 h	3 h	1 h	3 h	1 h	3 h
Without catalyst	36	85	89	73	11	25	0	2
p-TSA	100	100	10	8	57	55	33	37
Amb-15	97	100	35	31	54	57	11	12
Nb_2O_5 nH_2O	51	82	91	70	9	29	0	1
Ti10HMS	48	90	88	65	12	32	0	3
SZ-470	47	91	83	55	17	40	0	5
Dry-SZ	63	93	90	66	10	31	0	3
SAS	100	100	10	0	73	51	17	49
SSBA	100	100	15	5	74	62	11	33

adding reaction time from 1 h to 3 h is 20.5% and 101.4%. The highest increase in triacetin due to changes in time from 1 h to 3 h occurred in the use of SSBA catalysts, which reached up to 200%. The best catalyst used to produce triacetin with the highest percentage is 33% and 37% for reactions for 1 and 3 h is p-TSA.

Reddy et al. tried to examine the effect of temperature changes on glycerol conversion and selectivity of monoacetin, diacetin, and triacetin using zirconia-based solid acid catalysts, namely ZrO_2 or Z, TiO_2-ZrO_2 or TZ, WO_x/TiO_2-ZrO_2 or WTZ, and MoO_x/TiO_2-ZrO_2 or MTZ. The results of the research can be seen in Table 7.7 (Reddy et al. 2010).

Table 7.7 explains that glycerol conversion is higher if the reaction temperature is increased from 40 to 120 °C. The reaction temperature also affects the increase in selectivity of diacetin and triacetin. Experiments with a reaction temperature of 40 °C only produce monoacetin. When the reaction temperature is raised to 80 °C, it is formed to be diacetin, while triacetin products start to form when the reaction temperature was maintained at 120 °C. The highest selectivity of triacetin was reached at 120 °C with MTZ catalyst which was 7.52%.

The influence of the mol ratio of the reactants was also studied by Melero et al. The experiment was carried out using Pr-SBA-15 catalyst with glycerol/catalyst weight ratio of 25, the ratio of acetic acid and glycerol reactants varied from 3:1 to 9:1, reaction temperature of 150 °C, and reaction time of 4 h. The results can be seen in Table 7.8 (Melero et al. 2007).

Table 7.8 explains that the addition of acetic acid to the ratio of acetic acid: glycerol at the same temperature results in an increase in the glycerol conversion. It also affects the increase in selectivity of diacetin and triacetin, while the selectivity of monoacetin decreases. This is because some monoacetin changes to diacetin.

Different conditions and methods for esterification of glycerol with acetic acid have also been studied by Luque et al. (2008). The best triacetin results were achieved under 10 mmol glycerol, 30 mmol acetic acid, 0.2 g catalyst, which was reacted in a microwave 300 w, temperature of 130 °C and reaction time of 30 min

Table 7.7 Effect of temperature on acetylation of glycerol over Z, TZ, WTZ, and MTZ catalysts

Catalyst	Temperature (°C)	Conversion glycerol (%)	Selectivity		
			Monoacetin	Diacetin	Triacetin
Z	40	4.67	100	–	–
	80	30.96	95.44	4.52	–
	120	86.32	57.94	36.67	5.39
TZ	40	5.34	100	–	–
	80	38.72	92.27	7.73	–
	120	91.53	54.72	39.40	5.88
WTZ	40	12.61	100	–	–
	80	42.68	91.99	8.01	–
	120	99.02	53.21	40.01	6.78
MTZ	40	18.57	100	–	–
	80	50.72	88.87	11.13	–
	120	~100	52.03	40.45	7.52

Reaction conditions: molar ratio of acetic acid to glycerol = 6:1; catalyst amount = 5 wt.% (with respect to glycerol); reaction time = 3 h

Table 7.8 Effect of molar ratio of acetic acid and glycerol on modified mesostructured catalyst, Pr-SBA-15

No.	Acetic acid/ glycerol	Temperature, °C	Conversion glycerol, %	Selectivity, %		
				Monoacetin	Diacetin	Triacetin
1	3:1	100	63	45	42	13
2	3:1	125	57	58	33	9
3	3:1	150	47	49	40	11
4	6:1	100	78	37	41	22
5	6:1	125	75	26	50	24
6	6:1	125	78	28	47	25
7	6:1	125	74	31	44	25
8	6:1	150	72	22	48	30
9	9:1	100	78	20	52	28
10	9:1	125	78	17	44	39
11	9:1	150	69	7	50	43

with a starbon-400-SO3H catalyst. The conversion of glycerol is more than 99% with a selectivity of triacetin of 77%. This experiment also tried the use of different catalysts with less products compared to the use with starbon-400-SO3H as shown in Table 7.9 (Luque et al. 2008).

Each catalyst has different effects on glycerol conversion and yield selectivity. The best catalyst to get high glycerol conversion and selectivity of triacetin is Starbon-400-SO3H which is more than 99% and 77%. The method to synthesize triacetin can also be done in two steps to get high selectivity and conversion (Liao et al. 2009). The esterification of glycerol with acetic acid was carried out with

Table 7.9 Catalytic activity of different solid acids tested in the esterification of glycerol with acetic acid (10 mmol glycerol, 30 mmol acetic acid, microwave, 300 W, 130 °C, 30 min, 0.2 g catalyst)

Catalyst	Conversion, mol %	Selectivity, mol %		
		Monoacetin	Diacetin	Triacetin
No catalyst	<10	50	40	10
H_2SO_4	85	43	32	25
Sulfated zirconia	>90	15	37	48
Starbon-400-SO_3H	>99	8	15	77

various catalysts. In this experiment Amberlyst-35 was the best catalyst. The experiment was carried out under 105 °C, molar ratio of acetic acid/glycerol of 9:1, with a catalyst of 0.5 g. After reacting for 4 h at optimum conditions, 0.1 mol acetate anhydrous was added and after 15 min of reaction the triacetin selectivity was 100%.

7.2.5 Continuous Process

Production of triacetin in addition to a batch process has also been carried out with a continuous process. The process is carried out by mixing glycerol with acetic acid in the esterification reactor. The output from the esterification reactor was put into the acetylation reactor to be added with anhydrous acetate. Then, the output from the acetylation reactor goes into rectification to be separated into the main result of triacetine, anhydrous acetate which is returned to the acetylation reactor and acetic acid which is returned to the esterification reactor. The complete process can be seen in Fig. 7.2 (Bremus et al. 1983).

Figure 7.2 illustrates that the production of triacetin was continuously carried out using glycerol, acetic acid, and anhydrous acetate. The first reaction was carried out by the opposite direction process using an esterification column consisting of several plates. The reaction occurred between liquid glycerol and superheated acetic acid with a pressure of 0.2–30 bar and temperatures of 100–250 °C. The reaction time was 1 h. In this process, the use of several catalysts, namely p-toluene sulfonic acid, concentrated sulfuric acid, triflouroacetic acid, potassium hydrogen sulfate, aluminum sulfate or zinc acetate, was done. In this esterification column monoacetin and diacetin were produced as a result with the remaining acetic acid and a mixture of water and acetic acid as a result of the column. Then the bottom result of the esterification column was reacted with anhydrous acetate in the acetylation reactor to convert monoacetin and diacetin to triacetin.

Continuous processes made triacetin which was run on a column with a diameter of 1.5 cm and a length of 44 cm with Amberlyst-15 as filing material. Amberlyst-15, as catalyst, has porosity of 0.36, weight of 36.4 g and was used with a ratio of glycerol/acetic acid of 2:9. Process was carried out at a flow rate of 0.3 cm^3/min. In this research, acetic acid conversion was 50% (Gelosa et al. 2003). Continuous

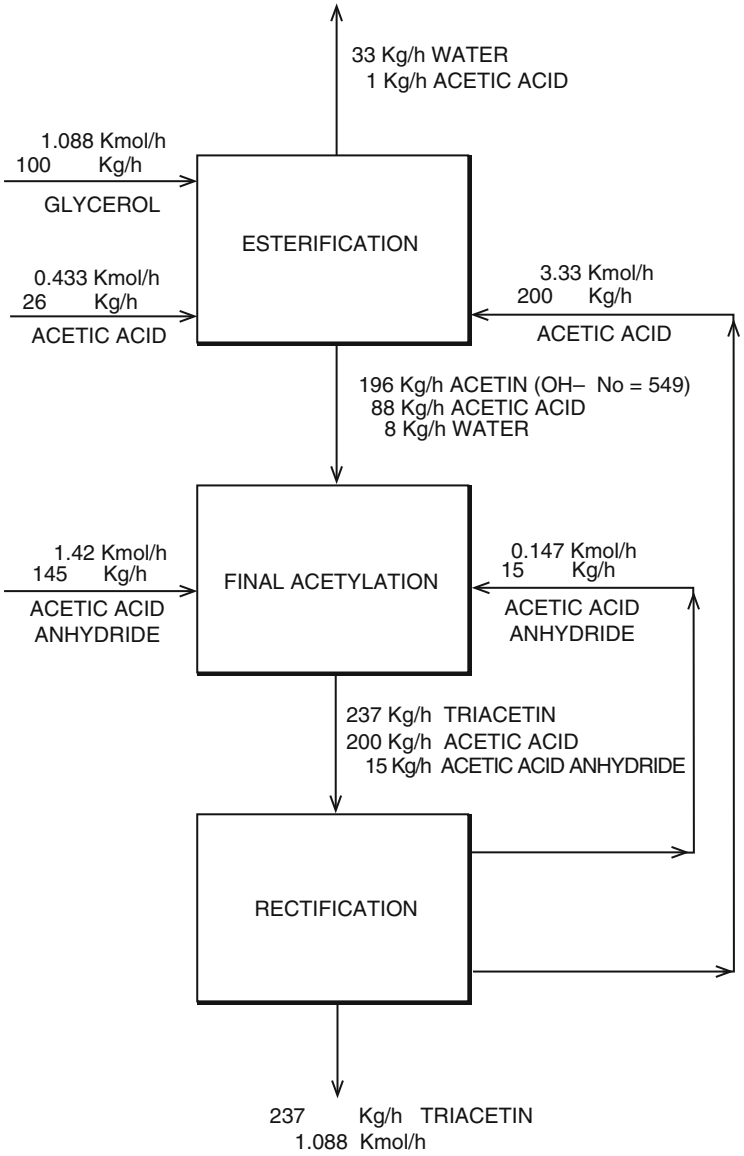


Fig. 7.2 Flow diagram process of acetylation of triacetin from glycerol, acetic acid, and acetic anhydride (Gelosa et al. 2003)

production of triacetin has also been investigated using a fixed bed reactor at 50 °C. The experiment was carried out at the ratio of acetic/glycerol acid of 1:1, 2:1, and 1:3. The catalyst used was Amberlist-16, the best ratio to get triacetin was 3:1. The equipment of this research can be seen in Fig. 7.3 (Fukumura et al. 2009).

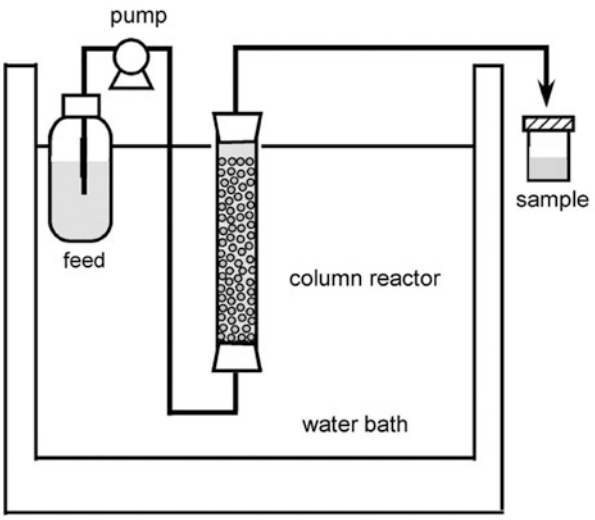


Fig. 7.3 Schematic diagram of the fixed bed reactor

The results obtained from the study with variations in the ratio of the concentration of acetic acid/glycerol and the amount of catalyst used in fixed bed reactor can be seen in Table 7.10 (Fukumura et al. 2009).

Table 7.10 shows that the addition of the amount of catalyst used twice as much as 75–150 g/dm^3 at the ratio of acetic acid and glycerol 1:1 resulted in a decrease in the concentration of monoacetin, diacetin, and triacetin. Reactive distillation can be used as a place of reaction and purification products in one place. The column was insulated to reduce heat loss and equipped with total condenser and partial reboiler. Figure 7.4 shows the schematic of a reactive distillation for triacetin production. Acetic acid (gas) and glycerol (liquid) feed to reactive distillation from different tray. The column is operated at atmospheric pressure, the temperature of acetic acid feed streams is 118 °C and glycerol feed streams is 100 °C.

Reactive distillation can separate water mixed with a little acetic acid to the reaction of distillate product around 75% of the main product of bottom results. The production using continuous reactive distillation resulted in glycerol conversion of 98.51% (Mufrodi et al. 2013, 2014).

7.3 Polyglycidyl Nitrate

7.3.1 The Process to PGN from Glycerol

The formation reaction of polyglycidyl nitrate (PGN) compounds from glycerol is a series reaction with three reaction steps. The first step is nitration of glycerol with nitric acid to produce 1,3-DNG. The second step is cyclization 1,3-DNG to glycidyl

Table 7.10 Equilibrium conversions and concentrations for various initial concentrations

Acetic acid mol/dm^3	Glycerol mol/dm^3	Catalyst g/dm^3	Equilibrium concentrations (mol/dm^3)					
			Acetic acid	Glycerol	Monoacetin	Diacetin	Triace tin	Water
7.67	7.67	75	2.04	2.04	3.49	1.01	0.036	5.44
4.92	9.83	75	0.72	0.72	3.26	0.42	0.007	4.01
12.3	4.09	75	6.25	6.25	1.81	1.65	0.183	5.46
7.67	7.67	150	1.85	1.85	3.45	0.98	0.034	5.18

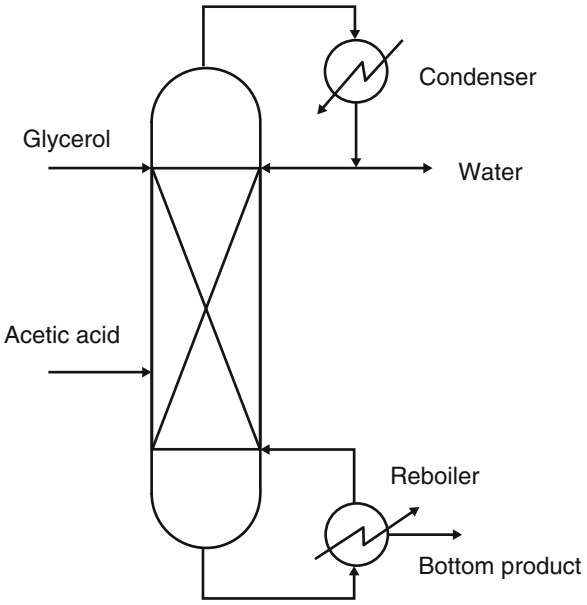


Fig. 7.4 Continuous reactive distillation column

nitrate. Cyclization is carried out in inorganic hydroxides such as sodium hydroxide, potassium hydroxide, and lithium hydroxide (Sanderson and Martins 2004). The third step is the polymerization of glycidyl nitrate to form PGN. This reaction one of the types of cation polymerization with the polyol initiator and acid catalyst (Highsmith et al. 2002). Glycidyl nitrate from the cyclization reaction undergoes a purification process in the form of extraction and filtration before being used as a monomer in a polymerization reaction. The production of PGN from glycerol can be carried out in batch processes (Sanderson and Martins 2004; Highsmith et al. 2002) and continuous processes (Highsmith and Johnston 2005).

7.3.1.1 Nitration

Nitration is defined as the reaction between a titrating agent and an organic compound, which includes one or more nitro groups (-NO_2). Nitration of glycerol with nitric acid produced five types of products: 1-MNG, 2-MNG, 1,3-DNG, 1,2-DNG, and TNG (Highsmith et al. 2002) (Fig. 7.5).

Research related to nitration of glycerol has been carried out by previous studies. The researchers carried out nitration of glycerol in batch process (Sanderson and Martins 2004; Highsmith et al. 2002). Highsmith and Johnston (2005) found a continuous process to produce glycidyl nitrate from glycerol with 1,3-DNG as an intermediate product. Nitration of glycerol with nitric acid is a parallel series reaction as shown in Table 7.11.

Table 7.11 shows that the reactions (1), (2), (6), and (7) have a negative reaction heat value. The reaction is an exothermic reaction. The reactions (3), (4), and (5) have positive reaction heat. Thus these reactions are endothermic reactions.

Glycerol nitration can be done with various titrating agents: nitric acid of 90%, mixed acids (nitric and sulfuric acid), acetyl nitrate, nitronium ion salts such as NO_2BF_4, NO_2ClO_4, and N_2O_5; TFAA with ammonium nitrate, nitric acid and/or Crivello reagent. The mol ratio between titrating agents and glycerol ranges from 4/1 to 5/1. Nitric acid concentration is 90%. The reaction takes place from 0 to 25 °C, usually chosen at temperatures of 10–20 °C. The reaction time is at least for a minimum of 4 h, usually for 6 h and above, and produces at least 50 mol of dinitroglycerin (Sanderson and Martins 2004; Highsmith et al. 2001, 2002). Batch nitration conversion usually ranges from 50% to 60%, while the minimum yield of

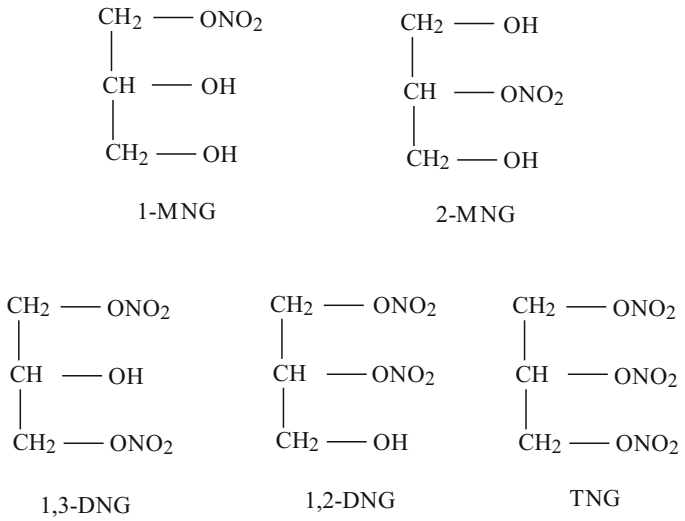


Fig. 7.5 Nitration results from glycerol and nitric acid

Table 7.11 Nitration reactions

Nomor reaksi	Reaksi (Mufrodi et al. 2013)	ΔH_R, kJ/mol (Sanderson and Martins 2004)
1	G + HNO_3 ⇄ 1-MNG + H_2O	−17.2 ± 1.3
2	G + HNO_3 ⇄ 2-MNG + H_2O	−8.8 ± 0.8
3	1-MNG + HNO_3 ⇄ 1,3-DNG + H_2O	15.9 ± 1.3
4	1-MNG + HNO_3 ⇄ 1,2-DNG + H_2O	20.1 ± 1.3
5	2-MNG + HNO_3 ⇄ 1,2-DNG + H_2O	11.7 ± 0.8
6	1,3-DNG + HNO_3 ⇄ TNG + H_2O	−6.3 ± 0.8
7	1,2-DNG + HNO_3 ⇄ TNG + H_2O	−10.5 ± 1.3

Table 7.12 Results of DNG (1,3-DNG and 1,2-DNG) in the first variation

No.	Reaction time, h	Product DNG, % mol from mix
1	1	36.3
2	2	39.0
4	4	44.5
5	5	71.5
6	6	73.8

continuous nitration was 50% and the highest was 90%. The concentration of TNG is expected to be less than 10% mol (Sanderson and Martins 2004; Highsmith et al. 2002; Sanderson et al. 2005).

Nitric acid is put into the reactor together with organic solvents such as dichloromethane (methylene chloride) or dichloro ethane. The function of the solvent is for process safety. Nitrogen or air is flowed into the reactor to help react and remove unwanted gases such as NOx, which may be formed from side reactions. From the reaction that was carried out, a mixture contain 1,3-DNG, water, and nitric acid (Highsmith and Johnston 2005). Batch nitration can be carried out in several ways. Highsmith et al. (2002) proposed seven variations of nitration reactions. The first, second, and seventh variations were studied again by Sanderson and Martins (2004). These three variations were stated again by Sanderson et al. (Sanderson et al. 2005). The nitration reaction is usually followed by a cyclization reaction.

The first variation was carried out with a mole ratio of nitric/glycerol of 4.1. The reaction is carried out at temperatures below 25 °C. The reaction is run on a triple neck flask equipped with a magnetic stirrer, thermometer, and nitrogen sparge. The three neck squash is placed in the cooler. Glycerol and methylene chloride are put in the same volume flask. Ninety percent nitric acid was added while the temperature was kept below 25 °C. The mixture is left for 4–18 h with vigorous nitrogen sparge. The results of 1,3-DNG and 1,2-DNG are listed in Table 7.12 (Highsmith et al. 2002).

Table 7.12 shows that the reaction time affects the 1,3-DNG and 1,2-DNG isomers produced. The longer the reaction time, the more isomers obtained. The second variation takes place with a mole ratio of nitric/glycerol of 4.1, volume ratio of glycerol/methylene chloride of 1.1, and reaction temperature equal to room temperature. Glycerol and methylene chloride are put into the same device with variation. A flask is placed in a coolant and the temperature is kept at 5 °C. Nitric acid with concentration of 90% as much as 150 mL was dripped for more than 30–45 min and then stirred at room temperature for 5–6 h. The seventh variation is carried out with mole ratio of nitric acid/glycerol of 4, volume ratio of glycerol/methylene chloride of 0.7, and a reaction temperature of −4 °C. A mixture of glycerol and methylene chloride is stirred at 0 °C with air being bubbled in the flask. Nitric acid drops for more than 2.5 h. After addition of nitric acid, the reactant mixture was cooled to −4 °C and left for 16 h.

The study which states the relationship between temperature and equilibrium constant in all reactions was determined by Kazakov et al. (1990b). The equilibrium constant is carried out at various reaction temperatures and nitric acid concentrations

and calculated based on activity. The equilibrium constant value is calculated based on experimental data and expressed as K_1 to K_7. Similarly the heat value of the reaction, Gibbs energy, enthalpy, and entropy are known (Highsmith et al. 2001). Rubstov and Kazakov (1997) state that the nitration equilibrium constant value of the hydroxyl group located on secondary carbon atoms is 3–10 times lower than the hydroxyl group equilibrium constant values located on primary carbon atoms in single-type compounds. Danov et al. stated that temperature increases have an impact on the increase in the reaction rate constant of the benzene nitration (Danov et al. 2010).

Fogler states that for some reactions which are series, parallel, or a combination of both, the reaction kinetics follows the reaction stoichiometric coefficient (Fogler 2006). Nitration of several compounds is modeled with one order against each reactant. Nitration of benzene and some reactive compounds in sulfuric acid (Coombes et al. 1968), nitration of benzene, chlorobenzene, toluene and di- and tri-methylbenzene in trifluoroacetic acid (Moodie et al. 1977) and nitration of the same reactants in perchloric acid (Moodie et al. 1978) is a one-order reaction to each reactant. The parameters of the nitroglycerine kinetic process of the Biazzi in a continuous stirred tank reactor were stated by Lu et al. (2008). The speed of the reaction of trinitroglycerin formation from glycerol and nitric acid in the Biazzi process can be expressed by the following equation:

$$-r_G = kC^n{}_G C^m{}_N = Ae^{-E/RT} C^n{}_G C^m{}_N \tag{7.1}$$

The values of n and m are 0.9350 and 1.117 (Lu et al. 2008).

The collision frequency factor (also known as pre-exponential factor, A_p) states the number of collisions between reactants and the possible interaction of the reactants to make a reaction. Activation energy (E_A) is the energy that blocks the occurrence of a reaction. Activation energy values are usually in order of 50–100 kJ/mol (Swaddle 1997). The activation energy of hydrogen iodide decomposition is 186 kJ/mol and ethylene decomposition is 254 kJ/mol (Missen et al. 1999). The value of the frequency factor and activation energy of nitration of some aromatic compounds is presented in Table 7.13.

The activation energy of the decomposition reactions of TNG, 1,3-DNG, and 1,2-DNG are listed in Table 7.14.

The mechanism of glycerol nitration is unknown. The mechanism and kinetics of the nitration can be analogous to the mechanism and kinetics of methanol nitration proposed by Kazakov et al. (1987a, b). Nitric acid balance is:

$$2HNO_3 \rightleftarrows NO_2{}^+ + NO_3{}^- + H_2O \tag{7.2}$$

$$NO_2{}^+ + NO_3{}^- \rightleftarrows N_2O_5 \tag{7.3}$$

$$HNO_3 + H_2O \rightleftarrows NO_3{}^- + H_3O^+ \tag{7.4}$$

Table 7.13 Impact frequency factor and activation energy of some aromatic compounds (Zaldivar et al. 1995)

	Ln A	$E_A/R \times 10^{-3}$
A. Calculated with H_R function		
Benzene	51.456	2.023
Toluena	62.362	22.83
Chlorobenzene	59.451	23.16
B. Calculated with M_C function		
Benzene	25.074	4.033
Toluene	32.264	4.989
Chlorobenzene	28.852	6.003

Table 7.14 Activation energy of formation of TNG, 1,3-DNG, and 1,2-DNG

No.	Compound	E_A, kJ/mol
1	TNG	87.7
2	1,3 DNG	91.2
3	1,2 DNG	72.3

Kazakov et al. explained that the reaction rate constant of nitration of methanol drops when using a lower concentration of nitric acid (Kazakov et al. 1987a).

Thermodynamic feasibility should be done before running experiments in the laboratory. Astuti et al. did thermodynamic feasibility to determine the parameters that affect nitration of glycerol and nitric acid and chose the operation condition. Many parameters were simulated to verify its possibility to experiment under conditions which would get the highest conversion of 1,3-dinitroglycerine and which was the ideal condition to get it. HYSYS was used to predict the effect of many parameters to the conversion of glycerol. The parameters that need to be studied to obtain the highest conversion of 1,3-dinitroglycerine were mole ratio of nitric acid/glycerol, reaction temperature, mole ratio of glycerol/dichloromethane, and pressure. The highest conversion was obtained in the range of mole ratio of nitric acid/glycerol between 2/1 and 5/1, reaction temperature of 5–25 °C, and pressure of 1 atm. The parameters that need to be studied further to obtain the highest conversion of 1.3 DNG are mol ratio of nitric acid/glycerol and reaction temperature (Astuti et al. 2015). Determinations were done with two methods: The HYSYS predictions and the laboratory experiment for the temperature effect on glycerol nitration processes have been studied (Astuti et al. 2014a). The comparison result between prediction method and laboratory experiment method can be observed in Fig. 7.6.

HYSYS exactly predicts temperature of nitration of glycerol. The difference in conversion between two methods is due to the equipment that was used in the experiments, procedure of experiments, and the accuracy of analysis. An increase in temperature has tendency to increase the reaction rate. The restriction of reaction temperature in nitration is the decomposition of TNG that occurred at temperature above 20 °C which is dangerous for safety. The nitration of glycerol should be carried out in low temperature (10–20 °C). Nitration of glycerol to 1,3-dinitroglycerin was studied in the temperature range 10–30 °C, the molar ratio of nitric acid to glycerol

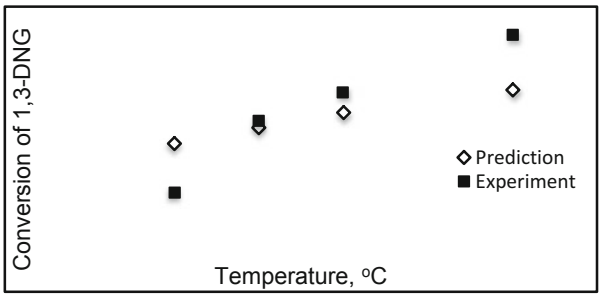


Fig. 7.6 Equilibrium conversion of 1,3-DNG at difference temperatures from the HYSYS predictions and experimental work

Table 7.15 Maximum yield of 1,3-dinitrogycerin from glycerol

Molar ratio	Temperature, °C	Maximum yield, %	Molar ratio	Temperature, °C	Maximum yield, %
1:1	10	1.64	5:1	10	1.57
	15	5.13		15	8.28
	20	11.05		20	22.26
	30	22.93		30	28.57
3:1	10	1.53	7:1	10	1.68
	15	6.15		15	13.42
	20	15.14		20	32.94
	30	25.09		30	38.70

1/1 to 7/1, and nitric acid concentration of 69% (Astuti et al. 2014b). The maximum yield of nitration is written in Table 7.15.

The seven reaction terms represent the reactions that occurred in the nitration of glycerol. The position of hydroxyl group in molecule causes difference in reaction rate. The primary hydroxyl group is more reactive than secondary hydroxyl group. The parameter values describe that the first, third, fifth, and seventh reaction rates are very fast. On the contrary, the second, fourth, and sixth reaction rates are slow. A kinetic model of nitration between glycerol and nitric acid was developed based on reaction that was proposed by Astuti et al. (2014c). The seven reaction terms represent the reactions that occurred in the nitration of glycerol (Table 7.16).

A simpler kinetic model of nitration between glycerol and nitric acid was proposed (Astuti et al. 2014c). The presented model describes that three controlling reactions model used elementary reactions consisting of three reversible reactions. The model utilizes first-order reaction according to each reactant. The nitration of glycerol was modeled by fitting the kinetic model with six parameters, the rate constant at an average temperature, and the activation energy. The reaction rate is assumed to be governed by three reactions, i.e., the formation of MNG (mononitroglycerin), the formation of DNG (dinitroglycerin), and the formation of TNG (nitroglycerin). This chapter compares two models: seven controlling reactions model and three controlling reactions model. Two models have the similar trend. The three controlling reactions model gives better fit than seven controlling reactions model (Table 7.17).

Table 7.16 Kinetic parameters for nitration of glycerol

	K_{ref}, m^3/mol,s $\times 10^{10}$				
No.	1/1	3/1	5/1	7/1	All
1	$1.03 \times 10^6 \pm 6.5 \times 10^7$	5.77 ± 0.34	15.24 ± 0.64	235.38 ± 19.03	0.5 ± 0.15
2	0.02 ± 0.20	0.05 ± 0.10	1.59 ± 0.30	4.17 ± 1.32	0.49 ± 0.14
3	$8.67 \times 10^4 \pm 3.13 \times 10^4$	80.18 ± 3.22	117.22 ± 5.72	89.97 ± 1.21	97.79 ± 7.82
4	3805.42 ± 8375.65	2.33 ± 3.65	4.90 ± 6.89	34.60 ± 8.38	26.17 ± 10.42
5	3.72 ± 156.23	59.91 ± 110.02	$1.08 \times 10^4 \pm 1.5 \times 10^4$	$2.4 \times 10^5 \pm 1.33 \times 10^5$	1319.12 ± 1727.00
6	56.82 ± 13.45	0.94 ± 0.72	2.28 ± 0.39	1.58 ± 0.06	30.77 ± 3.11
7	857.40 ± 282.03	2.44 ± 7.34	103.54 ± 33.07	716.19 ± 69.27	194.64 ± 34.00
	Activation energy, kJ/mol				
1	38.10 ± 3692.52	38.10 ± 4.21	38.15 ± 2.56	38.12 ± 5.52	38.11 ± 13.89
2	117.23 ± 1441.48	117.23 ± 105.65	117.23 ± 11.62	117.23 ± 2.57	117.23 ± 12.44
3	58.62 ± 332.69	58.62 ± 3.40	58.69 ± 3.97	58.64 ± 1.20	58.62 ± 6.68
4	31.40 ± 129.65	31.40 ± 67.81	31.40 ± 79.80	31.38 ± 12.44	31.40 ± 21.67
5	167.47 ± 3061.09	167.48 ± 147.73	167.48 ± 406.37	167.46 ± 880.47	167.48 ± 95.45
6	104.67 ± 32.10	104.53 ± 50.04	104.51 ± 14.36	104.53 ± 2.97	104.53 ± 10.07
7	71.18 ± 31.58	71.89 ± 192.41	71.90 ± 24.04	71.84 ± 6.61	71.89 ± 14.92

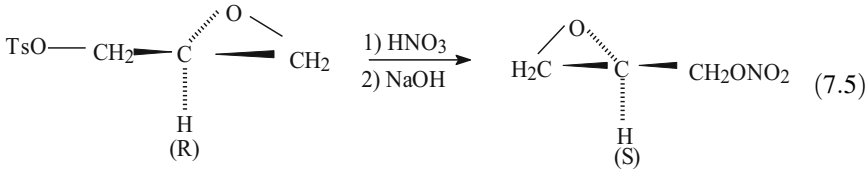
Table 7.17 Kinetic parameters for nitration of glycerol: three controlling reactions method

	K_{ref}, m^3/mol,s $\times 10^{10}$			
No.	1/1	3/1	5/1	7/1
1	$1.03 \times 10^4 \pm 6.53 \times 10^4$	5.77 ± 0.34	15.24 ± 0.64	235.38 ± 19.03
2	0.02 ± 0.20	0.05 ± 0.10	1.59 ± 0.30	4.17 ± 1.32
3	86,632.50 ± 313,434.38	80.18 ± 3.22	117.22 ± 5.72	89.97 ± 1.21
4	3805.42 ± 8375.65	2.33 ± 3.65	4.90 ± 6.89	34.60 ± 8.38
5	3.72 ± 156.23	59.91 ± 110.02	$1.08 \times 10^4 \pm 1.50 \times 10^4$	$246{,}130.55 \pm 1.33 \times 10^4$
6	56.82 ± 13.45	0.94 ± 0.72	2.28 ± 0.39	1.58 ± 0.06
7	857.40 ± 282.03	2.44 ± 7.34	103.54 ± 33.07	716.19 ± 69.27
	Activation energy, kJ/mol			
1	38.10 ± 3692.52	38.10 ± 4.21	38.15 ± 2.56	38.12 ± 5.52
2	117.23 ± 1441.48	117.23 ± 105.65	117.23 ± 11.62	117.23 ± 2.57
3	58.62 ± 332.69	58.62 ± 3.40	58.69 ± 3.97	58.64 ± 1.20
4	31.40 ± 129.65	31.40 ± 67.81	31.40 ± 79.80	31.38 ± 12.44
5	167.47 ± 3061.09	167.48 ± 147.73	167.48 ± 406.37	167.46 ± 880.47
6	104.67 ± 32.10	104.53 ± 50.04	104.51 ± 14.36	104.53 ± 2.97
7	71.18 ± 31.58	71.89 ± 192.41	71.90 ± 24.04	71.84 ± 6.61

Nitration of glycerol to 1,3-dinitroglycerin was studied in the temperature range 10–30 °C, the molar ratio of nitric acid to glycerol 1/1 to 7/1, and nitric acid concentration of 69% to get optimum operating conditions (Astuti et al. 2016a). Nitration of glycerol to 1,3-dinitroglycerin was studied in the temperature range of 10–30 °C, the molar ratio of nitric acid to glycerol 1/1 to 7/1, and nitric acid concentration of 69%. From experiments it is known that the optimum conditions of nitration of glycerol with nitric acid are a reaction temperature of 20 °C, the mole ratio of nitric acid/glycerol of 5/1, and the concentration of nitric acid of 69%.

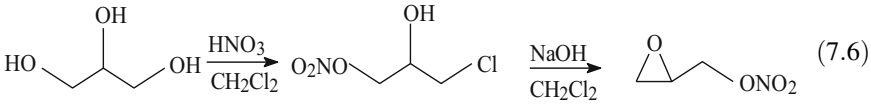
7.3.1.2 Cyclization

Glycidyl nitrate is produced from various processes. Willer et al. (1992b) make glycidyl nitrate from glycidyl tosylate. Glycidyl tosylate was reacted with 70% nitric acid solution at 10 °C, and then reacted with sodium hydroxide solution at 10 °C or less. The reaction is:



(7.5)

The production of glycidyl nitrate from hydroxyalkyl cyclic ether substitutes such as glycidol and nitrogen pentoxide (N_2O_5) was proposed by Millar et al. (1992) and Paul et al. (1992) with reactions:



(7.6)

The cyclization reviewed in this study was a continuation of the glycerol nitration. Nitration solutions consist of residual glycerol, residual nitric acid, solvents in the form of dichloro methane or dichloro ethane, and nitration products in the form of 1-MNG, 2-MNG, 1,3-DNG, 1,2-DNG, and TNG. In the cyclization reaction, the nitration solution was reacted with sodium hydroxide with concentration of 25–50%.

The cyclization of dinitroglycerin to glycidyl nitrate is carried out in the presence of inorganic hydroxides such as sodium hydroxide, potassium hydroxide, and lithium hydroxide (Sanderson and Martins 2004; Highsmith et al. 2002). The cyclization reaction can be run at a temperature of 0–25 °C, chosen at 24 °C (Highsmith and Johnston 2005). The process can be carried out in batch (Highsmith et al. 2002) or continuously (Highsmith and Johnston 2005) with various combinations of vessels and decanters. The cyclization reaction can be carried out in various ways (Sanderson and Martins 2004; Highsmith et al. 2002; Kazakov et al. 1990a). The difference lies in the reaction temperature, the mole ratio of sodium hydroxide/

glycerol, reaction time, and pH when adding sodium hydroxide. Sodium hydroxide was added in nitration solution twice. The first addition of sodium hydroxide aims to neutralize residual nitric acid. The second addition was made to react with 1,3-DNG to produce glycidyl nitrate. Additions were carried out at pH 7 or 14 with temperatures kept below 25 °C. The reaction time ranges from 0.5 to 3 h.

Seven variations of the cyclization reaction which was a continuation of the nitration were delivered by Highsmith et al. (2002). The first, second, and seventh variations were repeated by next research (Highsmith et al. 2002; Kazakov et al. 1990a). The first variation of cyclization was as follows: after the nitration was carried out, 50% sodium hydroxide was added while the temperature was kept below 25 °C. After the neutral mixture is carried out the addition of solid sodium hydroxide with the molar amount equivalent to glycerol slowly to keep the temperature below 25 °C. After stirring for 1–3 h at room temperature, the mixture was extracted three times with ether, methylene chloride or MTBE. The combined organic phase was dried with magnesium sulfate, then filtered and evaporated in a vacuum. This reaction produced about 50 mol of pure glycidyl nitrate.

The second variation of the cyclization used 30% sodium hydroxide solution and mole ratio of sodium hydroxide/glycerol of 2.7. The solution was added to the nitration solution and the temperature was kept below 25 °C. After the mixture has pH 14, 30% sodium hydroxide solution is slowly added to keep the temperature below 25 °C. Then the mixture is left for ½ h, and then extracted three times with ether. The resulting organic combined phase was dried with magnesium sulfate filtrated and evaporated under vacuum and pure glycidyl nitrate produced as much as 63% by weight of initial glycerol.

The third variation of cyclization was carried out by adding a 28% salt solution to the nitration solution, and the reaction temperature was maintained at 0 °C until the mixture was neutral. After a neutral condition was reached, stirring was stopped and the two phases were separated. The aqueous phase was separated and the colorless organic phase was analyzed by NMR. The analysis showed that there were more than 90% dinitroglycerin with a small amount of trinitroglycerin, methylene chloride, and mononitroglycerin. Then the organic phase was mixed with 65 mL of methylene chloride and 30% sodium hydroxide solution was added for more than 20 min. The mole ratio of sodium hydroxide/glycerol was 2.1. The reaction temperature was maintained at a temperature of 20–25 °C. After addition, the mixture was stirred for 10 min. Then stirring was stopped and two phases were left to separate. The organic phase was washed once with a salt (brine) solution and dried with magnesium sulfate so that 82% by weight of glycidyl nitrate was obtained from glycerol.

The 1,3-DNG cyclization variation proposed by several researchers (Sanderson and Martins 2004; Highsmith et al. 2002; Kazakov et al. 1990a) used different operating conditions so that the amount of glycidyl nitrate results was also different. Table 7.18 presents the operating conditions and the results of glycidyl nitrate from several variations carried out by Highsmith et al. (2002).

The biggest results of glycidyl nitrate were obtained from the seventh variation. But the first variation produces glycidyl nitrate of 50 mol% with the amount of molar

Table 7.18 The cyclization results of 1,3-DNG were delivered by Highsmith et al. (2002)

No.	Glycerol, g	NaOH		T, °C	t, h	Glycidyl nitrate
		%	Weight, g			
1	140	50	Molar equivalent with glycerol	<25	1–3	50 %mol
2	70	30	275	<25	0.5	44.0 g
3	70	30	107	<25	1	30.1 g
4	70	Solid[a]	30	20	1	42.0 g
5	70	Solid[a]	50	20	1	42.7 g
6	3	KOH[b]	75	20	n/a	56.5 g
7	92	30	282	20–25	1/3	75.3 g

[a]Added directly to the solution without neutralization
[b]Made from solid KOH of 31 g

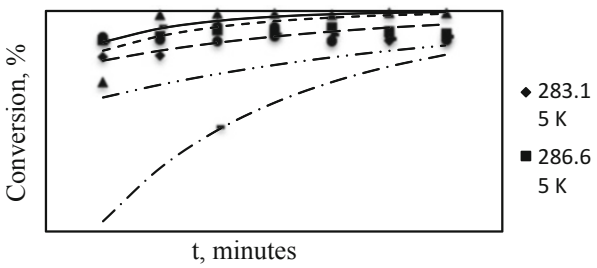


Fig. 7.7 The 1,3-DNG conversion into glycidyl nitrate at various reaction temperature

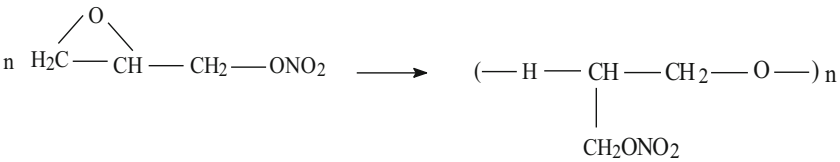
sodium hydroxide equivalent to glycerol. The use of sodium hydroxide in the first variation was far less than the seventh variation. The seventh variation was only carried out with a reaction time of 20 min. This indicated that the cyclization reaction run fast in minute orders.

The optimum conditions of cyclization were studied by Astuti et al. (2016b). The cyclization was carried out in a 5 ml reactor and equipped with Hickman distillation head and nitrogen purge with variations: the variables are mole ratio of sodium hydroxide/glycerol of 1/1 to 1.5/1, reaction temperature of 10–20 °C, and sodium hydroxide concentration of 15% (Fig. 7.7).

The data of experiments prove that optimum conditions of cyclization are temperature of 15 °C and the mole ratio of sodium hydroxide/glycerol of 1.5.

7.3.1.3 Polymerization

The glycidyl nitrate polymerization was carried out to obtain PGN. This reaction was cation polymerization with the initiator of the polyol and Lewis acid catalyst (Highsmith et al. 2002; Paraskos et al. 2004). The reaction was carried out by a solution polymerization method using organic solvents such as dichloromethane (methylene chloride) or dichloro ethane. The reaction that occurs can be seen in Eq. (7.5). The mechanism of the polymerization reaction was studied by many researchers (Desai et al. 1996; Paraskos et al. 2004; Colclough et al. 1993; Provatas 2000).



glycidyl nitrate

poly glycidyl nitrate

(7.7)

Lewis acid catalysts were BF_3 (boron trifluoride), HBF_4, and TEOP (triethyloxonium hexafluorophosphate), and BF_3Et_2O (boron tri fluoride etherate), BF_3:THF (boron trifluoride tetrahidrofuran), BF_3, PF_5 and SbF_5 (Highsmith et al. 2002; Millar et al. 1992; Astuti et al. 2016b). Lewis acid catalysts form a catalyst–initiator complex with polyols. For example, butanadiol forms a complex with BF_3. The mole ratio between Lewis acid and the hydroxyl group in the initiator was less than 1/1; it is recommended to range between 0.4/1 and 0.8/1 (Highsmith et al. 2002).

The polyol initiator used generally has a hydroxyl group of unhindered polyols. Polyol was preferably a diol such as ethylene glycol, propylene glycol, 1,3-propanediol, and 1,4-butanediol. Triol is in the form of glycerol, trimethylol propane, and 1,2,4-butanetriol. While the example of tetrol is 2,2 dihydroxymethyl, 1,3 propanadiol. The relatively low molecular weight of the polyol, preferably less than 500, more preferably below 300, is particularly preferred under 150 (Highsmith et al. 2002; Astuti et al. 2016b). According to Higsmith et al. (2002) the initiator had at least two functional hydroxyls, preferably between 2 and 4, preferably between 2 and 3, having at least two unhindered hydroxyl groups. Whereas Sanderson and Martins (2004) argue that the polyol initiator preferably has at least three unhindered hydroxyl groups and preferably had four functional hydroxyls such as tetraol. The initiator was expected to be a liquid at ambient temperature. Examples of liquid triols were glycerol, while solid triols were trimethylol propane and butanatriol, which were dissolved in suitable solvents such as methylene chloride.

The reaction between the catalyst and the initiator produces an alkoxide group (alkoxide group) formation in the catalyst–initiator complex. One example of the formation of an alkoxide group component was the reaction between boron trifluoride etherate and 1,4 butanediol to produce PGN which has low functionality. The reaction between the catalyst and the initiator will not form a product containing an alkoxide group if the boron trifluoride gas replaces boron trifluoride etherate, so that the reaction between the catalyst and the initiator and the removal of the potential component of the alkoxide group is not necessary. Table 7.12 shows the experiments conducted by Willer et al. (1992a) using the catalyst/initiator mole ratio of 0.5/1 to 2/1. Most experiments were carried out with the catalyst/initiator mole ratio 1/1. One of the glycidyl nitrate polymerization variations proposed by Highsmith et al. (2002) uses a catalyst/initiator mole ratio of 1/1.

The cationic polymerization reaction took place with non-protic, non-ether, inert solvent organic solvents. Examples were methylene chloride; chloroform; and 1,2-dichloroethane (Sanderson and Martins 2004; Highsmith et al. 2001; Willer et al. 1992a, 1993). Monomers (glycidyl nitrate) were added to the mixture at a speed where the monomer reacts as soon as it was added. The temperature was maintained between 10 and 25 °C, preferably between 11 and 17 °C; it was strongly recommended that it be from 13 to 15 °C. The faster the heat was taken from the mixture, the faster glycidyl nitrate can be added to the mixture. The hydroxyl equivalent weight of PGN was produced between 1000 and 1700, preferably between 1200 and 1600 (Willer et al. 1992a, 1993). Table 7.12 presents the results of glycidyl nitrate polymerization by Willer et al. (1992a). The polymerization was carried out by initiator 1,4-butanadiol. Most of the reactions used the BF_3THF catalyst.

Experiments on polymerization were also put forward by Highsmith et al. (2002). They delivered two variations of glycidyl nitrate polymerization. The first polymerization was carried out by initiator 1,4-butanadiol mixed with calcium hydride and catalyst BF_3THF. The polymerization was carried out with the catalyst and hydroxyl mole ratio in the initiator was 0.48/1 and the reaction time was 1 h. The yield produced was 85% by weight. The second polymerization variation differed from the first polymerization in terms of how to mix the initiator and catalyst as well as the mixing time. The second polymerization was carried out with the initiator of calcium hydride and the BF_3THF catalyst. The catalyst and catalyst mole ratio was 0.28/1 and the reaction time was 16 h. This polymerization also got a yield of 85% by weight.

Sanderson et al. (Sanderson and Martins 2004) also conveyed two variations of glycidyl nitrate polymerization to form PGN. The first polymerization variation used a mixture of initiator trimethylol propane and calcium hydride as well as BF_3THF catalyst. The reaction time was 16 h. The second polymerization variation used 1,4-butanadiol initiator and BF_3THF catalyst with a reaction time of 5 h.

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Chapter 8
Potential Application of Native Fruit Wastes from Argentina as Nonconventional Sources of Functional Ingredients

María Inés Isla, Florencia Cattaneo, María Eugenia Orqueda, María Alejandra Moreno, Jorgelina Pérez, Ivana Fabiola Rodríguez, Florencia María Correa Uriburu, Sebastián Torres, and Iris Catiana Zampini

Abstract The disposal of a large number of waste materials results in high costs for the food industry and can have a negative environmental impact. Metabolites, such as phenolic compounds, fibers, and proteins obtained from vegetable by-products or waste biomass could be used as ingredients in the formulation of new functional foods. Argentine native fruits (chilto, algarrobo, and mistol) were used as food (edible fleshy fruits, sweets, flours, juices, pulp, and beverages) by different local communities and some of them have now been industrialized. In fact, fruit industrial processing has, as a consequence, the production of large amounts of wastes, mainly peels or skin, pomace, and seeds. Phenolic enriched extracts (benzoic and cinnamic acids and derivatives; phenylpropanoid acids; C-glycosyl flavones; anthocyanins, among others) obtained from Argentinean native fruit wastes were able to modulate the metabolism of lipids and carbohydrates in the gastrointestinal tract (GT) through enzymes inhibition (lipase, amylase, and glucosidase), regulate oxidative processes and inflammatory pathologies, so these extracts could be considered functional ingredients. Furthermore, these phenolic extracts were used to develop zein matrix microcapsules and coating structures based on zein fibers that could be optimized to food package. Proteins and protein hydrolysates obtained from carob tree seeds were also antioxidants and inhibitors of pro-inflammatory enzymes and improve vascular function in a rabbit model of high fat diet-induced metabolic syndrome. Thus, Argentinean native fruit wastes have the potential to be a novel renewable, sustainable, and low-cost raw material for the production of several value-added products.

M. I. Isla (✉) · F. Cattaneo · M. E. Orqueda · M. A. Moreno · J. Pérez · I. F. Rodríguez · F. M. C. Uriburu · S. Torres · I. C. Zampini (✉)
Facultad de Ciencias Naturales e Instituto Miguel Lillo, Instituto de Bioprospección y Fisiología Vegetal (CONICET-UNT), Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina
e-mail: misla@csnat.unt.edu.ar; misla@tucbbs.com.ar; zampini@csnat.unt.edu.ar

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Keywords Argentine native fruits · By-products · Fibers · Functional ingredients · Phenolic compounds

8.1 Introduction

Nowadays, all the food-related substrates, which are lost within the food supply chain, are considered as wastes or *by-products*. Some food wastes are processed into low-market-valued products, such as fertilizers or animal food. Nevertheless, these food wastes contain valuable substances, and their processing as cheap products does not guarantee the efficient use of these natural resources. Thus, a more attractive approach from the economic point of view is to recover the high-added-valued constituents present in *food processing wastes principally in fruits processing wastes or fruits by-products*. Primary and secondary metabolites are valuable substances that have been extracted from fruit wastes (Figuerola et al. 2005; Schieber et al. 2003; Gorinstein et al. 2001). Phenolic compounds (hydrolyzable and condensed tannins, lignans, phenolic acids, flavonoids, anthocyanins), alkaloids, glycosides, volatile oils are some of the examples of secondary metabolites. Many of secondary metabolites have shown strong antioxidant properties and have a wide range of action which includes anti-obesity, anti-inflammatory, antimicrobial, antitumor, cardioprotective, etc. Fruit wastes may be an important source for recovery of cellulose, hemicellulose, and lignin. Cellulose can be turned into sugars and further to biofuels and biochemicals products (Sindhu et al. 2016; Joglekar et al. 2019). Additionally, some fruit wastes showed high protein content (Pfaltzgraff et al. 2013; Fasoli and Righetti 2015). Proteins extracted from these wastes may be nutritionally valuable, and the peptides obtained by hydrolysis from these proteins could be useful in biomedicine showing several health benefits (Banerjee et al. 2017). Indeed, the potential of food wastes to create new opportunities and markets has been underestimated. Nowadays, at least 50 companies around the world recover valuable compounds from food wastes (e.g., natural preservatives, functional compounds) (Galanakis 2012, 2013, 2018; Galanakis et al. 2016; Ferrentino et al. 2018). Food products must be politically, economically, environmentally, socially, and technologically sustainable.

8.2 Fruit Wastes as Ingredient for New Functional Foods with the Ability to Modulate Oxidative Processes, Inflammatory Processes, and Obesity Associated with Metabolic Syndrome

Diabesity is the leading cause of modern, chronic disease. In 2012, diabetes killed 1.2 million people worldwide and its global prevalence exceeded 347 million people, and it is expected that it will increase to 540 million by 2030 (Martínez Leo et al. 2016; Naveen and Baskaran 2018). Diabetes epidemic is also linked to increasing obesity, and both became a public health imperative. The role of the inflammatory process and oxidative stress in the pathophysiology of these diseases is widely documented (Martínez Leo et al. 2016; Pistollato and Battino 2014). Obesity is accompanied by low-grade chronic inflammation in different tissues such as adipose tissue, liver, pancreas, muscle, and brain (Troncon Rosa et al. 2012). The inflammatory response associated with obesity provokes the activation of cytokines and transcriptional factors and the macrophages infiltration in the adipose tissue, resulting in an unresolved response associated with insulin resistance, endothelial dysfunction, and subsequent cardiovascular disorders (Chung et al. 2009). In this context, new, safer, economically viable, effective and useful food strategies to counteract these states are necessary. Functional foods able to modulate oxidative stress and energy homeostasis are promising tools to control inflammatory status and body weight gain (Siriwardhana et al. 2013).

8.2.1 Polyphenols as Functional Ingredients

Energy reduction could be a solution and it could be obtained from some natural compounds mainly from *polyphenols* which inhibit digestive enzymes, molecules able to limit both bioaccessibility and bioavailability of carbohydrates, fats, and proteins along the GT.

The chemical structure of polyphenols, their linkage with other food components (carbohydrates, proteins, fats and fiber), as well as their arrangement in the food matrix influence their bioaccessibility in GT (González-Aguilar et al. 2017; Renard et al. 2017; Jakobek 2015; Dufour et al. 2018). Thus, the action of digestive and bacterial enzymes, by breaking up the food matrix and delivering polyphenols in the GT, is fundamental for phenolic compounds to act locally and systematically. They can scavenge free radicals continuously forming in the GT, thus counteracting both subclinical oxidative stress and intestinal fat-high content diet-induced inflammation, which are both correlated with obesity exacerbation and insulin resistance (Van Den Ende et al. 2011). In the GT, phenolic compounds may influence the activity of digestive enzymes such as pancreatic α-amylase, brush-border α-glucosidase and pancreatic lipase, thus modulating nutrient bioavailability and neurohormonal signals being important to appetite mechanisms and body weight (De La Garza et al.

2011; Tucci et al. 2010; Xiao et al. 2013a, b). It was widely demonstrated that various classes of polyphenol compounds show different enzyme inhibitory capacities and that such differences are linked to specific features in their chemical structures such as number and position of hydroxyl and/or galloyl groups, degree of polymerization, glycosylation and/or methylation. For example, concerning lipase inhibition, both for flavonoids and phenolic acids, a higher number of hydroxyl groups and galloyl moieties increases inhibitory effects. Moreover, hydroxybenzoic acids inhibit less powerfully pancreatic lipase than hydroxycinnamic acids (Buchholz and Melzig 2015). Similarly, a high number of galloyl and hydroxyl groups in molecules increases the polyphenol inhibitory capacity on α-glucosidase and α-amylase while methylation, methoxylation, hydrogenation, and glycosylation usually diminish their activity (Xiao et al. 2013a, b). However, phenolic compounds can undergo several chemical transformations during food processing and digestive processes. As a result, their inhibitory capacity may change with respect to initial pure compounds. In order to determine bioaccessibility in the GT and simulate the ability of polyphenol compounds to inhibit digestive enzymes after chemical transformations that occur during the digestive process, in vitro enzyme inhibition assays can couple to in vivo digestion models (Costamagna et al. 2016). On the other hand, the ability of polyphenols is considered to scavenge free radicals and inhibit the production of inflammatory mediators since, as indicated above, the metabolic syndrome is a condition associated with oxidative stress and chronic inflammation. In this context, new useful food strategies to counteract these states are necessary.

8.2.2 Proteins and Peptides as Functional Ingredients

Fruit wastes, particularly seeds, are protein-rich and could represent a nutritionally relevant protein source contributing to the production of essential amino acids (Banerjee et al. 2017; Prandi et al. 2019). Furthermore, bioactive peptides, obtained from protein hydrolysis, were found to exhibit various pharmacological properties such as antihypertensive to anti-inflammatory (Banerjee et al. 2017; Martínez Leo et al. 2016; Udenigwe and Aluko 2012). Amino acids like histidine and aromatic amino acids like phenylalanine or tyrosine were also found to be effective in scavenging metal ions, reactive oxygen species and as antioxidants (Deng et al. 2012; Matsui et al. 2018).

8.2.3 Sugars and Derivatives as Functional Ingredients

In the last recent years, there has been an increased research interest in lignocellulosic biomass obtained from wastes as a renewable source of polysaccharides. Cellulose and its derivative forms are used in medicine due to their sugar lowering effects, hydrogel-forming ability, water retention, and biodegradability (Liu et al.

2015). Hemicellulose comprises xyloglucans, xylans, mannans, and glucomannans (Scheller and Ulvskov 2010). Xylo-oligosaccharides belong to a class of dietary fibers which are resistant to human gut enzymes and pass undigested into the colon, wherein they are fermented by colonic microbiota (Singh et al. 2015). Fermentation of such oligosaccharides produces short-chain fatty acids (Singh et al. 2015), which provide a range of health benefits including maintenance of gut health and microbiota, antioxidant and antitumor activity, glucose metabolic control and lipid profile and immunomodulation. Resistant starch already constitutes another class of dietary fiber; this refers to the portion of starch that resists digestion in the GT and is partially or completely fermented in the colon producing various health benefits (Fuentes-Zaragoza et al. 2010). Dietary fiber and its concentrates have been promoted as a nutritional supplement for hypertension, obesity, and antidiabetic treatment (Panja 2017; Vanda et al. 2018).

8.2.4 *Procedures for Bioactive Compounds Extraction*

Figure 8.1 shows that a complete scheme for a biorefinery able to process a variety of fruit wastes. The wastes are dried and size-reduced for further processing. Based on the type of waste available for processing, extraction is carried out to obtain oil, sugar, proteins, phytochemicals (phenolics, anthocyanins, carotenoids, among

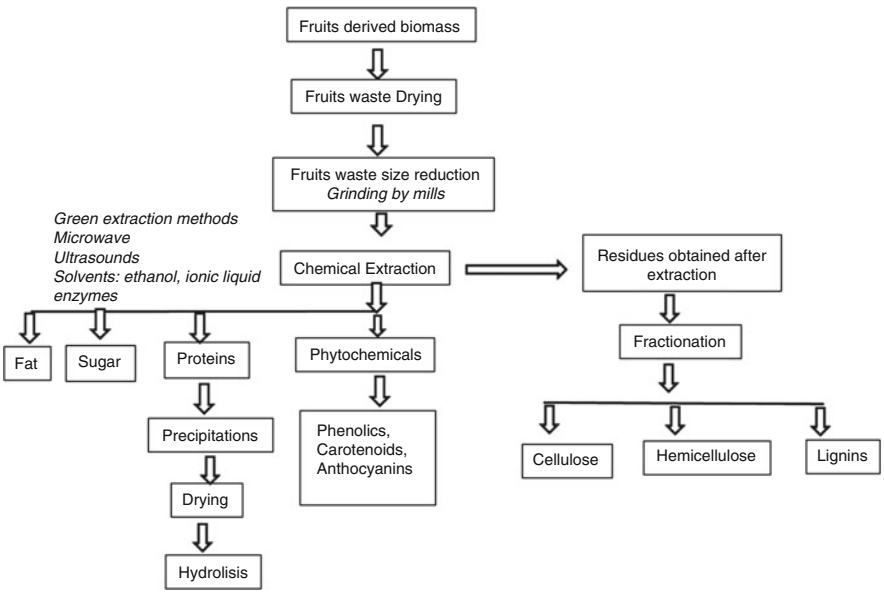

Fig. 8.1 Scheme of an integral processing of fruit-derived wastes to produce value-added products linked to biorefinery concept

others), cellulose, hemicellulose, and lignin. The economic feasibility of the extraction method depends on the maximum recovery of bioactive compounds from fruit wastes. Alternative/novel green extraction methods are more sustainable compared to conventional methods and may be an environment-friendly replacement for organic solvent-based methods. Microwave and ultrasounds are widely accepted as compared to the former owing to their ease of applicability for high moisture substrates (Nawirska and Kwaśniewska 2005). The preferred solvent among alcohols is ethanol due to its lower boiling point, quick recovery, and the "generally regarded as safe" status as defined by the US FDA. However, alcohol as a solvent may not always be useful since many compounds such as carotenoids are more soluble in aprotic solvents. The yield of hydroxylated components, which are commonly present in fruit wastes, was found to be optimum with ethanol, while methoxylated components, which are present in peel or skin, required less polar solvents such as acetone. Ionic liquids are a novel medium for extraction of bioactive compounds (Garcia-Amezquita et al. 2018).

8.3 Native Fruit Wastes from Argentina

In Argentina there are several phytogeographical regions that coincide with arid and semiarid, and tropical and subtropical regions. The Argentinean northwest is formed by the Chaco Forest region, the rainforest region, and the Andean region and is characterized by its extraordinary biodiversity with numerous fruit plant species. Most of them are not commercially exploited because their potential as food, dietary supplement, functional food, or nutraceutical is completely unknown, whereas many of them are overexploited by local communities. For example, the Chaco forest region is characterized by the presence of *Geoffroea decorticans*, popularly known as chañar, *Ziziphus mistol* (mistol), and *Prosopis*, both *P. alba* and *P. nigra*, known as white and black carob tree, respectively. Between the native fruit species that grow in the Argentine rainforest region are arrayan (*Eugenia uniflora*), mato (*Eugenia* mato), chilto (*Solanum betaceum*), and other fruits are consumed by the local people as fresh fruits or as preparations obtained from them, like candies, alcoholic and non-alcoholic beverages or flour. The industrial processing of these native fruits generates fruit wastes, mainly peels or skin, pomace, and seeds which could represent important natural sources of essential nutrients and chemicals beneficial for human health.

8.3.1 Solanum betaceum

Solanum betaceum Sendt ex *Cyphomandra betacea* Sendt. (common name: chilto or tree tomato), belongs to *Solanaceae* family. This is a native food species that grows in the humid forest of the eastern Andean slopes from Northwestern Argentina.

Fig. 8.2 Pictures of (**a**) *Solanum betaceum* (Chilto) fruits; (**b**) *Prosopis alba* (algarrobo) fruits (**c**) *Ziziphus mistol* fruits

Chilto or tree tomato fruit is a fleshy berry with ovoid to ellipsoid in shape, singly or in groups of 3–12. The skin can be classified into three main types: red, yellow, and purple. The pulp presents firm texture with different colors and has a jelly with seeds that are orange or intense red. The size of mature fruit ranges between 4 and 10 cm in length, 3 and 6 cm in cross-section, and weigh between 45 and 80 g (Fig. 8.2a). The ripe chilto fruits are consumed mainly in salads, jams, juice, and liquors, and commercialized in regional markets from Northwestern Argentina (Ordóñez et al. 2010, 2011; Ordoñez et al. 2012). "Chilto" is included in the Argentine Food Code. During processing to obtain pulp and juices, the *skin* and *seeds* are discarded.

Seed and Skin Powder The freeze-dried powders obtained from these wastes (Fig. 8.3) have low carbohydrate and sodium content and are a source of vitamin C, carotenoid, phenolic compounds, potassium, and fiber (Table 8.1) (Orqueda et al. 2017a).

Seed, Skin, and Pomace Polyphenol-Enriched Extracts The HPLC-MS/MS analysis of polyphenol-enriched extracts allowed the identification of 11 caffeic acid derivatives and related phenolic compounds, 8 rosmarinic acid derivatives, and 5 flavonoids (Fig. 8.3). Polyphenol-enriched extracts inhibited enzymes associated with metabolic syndrome, including α-glucosidase, amylase, and lipase and exhibited antioxidant activity by different mechanisms and anti-inflammatory activity (Ordóñez et al. 2010; Orqueda et al. 2017a). Indeed, chilto seeds and skin polyphenols could scavenge the free radicals continuously forming in GT, thus counteracting both subclinical oxidative stress and intestinal high-fat diet-induced inflammation. Polyphenols could also influence the activity of digestive enzymes such as pancreatic amylase, brush-border glucosidase, and pancreatic lipase, thus modulating nutrients bioavailability and the body weight in the long term. This by-product is a promising resource that could be used as a functional food or as a functional ingredient in food formulations or as a nutraceutical.

Polyphenol Films Obtained from Seed and Skin Zein fibers loaded with polyphenol-enriched extracts from seed and skin of chilto were placed on polyhydroxyalkanoate (PHA) films through the electrospinning technique for their

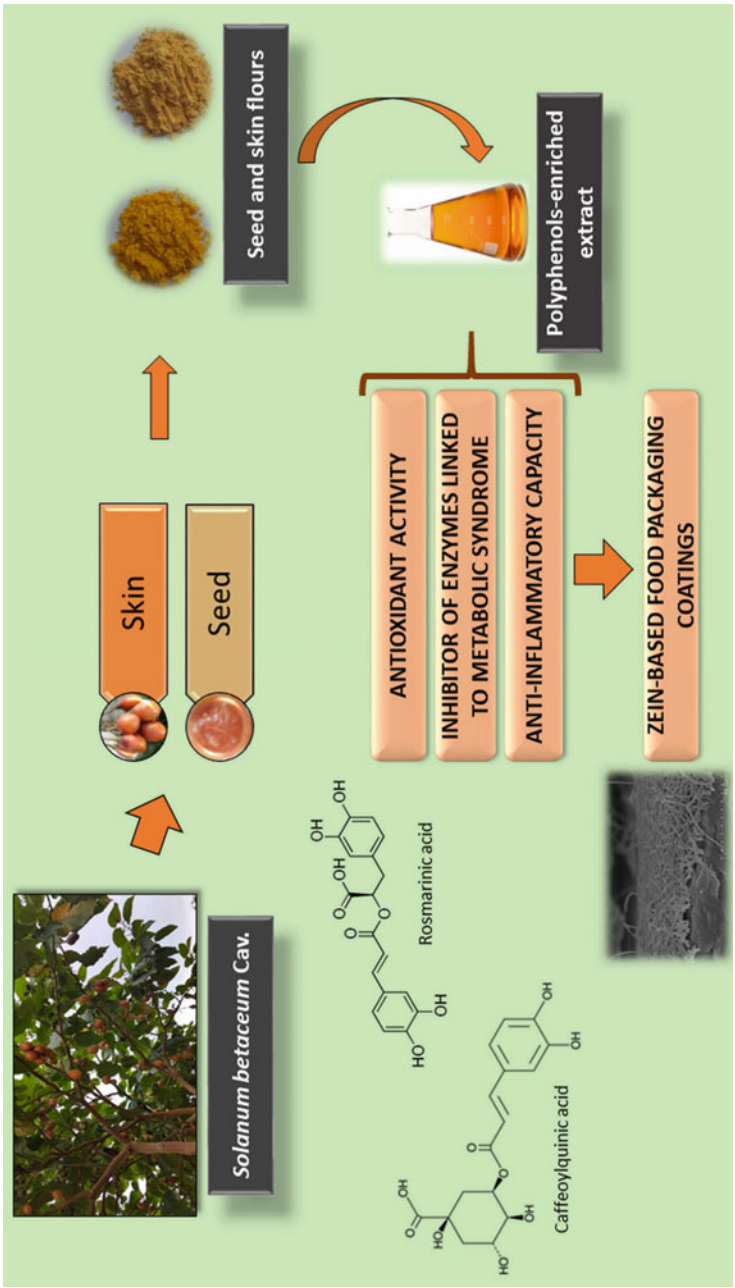

Fig. 8.3 Bioactive polyphenols obtained from skin and seed powder of *Solanum betaceum*

Table 8.1 Nutritional and phytochemical composition of Argentinean native fruit seeds

Phytochemical content of powder or flour	Chilto seed	Algarrobo seed without cover	Mistol seed
Total phenolic (mg GAE/100 g powder)	179 ± 3	1150 ± 20	425 ± 6
Flavone and flavonol (mg QE/100 g powder)	175 ± 3	396 ± 10	444 ± 4
Condensed tannins (mg procyanidin-B_2/100 g powder)	<DL	175 ± 15	153 ± 6
Hydrolyzable tannins (mg GAE/100 g powder)	<DL	<DL	<DL
Anthocyanins (mg C-3GE/100 g powder)	<DL	<DL	<DL
Ascorbic acid (mg AA/100 g powder)	56.80 ± 2.30	0.33 ± 0.01	36.50 ± 1.00
Carotenoids (g β carotenoid/100 g powder)	3.65 ± 0.60	10.55 ± 0.05	<DL
Total sugar (g GE/100 g powder)	11.91 ± 2.00	2.57 ± 0.35	2.10 ± 0.10
Total protein (g /100 g powder)	20.9 ± 1.8	62.1 ± 6.2	2.5 ± 0.2
Fat (g/100 g powder)	0.31 ± 0.03	1.22 ± 0.05	18.50 ± 1.50
Fiber (g/100 g powder)	28.4 ± 3.2	9.1 ± 1.0	18.8 ± 1.6
Yield (%, g powder/100 g Seed)	13.3 ± 1.8	6 ± 0.5	42.5 ± 2.1

GAE Gallic acid equivalents, *QE* Quercetin equivalents, *C-3 GE* Cyanidin-3 glucoside equivalents, *AA* Ascorbic acid, *GE* Glucose equivalents

potential use as bioactive internal coatings for food packaging applications. Zein fibers were characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, and thermogravimetric analysis (Fig. 8.3). The mechanical properties and stability in aqueous environments of zein fibers were improved by crosslinking with glutaraldehyde vapors. Encapsulation efficiency of bioactive phenolic compounds was higher than 90%. Encapsulation in zein fibers improved the thermostability of phenolic compounds. Two food simulants (50% ethanol and 3% acetic acid) were used to evaluate phenolic compound release from crosslinked zein fibers. Crosslinking was observed to allow a gradual release of phenolic compounds (rosmarinic acid, caffeic acid, and their derivatives) in both solvents, their antioxidant properties being kept. Therefore, the potential of zein-based encapsulation structures was demonstrated to contain polyphenolic extracts to be applied as antioxidant coatings in food packaging structures in order to contribute to hydrophilic and lipophilic food product preservation, which could result in added value applications of these fruits, as well as in skin and seed valorization (Moreno et al. 2019).

The Direction of Non-timber Forest Products from Argentina promotes the chilto crop in its natural environment in the Yungas as a commercial crop for a sustainable management of the montane forests.

Protein from Chilto The antioxidant activities in free-radical-mediated oxidative systems and the genotoxic/antigenotoxic effects of proteins with a molecular mass around 17 kDa, purified from *Solanum betaceum* fruits (cyphomine) were

investigated (Ordoñez et al. 2011) . These proteins were xanthine oxidase enzyme inhibitors preventing uric acid formation and were able to reduce oxidative damage by scavenging hydroxyl radicals and superoxide anion in a dose-dependent manner. Cyphomine was able to retain their antioxidant activity after heat treatment at 80 °C. No genotoxic and mutagenic effects were detected. These proteins are promising ingredients for the development of functional foods with a beneficial impact on human health and an important source for the production of bioactive peptides.

8.3.2 Prosopis alba

Prosopis species are characteristic of the Monte phytogeographical region, from Salta to Chubut province in Argentina. *Prosopis alba* Griseb. common name white algarrobo (carob tree) are extensively distributed in Northwestern Argentina and grow in arid and semiarid regions. The National Program of Algarrobo (NPA) promotes the cultivation of these species in order to decrease the pressure on the native forests, thus contributing to regional development under a social, environmental, and economic sustainability approach. Fruits are linear, arched, or annular, 12–25 cm long, 1.2–1.8 cm wide, and 0.5 cm thick, straw yellow color, very compressed, with parallel edges, with seeds that are identified in the lateral faces, and sweet pulp. The seeds (15–30 per pod) are ovoid, 6–7 mm long, 4–5 mm wide, and brown (Fig. 8.1b). The white algarrobo pods are used to prepare fermented and non-fermented beverages (aloja and añapa) and food products such as syrup, flour, and sweets (arrope, patay, jam) (Cardozo et al. 2010; Pérez et al. 2014; Cattaneo et al. 2014). Nutritional and biological properties of mesocarp flour (Cardozo et al. 2010; Pérez et al. 2014) were reported. The seeds plus endocarp of *Prosopis* pods are considered fruit wastes. If we consider that in Chaco Park there are approximately 26,000,000 ha cultivated with *Prosopis* and that for each hectare of crops, 10,000 kg of pods are recovered, 48% represents seeds, the amount of material available for use is evident. To suggest a potential use for *Prosopis alba* seeds is necessary to determine the nutritional, phytochemical, and functional quality of the flour of seeds.

Seed Flour Crude protein fractions from cotyledon tissue flour represent 62.1 ± 6.2% (Table 8.1) (Cattaneo et al. 2014). Protein values from *P. alba* cotyledon flour were higher than those of soybeans (34.6%), broad beans (23.7%), lentils (25.4%), peas (22.9%), common beans (21.8%), and chickpeas (18.5%) (Cosiansi et al. 2002). These results suggest that due to its high protein content, *P. alba* cotyledon flour (currently discarded) could be considered a new alternative in food formulation or food supplements for both humans and animals, alone or combined with cereal proteins (cereal bars, cookies, coffee substitutes). This flour showed low content of carbohydrate and fat. Free polyphenols (1150 ± 20 mg GAE/100 g flour) and carotenoids (10 ± 0.05 mg β-CE/100 g flour) were the major phytochemicals (Table 8.1). The effects of dietary supplementation with *Prosopis alba* seed flour on a high-fat diet-induced rabbit model of metabolic syndrome were assayed. The dietary

supplementation with algarrobo seed flour has vascular protector properties and could be used to prevent vascular alterations characterizing the metabolic syndrome (Cattaneo et al. 2019).

Polyphenol-Enriched Extract Obtained from Seed The main polyphenolic constituents identified in *P. alba* seed extract were C-glycosyl flavones (Fig. 8.4), including schaftoside, isoschaftoside, vicenin II, vitexin, and isovitexin (Cattaneo et al. 2016). Polyphenolic-enriched extracts exhibited $ABTS^{\bullet+}$ reducing capacity and H_2O_2 scavenging activity and was able to inhibit phospholipase, lipoxygenase and cyclooxygenase, three pro-inflammatory enzymes (Cattaneo et al. 2016). According to these results, the *P. alba* cotyledon flour could be proposed as a new alternative in the functional foods or food supplement formulation or a substrate for the recovery of functional compounds.

Protein Hydrolysate from Seed According to their amino acid profile and chemical score (>100%), the *P. alba* seed proteins are not deficient in essential amino acids considering the amount of amino acid necessary for adults (Cattaneo et al. 2014). From seed flour a protein isolate was obtained. This isolate showed a good solubility (pH 7.4–9), emulsifying capacity, oil binding capacity, and water adsorption capacity.

In order to simulate the effect of proteolytic enzymes normally produced during the digestion process, two intestinal proteases—pepsin and pancreatin—were put in contact with the protein isolate obtained from cotyledon flour for 4 h to obtain a protein hydrolysate (Cattaneo et al. 2014). Then, the peptides of different molecular weight were separated by ultrafiltration in two fractions: one with MW >3 kDa and another with MW <3 kDa. The antioxidant ability of the proteins was significantly increased with hydrolysis (SC_{50} values: 50–5 μg/mL, respectively). Proteins and protein hydrolysates were also inhibitors of pro-inflammatory enzymes (lipoxygenase and phospholipase), Fig. 8.4. Results suggest that *P. alba* cotyledon flour or protein isolate or protein hydrolysates could be new alternatives in the formulation of food for humans due to not only its nutritive value and bioactivity but also their functional properties such as emulsifying activity, water absorption capacity, oil binding capacities, and solubility (Cattaneo et al. 2014). This information is relevant for the development of value-added products in the region, particularly as ingredients for functional foods.

8.3.3 Ziziphus mistol

Ziziphus genus (Rhamnaceae) comprises approximately 170 pantropical species, 25 of which are native to America and the Caribbean (Islam and Simmons 2006). *Ziziphus* species are important fruit trees with food and medicinal value (Mizrahi et al. 2002). The *Ziziphus mistol* Grisebach tree is distributed in semiarid areas of Brazil, Paraguay, and Argentina (Tortosa 1995). Its fruits, known as mistol, have been used as food, forage for cattle, and in traditional medicine (Scarpa 2004). Mistol fruit is a drupe with 1.5 cm in diameter and reddish-brown colored when

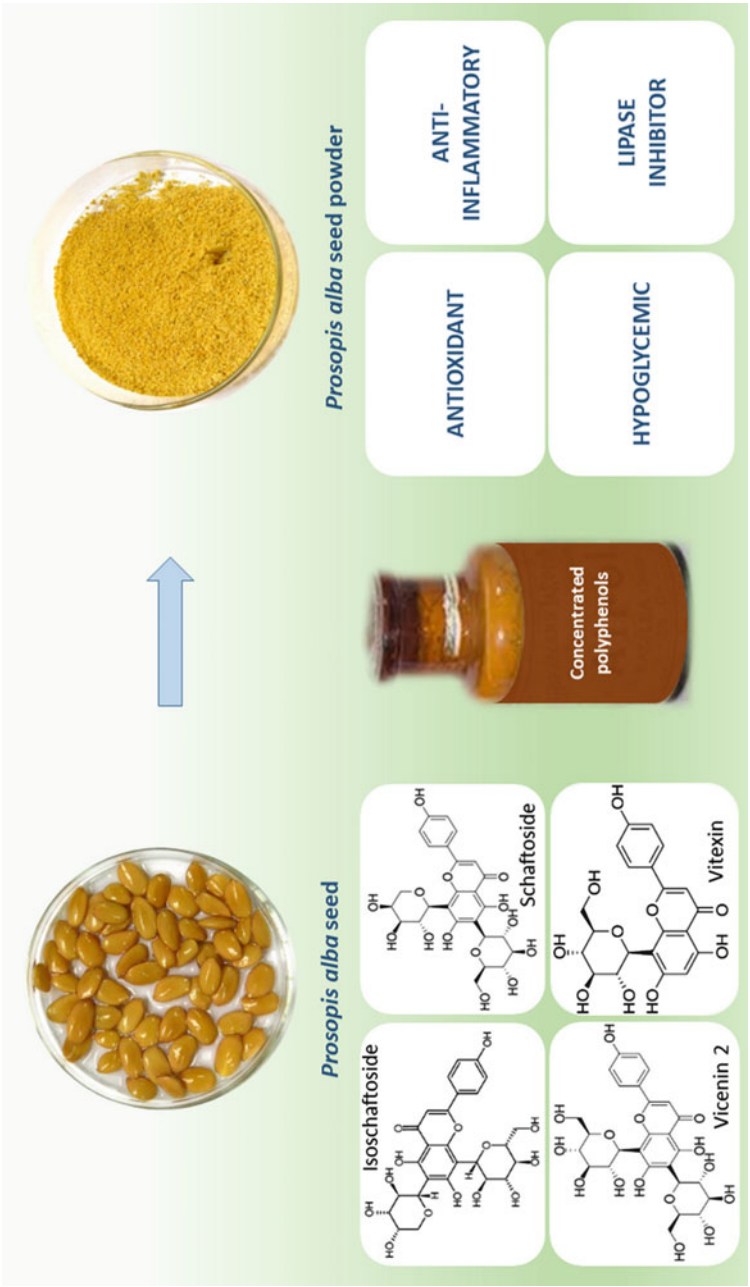

Fig. 8.4 Bioactivity of polyphenol-enriched extract of *Prosopis alba* seed

mature. The fruit has a pasty and sweet pulp, and can be eaten fresh, sundried (mistol dried fruit), or boiled. Cardozo (Cardozo et al. 2011) reported the nutritional composition and functional properties (antioxidant and anti-inflammatory activities) of aqueous and alcoholic extracts from ripe *Ziziphus mistol* fruit pulp. Aqueous extracts of *Z. mistol* fruits have been demonstrated to have potential application for reducing the severity of the chronic evolution of hemolytic uremic syndrome produced by *Escherichia coli* (Albrecht et al. 2011). This pathology has been associated with the oxidative stress of diverse tissues, and mainly with the oxidative damage of blood components and kidneys (Albrecht et al. 2011).

Seed and Skin Powder Powder from mistol wastes, such as skin and seed, have moderate carbohydrate content and are an important source of flavonoids, fiber, potassium, magnesium, and calcium (Table 8.1). Mistol seed oil contains 22% of its fatty acids as 18:3, n-3 (alpha-linolenic acid) and 12% as linoleic acid. Eynard (Eynard et al. 1992) has shown that mistol fruit oil is a good source of edible fats for growing and reproducing mice. The modulating effect of dietary *Z. mistol* seed oil on two murine mammary gland adenocarcinomas having low and high metastatic abilities was reported. These results suggested that *Z. mistol* seed oil has potential value in nutritional oncology (Muñoz et al. 1995, 1999).

Seed and Skin Polyphenol-Enriched Extracts The HPLC–ESI-MS/MS analysis of polyphenol-enriched extracts allowed the identification of 17 compounds including 16 flavonoids and a procyanidin (Fig. 8.5). Extracts showed antioxidant capacity and were able to inhibit α-glucosidase, α-amylase and pancreatic lipase, enzymes related to the metabolic syndrome (Orqueda et al. 2017b). These results suggest the potential of powder and extracts obtained from mistol skin and seed as a functional food or dietary supplement in the prevention or treatment of diseases associated with the metabolic syndrome.

8.4 Conclusion

This report analyzes bioproducts (phenolic compounds and proteins) extracted from wastes or products derived from Argentine native fruits. These bioactive compounds have a great potential to be used as functional ingredients. The exploitation of native fruit wastes from Argentina as a source of functional compounds and their application in food is a promising field.

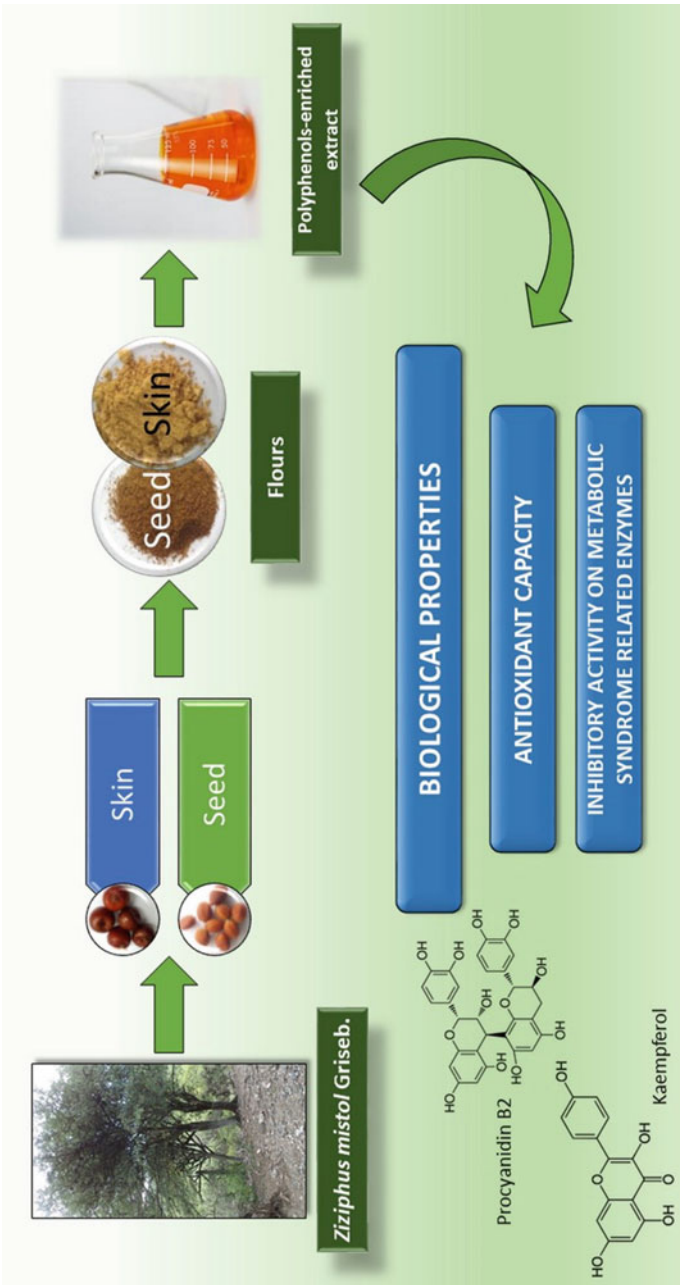

Fig. 8.5 Bioactivity of polyphenol-enriched extract of *Ziziphus mistol* seed and skin

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Chapter 9
Conventional and Alternative Strategies of Pretreatment of Chili Postharvest Residue for the Production of Different Value-Added Products

Raveendran Sindhu, Parameswaran Binod, and Ashok Pandey

Abstract Agricultural and crop residues serve as a renewable source for the production of bioethanol and other value-added chemicals. The residue which is left out after harvesting chili from chili plants constitute chili postharvest residue (CPHR). It is an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass. Presently this is considered as a waste and disposed by burning after harvest. Hence this could be a viable biomass for second-generation biofuel production as well as for the production of other value-added products like biopolymer and industrial enzymes. Similar to other lignocellulosic biomass, chili postharvest residue also requires some kind of pretreatment for better enzymatic saccharification of cellulose by enzymes. The objective of the present study was to evaluate pretreatment liquor as well as the enzymatically saccharified biomass for second-generation biofuel, xylanase and biopolymer production.

Keywords Biomass · Pretreatment · Biorefinery · Biofuel · Saccharification · Fermentation

9.1 Introduction

Agro-residues serve as a potential feedstock for the production of bioethanol (Sukumaran et al. 2010). Chili is one of the most important commercial spice crops of India. Chili is cultivated in almost all states of India. Andhra Pradesh is

R. Sindhu · P. Binod (✉)
Microbial Processes and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram, Kerala, India

A. Pandey
Centre for Innovation and Translational Research, CSIR- Indian Institute for Toxicology Research (CSIR-IITR), Lucknow, Uttar Pradesh, India

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the largest chili-producing state in India accounting for more than 50% of the total chili production in India followed by Karnataka which accounts for around 10–15% of the total chili production in the country (https://agmarknet.gov.in/Others/preface-chhilli.pdf).

CSIR-NIIST, India has published a report on biomass availability in India (Pandey et al. 2009). This provides an authenticated report on generation and availability of different biomass residues. The study revealed that crop residues generated during chili cultivation accounts for around 0.6 million tons. Crop residues from chili are generated in surplus since these residues do not have much use other than fuel (Pandey et al. 2009). Residue which is left out after harvesting of chili constitutes chili postharvest residue which consists of stalks, leaves, and roots. To utilize this biomass for the production of bioethanol and other value-added products, some kind of pretreatment is to be carried out for the removal of hemicelluloses and lignin. Though several conventional as well as alternative strategies of pretreatment are available for different feedstocks, till date no reports were available for chili postharvest residue. A common pretreatment cannot be used as a universal one for all biomass, since the pretreatment conditions have to be fine-tuned based on the composition of the biomass. Hence Centre for Biofuels Group of CSIR-NIIST has evaluated conventional as well as different alternative strategies for pretreatment of chili postharvest residue.

The present chapter compares the merits and limitations of conventional and alternative strategies of pretreatment adopted for the production of bioethanol and other value-added products from chili postharvest residue.

9.2 Pretreatment Strategies Adopted for Chili Postharvest Residue

Conventional as well as different hybrid/alternative strategies were adopted for pretreatment of CPHR. Conventional strategies adopted include dilute acid and dilute alkali pretreatment (Preeti et al. 2012). Hybrid/alternative strategies adopted includes crude glycerol-assisted surfactant pretreatment (Sindhu et al. 2015), Sono-assisted acid pretreatment (Sindhu et al. 2016a), microwave-assisted surfactant pretreatment (Sindhu et al. 2016b), ultrasound-assisted hydrothermal pretreatment (Sindhu et al. 2017a), ultrasound-assisted alkali pretreatment (Sindhu et al. 2017b), surfactant-assisted transition metal pretreatment (Sindhu et al. 2017c), dilute acid-assisted lime pretreatment (Sindhu et al. 2018a), surfactant-assisted hydrothermal pretreatment (Sindhu et al. 2018b), and microwave-assisted acid pretreatment (Sindhu et al. 2018c). Details are presented in Table 9.1.

Table 9.1 Different strategies adopted for pretreatment of Chili postharvest residue

Pretreatment strategy	Conditions of pretreatment	References
Acid pretreatment (high temperature)	Biomass loading—5% w/v Acid conc.—4% w/v Temperature—140 °C Pretreatment time—60 min	Preeti et al. (2012)
Alkali pretreatment (high temperature)	Biomass loading—5% w/v Alkali conc.—3.5% w/v Temperature—180 °C Pretreatment time—15 min	Preeti et al. (2012)
Crude glycerol-assisted surfactant pretreatment (CGASP CPHR)	Biomass loading—25% w/w Crude glycerol conc.—0.55% w/w Surfactant conc.—4.5% w/w Pretreatment time—60 min	Sindhu et al. (2015)
Sono-assisted acid pretreatment (SAAP CPHR)	Biomass loading—20% w/w Sonication time—4 min Acid conc. = − 4.0% w/w Pretreatment time—60 min	Sindhu et al. (2016a)
Microwave-assisted surfactant pretreatment (MWASP CPHR)	Biomass loading—5% w/w Surfactant conc.—4% w/w Microwave time—1 min Microwave power—450 W Pretreatment time—45 min	Sindhu et al. (2016b)
Ultrasound-assisted hydrothermal pretreatment (USAHTP CPHR)	Biomass loading—25% w/w Sonication time—10 min Pretreatment time—15 min	Sindhu et al. (2017a)
Ultrasound-assisted alkali pretreatment (USAAP CPHR)	Biomass loading—15% w/w Alkali conc.—4% w/w Sonication time—30 min Pretreatment time—15 min	Sindhu et al. (2017b)

(continued)

Table 9.1 (continued)

Pretreatment strategy	Conditions of pretreatment	References
Surfactant-assisted transition metal pretreatment (SATMP CPHR)	Biomass loading—5% w/w Surfactant conc.—1% w/w $FeSO_4$ conc.—2% w/w Pretreatment time—20 min	Sindhu et al. (2017c)
Dilute acid-assisted lime pretreatment (DAALP CPHR)	Biomass loading—10% w/w $Ca(OH)_2$ conc.—2% w/w Acid conc.—5% w/w Pretreatment time—30 min	Sindhu et al. (2018a)
Surfactant-assisted hydrothermal pretreatment (SAHTP CPHR)	Biomass loading—20% w/w Surfactant conc.—3% w/w Pretreatment time—60 min	Sindhu et al. (2018b)
Microwave-assisted acid pretreatment (MWAAP CPHR)	Biomass loading—5% w/w Acid conc.—3% w/w Microwave power—550 W Microwave time—3 min	Sindhu et al. (2018c)

9.2.1 Comparison of Different Pretreatment Strategies Adopted for Chili Postharvest Residue

Details are depicted in Tables 9.1 and 9.2. Among the different pretreatment strategies adopted for CPHR, maximum reducing sugar yield (0.882 g/g) was observed with alkali pretreatment (Preeti et al. 2012). Here the pretreatment was carried out in a high-pressure reactor with low biomass loading (5% w/v) and high temperature (180 °C). Conventional pretreatment strategies like acid and alkali pretreatment of CPHR were carried out in high-pressure reactor (Amar Equipments Pvt. Limited, New Delhi, India). One of the main drawbacks of acid and alkali pretreatment is that it was carried out with very low biomass loading (5% w/w) which makes the process economically nonviable. Since the pretreatment is carried out in a high-pressure reactor at high temperature, charring occurred with high biomass loading (Preeti et al. 2012).

All alternative strategies adopted for CPHR, except for microwave-assisted surfactant pretreatment, microwave-assisted acid pretreatment, and ultrasound-assisted hydrothermal pretreatment, were carried out at low temperature (121 °C) in a laboratory autoclave. A study conducted by Vani et al. 2012 revealed that the

Table 9.2 Reducing sugar profile obtained after enzymatic saccharification of different pretreated Chili postharvest residue

Pretreatment strategy	Reducing sugar yield (g/g)	References
Acid pretreatment (high temperature)	0.548	Preeti et al. (2012)
Alkali pretreatment(high temperature)	0.882	Preeti et al. (2012)
Crude glycerol-assisted surfactant pretreatment (CGASP CPHR)	0.451	Sindhu et al. (2015)
Sono-assisted acid pretreatment (SAAP CPHR)	0.465	Sindhu et al. (2016a)
Microwave-assisted surfactant pretreatment (MWASP CPHR)	0.316	Sindhu et al. (2016b)
Ultrasound-assisted hydrothermal pretreatment (USAHTP CPHR)	0.436	Sindhu et al. (2017a)
Ultrasound-assisted alkali pretreatment (USAAP CPHR)	0.402	Sindhu et al. (2017b)
Surfactant-assisted transition metal pretreatment (SATMP CPHR)	0.245	Sindhu et al. (2017c)
Dilute acid-assisted lime pretreatment (DAALP CPHR)	0.622	Sindhu et al. (2018a)
Surfactant-assisted hydrothermal pretreatment (SAHTP CPHR)	0.445	Sindhu et al. (2018b)
Microwave-assisted acid pretreatment (MWAAP CPHR)	0.205	Sindhu et al. (2018c)

energy requirement for microwave pretreatment is five times lesser than high-pressure reactor treatment. Among all the alternative strategies adopted for CPHR highest reducing sugar yield was observed with integrated dilute acid lime treatment (Sindhu et al. 2018a) followed by Sono-assisted acid pretreatment (Sindhu et al. 2016a), where the reducing sugar yields were 0.622 g/g and 0.465 g/g, respectively.

In pretreatment strategies like crude glycerol-assisted surfactant pretreatment (Sindhu et al. 2015), microwave-assisted surfactant pretreatment (Sindhu et al. 2016b), ultrasound-assisted hydrothermal pretreatment (Sindhu et al. 2017a), and surfactant-assisted hydrothermal pretreatment (Sindhu et al. 2018b) the pretreated samples can be directly used for enzymatic saccharification without any neutralization, washing and drying. Elimination of multiple steps in the biomass to bioethanol and other value-added products production will make the process economically viable as well as eco-friendly by eliminating effluent generation during neutralization and washing steps.

Though there are several advantages for alternative strategies of pretreatment, cost of chemicals is one of the major challenges in scale-up of the process. Most of the pilot plants for biofuel production are using acid pretreatment. But it has several drawbacks like requirement of corrosion-resistant reactor for carrying out pretreatment, generation of solid waste during neutralization, and treatment of

effluent generated during the pretreatment process (Sharma and Kumar 2013). In comparison with acid pretreatment, alkali pretreatment has several advantages like better hydrolysis efficiency due to better delignification. Disadvantages include inhibitor generation and salt formation during neutralization (Prado et al. 2012). Several studies are going on in this direction, to improve the conventional strategies as well as to make the alternative strategies economically viable.

9.2.2 Compositional Data of Native and Pretreated CPHR

Compositional data of native and pretreated CPHR are depicted in Table 9.3. Comparison of compositional data of native as well as pretreated CPHR revealed that effective hemicelluloses and lignin removal occurred with all alternative strategies of pretreatment in comparison with conventional pretreatment strategies like acid and alkali pretreatment of CPHR. Acid pretreatment removed hemicelluloses while the alkali pretreatment removed lignin. There was 30–40% of weight loss observed in all pretreatment strategies adopted for CPHR.

Table 9.3 Compositional data of native and pretreated chili postharvest residue

Pretreatment strategy	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Native CPHR	38.76	14.62	27.55	Preeti et al. (2012)
Acid pretreatment (high temperature)	44.65	–	9.57	Preeti et al. (2012)
Alkali pretreatment (high temperature)	58.72	5.57	3.68	Preeti et al. (2012)
Crude glycerol-assisted surfactant pretreatment (CGASP CPHR)	51.54	10.17	7.29	Sindhu et al. (2015)
Sono-assisted acid pretreatment (SAAP CPHR)	49.32	10.25	11.13	Sindhu et al. (2016a)
Microwave-assisted surfactant pretreatment (MWASP CPHR)	41.05	16.79	24.11	Sindhu et al. (2016b)
Ultrasound-assisted hydrothermal pretreatment (USAHTP CPHR)	44.21	10.01	20.21	Sindhu et al. (2017a)
Ultrasound-assisted alkali pretreatment (USAAP CPHR)	48.22	11.12	9.11	Sindhu et al. (2017b)
Surfactant-assisted transition metal pretreatment (SATMP CPHR)	42.11	11.23	12.13	Sindhu et al. (2017c)
Dilute acid-assisted lime pretreatment (DAALP CPHR)	43.89	10.27	14.37	Sindhu et al. (2018a)
Surfactant-assisted hydrothermal pretreatment (SAHTP CPHR)	57.23	13.15	11.79	Sindhu et al. (2018b)
Microwave-assisted acid pretreatment (MWAAP CPHR)	42.11	12.21	19.12	Sindhu et al. (2018c)

9.2.3 Enzymatic Saccharification

Enzymatic saccharifications of the pretreated biomass were carried out as per the standard protocol (Preeti et al. 2012). Reducing sugar analysis of the pretreated biomass after enzymatic saccharification was carried out by 2,5-dinitrosalicylic acid method (Miller 1959).

9.2.4 Inhibitor Profile of Hydrolysate Obtained After Enzymatic Saccharification of Different Pretreated Chili Postharvest Residue

Analysis of fermentation inhibitors like furfural, 5-hydroxymethylfurfural, and organic acids like citric acid, acetic acid, formic acid, succinic acid and propionic acid in the hydrolysate was carried out by HPLC (Sindhu et al. 2015). The study revealed that for hydrolysate obtained after enzymatic saccharification of alternative pretreated CPHR is devoid of major fermentation inhibitors like furfural, 5-hydroxymethylfurfural, and organic acids. Details are presented in Table 9.4. Earlier studies conducted by Sandhya et al. 2013 revealed that the acid pretreatment generates inhibitors like furfural, 5-hydroxymethylfurfural, and organic acids like formic acid and acetic acid which will inhibit fermentation. Many yeast strains like *Saccharomyces cerevisiae* and xylose fermenting yeasts like *Pichia stipitis* and *Candida shehatae* were inhibited by furfural (Palmqvist et al. 1999), hence a detoxification process to be carried out for inhibitor removal using adsorbents like styrene divinylbenzene (XAD 4), acrylic ester (XAD 7) and Polystyrene, a polar (XAD 12). One of the main advantages of these alternative strategies of pretreatment is that a detoxification step is not required since major fermentation inhibitors like furfural, 5-hydroxymethylfurfural, and organic acids were absent or present in small amount which is tolerable for fermentation using yeast. Hence the hydrolysate can be directly used for fermentation (Sindhu et al. 2017a, 2018b).

9.2.5 Fermentation of the Hydrolysate for the Production of Various Value-Added Products from CPHR

The hydrolysates obtained after enzymatic saccharification of different pretreated CPHR were evaluated for the production of various value-added products like bioethanol, biopolymer (poly-3-hydroxybutyrate), and xylanases (Sindhu et al. 2018c). Table 9.5 depicts the different value-added products produced by fermentation of the hydrolysate obtained from different pretreated CPHR. In all studies non-detoxified hydrolysate was used for the production of different value-added products. The study revealed that the hydrolysate obtained after enzymatic

Table 9.4 Removal of hemicelluloses and lignin from the pretreated biomass as well as inhibitor profile of hydrolysate obtained after enzymatic saccharification of different pretreated chili postharvest residue

Pretreatment strategy	Hemicelluloses removal	Lignin removal	Inhibitor profile	Washing and neutralization before enzymatic saccharification	Reference
Acid pretreatment (high temperature)	+	–	+	+	Preeti et al. (2012)
Alkali pretreatment (high temperature)	–	+	+	+	Preeti et al. (2012)
Crude glycerol-assisted surfactant pretreatment (CGASP CPHR)	+	+	–	–	Sindhu et al. (2015)
Microwave-assisted surfactant pretreatment (MWASP CPHR)	+	+	–	–	Sindhu et al. (2016b)
Ultrasound-assisted hydrothermal pretreatment (USAHTP CPHR)	+	+	–	–	Sindhu et al. (2017a)
Ultrasound-assisted alkali pretreatment (USAAP CPHR)	+	+	–	+	Sindhu et al. (2017b)
Surfactant-assisted transition metal pretreatment (SATMP CPHR)	+	+	–	+	Sindhu et al. (2017c)
Dilute acid-assisted lime pretreatment (DAALP CPHR)	+	+	–	+	Sindhu et al. (2018a)
Surfactant-assisted hydrothermal pretreatment (SAHTP CPHR)	+	+	–	–	Sindhu et al. (2018b)
Microwave-assisted acid pretreatment (MWAAP CPHR)	+	+	–	+	Sindhu et al. (2018c)

saccharification of the pretreated CPHR is suitable for the production of different value-added products like bioethanol, biopolymer (poly-3-hydroxybutyrate), and xylanases (Sindhu et al. 2016b, 2018c).

Table 9.5 Profile of value-added products obtained from the hydrolysate of different pretreated chili postharvest residue

Pretreatment strategy	Microorganism	Value-added products	References
Acid pretreatment (high temperature)		–	Preeti et al. (2012)
Alkali pretreatment (high temperature)		–	Preeti et al. (2012)
Crude glycerol-assisted surfactant pretreatment (CGASP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2015)
Sono-assisted acid pretreatment (SAAP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2016a)
Microwave-assisted surfactant pretreatment (MWASP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2016b)
	Bacillus firmus NII 0830	Biopolymer (poly-3-hydroxybutyrate)	
Ultrasound-assisted hydrothermal pretreatment (USAHTP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2017a)
Ultrasound-assisted alkali pretreatment (USAAP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2017b)
	Streptomyces sp. *Bacillus pumilus*	Xylanases	
Surfactant-assisted transition metal pretreatment (SATMP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2017c)
Dilute acid-assisted lime pretreatment (DAALP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2018a)
Surfactant-assisted hydrothermal pretreatment (SAHTP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2018b)
Microwave-assisted acid pretreatment (MWAAP CPHR)	*Saccharomyces cerevisiae*	Bioethanol	Sindhu et al. (2018c)
	Bacillus firmus NII 0830	Biopolymer (Poly-3-hydroxybutyrate)	
	Streptomyces sp. *Bacillus pumilus*	Xylanases	

9.3 Conclusion and Future Perspectives

CPHR represents one of the underutilized surplus available biomass of India. Till date only few reports were available on the utilization of this biomass for the production of bioethanol and other value-added products. Implementing proper strategies for collection, transportation, and storage of biomass as well as developing new economically viable technologies and fine tuning of existing technologies and focusing on the production of multiple products including low volume high value-added products like amino acids, itaconic acids, fine chemicals etc. can make the process a reality in the coming decades. To the best of our knowledge all reports related to CPHR were from our Centre for Biofuels group at CSIR-NIIST, Trivandrum, Kerala, India.

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Chapter 10
Valorization of Sugarcane-Based Bioethanol Industry Waste (Vinasse) to Organic Fertilizer

Ratna Dewi Kusumaningtyas, Dhoni Hartanto, Hasan Abdul Rohman, Mitamaytawati, Nur Qudus, and Daniyanto

Abstract Indonesia is among the top ten sugarcane-producing countries in the world. Among the important sugarcane-based industry is bioethanol production. Bioethanol is recently experiencing significant growth due to the increase in need of renewable energy. However, this industry faces a challenge since it produces a huge amount of liquid waste, namely vinasse. The production of 1 L of bioethanol generates 12 L of vinasse. Vinasse is pollutant due to its high value of chemical oxygen demand (COD) and biological oxygen demand (BOD), high salt content, unpleasant odor, high acidity, and dark color. Therefore, it should be treated before releasing to the environment. However, pretreatment of vinasse is not economical. The more feasible way to handle vinasse is shifting it into valuable product. Vinasse contains nutrients which are necessary for improving soil fertility and useful for plant fertilization. There are some methods to convert vinasse to organic fertilizer. This chapter shows one case study of formulating vinasse with filter cake of sugar factory, and agricultural wastes to produce liquid organic fertilizer (LOF). LOF was synthesized via anaerobic fermentation of vinasse in the presence of promoting microbes and formulation of fermented vinasse with filter cake, lead tree leaves, and banana peel to produce LOF. The LOFs were characterized to determine the values of organic C, C/N ratio, and the contents of N, P, and K elements. LOFs were applied on the tomato plant to enhance plant growth. The more advanced process of vinasse valorization is converting it into slow release solid organo-mineral fertilizer (SR-OMF).

R. D. Kusumaningtyas (✉) · D. Hartanto · H. A. Rohman · Mitamaytawati
Department of Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang, Indonesia
e-mail: ratnadewi.kusumaningtyas@mail.unnes.ac.id

N. Qudus
Civil Engineering Department, Faculty of Engineering, Universitas Negeri Semarang, Gunungpati, Semarang, Indonesia

Daniyanto
PT Perkebunan Nusantara (PTPN) XI, Surabaya, Indonesia

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Keywords Vinasse · Filter cake · Organic fertilizer · Tomato plant · Slow release organo-mineral fertilizer

10.1 Introduction

Indonesia is among the major sugarcane producing countries in the world. In Indonesia, sugarcane (*Saccharum officinarum*) is mostly utilized as raw material in sugar industry. There are 62 sugar industries for sugarcane, and 10 refined sugar industries. In Manufacture Year 2017/2018, Indonesian white sugar production increased to 2.2 MMT, while sugarcane production was stable at 28.0 MMT (Wright and Meylinah 2014). Among the major residues of sugar refinery is filter cake and molasses. Filter cake is a waste of sugarcane juice filtration, which contains organic compounds and phosphorus. Hence, this waste is potential to be converted into fertilizer (Prado et al. 2013). On the other hand, molasses is usually used for the production of ethanol (Obono et al. 2016). Sugarcane ethanol is a well-established industry and it is recently experiencing significant growth due to the increase in need of renewable energy. However, this industry faces a challenge since it produces a huge amount of liquid waste, namely vinasse.

Vinasse is the bottom effluent of distillation column in sugarcane ethanol purification process. The production of 1 L of ethanol from sugarcane generates up to 12 L of vinasse (Cassman et al. 2018). Vinasse is pollutant to the environment due to its high value of Chemical Oxygen Demand (COD) up to 140 g/L, high value of Biological Oxygen Demand (BOD), high salt content, strong unpleasant odor, very high acidity (pH 3.5–5), and heavy dark color (Hoarau et al. 2018). Therefore, it should be treated before releasing to the environment, or otherwise it will pollute surface and groundwater, as well as harm aquatic biota. However, pretreatment process of vinasse waste prior to its disposal is not economical since it is produced abundantly. The more feasible way to handle vinasse is shifting this liquid waste into valuable product.

Despite its polluting characteristic, vinasse contains nutrients which is necessary for improving soil fertility, such as nitrogen (up to 4.2 g/L), phosphorus (up to 3.0 g/L), or potassium (up to 17.5 g/L) as reported by Hoarau et al. (2018). Vinasse also comprises magnesium, calcium, and organic matters (organic acids, amino acids, sugars, polysaccharides, and proteins) which are useful for plant fertilization. This feature demonstrates that valorization of vinasse to organic fertilizer is prospective. Application of vinasse for organic fertilizer of several types of plants (sugarcane, corn, tomato, watermelon) has been studied previously.

There are some methods of applying vinasse as organic fertilizer, such as fertigation or direct application of untreated vinasse to the soil (Jiang et al. 2012), application of concentrated vinasse (Lourenço et al. 2019), formulation of vinasse-based organo-mineral fertilizer (Kusumaningtyas et al. 2017). Fertigation is simple and easy to apply. However, the direct application of unprocessed vinasse to the land shows several drawbacks to the environment, such as the increasing salt level in the

soil, groundwater pollution, and greenhouse gas emissions. Besides that, untreated vinasse contains high amount of water. Thus, fertilization needs large volume of untreated vinasse. This condition causes costly expense of vinasse transportation (Bettani et al. 2019). To overcome this obstacle, vinasse is concentrated through vaporization before being applied as organic fertilizer. This is the efficient way to lessen the volume of vinasse in order to decrease transportation cost without losing its nutrients (Lourenço et al. 2019). However, in some countries, characteristic of solitary concentrated vinasse fertilizer does not fulfill the national standard parameter of organic fertilizer properties, such as nitrogen (N), phosphorus (P), and potassium (K) contents, as well as C/N (carbon/nitrogen) ratio value. Hence, concentrated vinasse should be combined with other material to improve its characteristic as organic fertilizer. In the previous work, concentrated vinasse was blended with 3, 6, and 9% commercial NPK fertilizer to produce vinasse-based organo-mineral fertilizer (OMF). OMF was successfully employed for improving the growth of tomato and watermelon plant.

In this work, vinasse was combined with other sugar industry waste (filter cake) and agricultural wastes to produce liquid organic fertilizer (LOF) through microbiological process. Microbiological process was applied to help the decomposition of solid organic matters and transform them into organic fertilizers which are useful for improving soil fertility and plant productivity. Filter cake and agricultural wastes (lead tree leaves and banana peel) were added in formulation to enhance the quality of vinasse-based fertilizer. Filter cake is a good source of phosphorus. Phosphorus is important for supporting photosynthesis process and stomatal conductance, as well as promoting root growth (Vasconcelos et al. 2017). The improved stomatal conductance is essential for increasing transpiration rates. On the other hand, the optimum growth of root will enhance the root exploration capability, leading to increased water absorption.

Lead tree (*Leucaena leucocephala*) is a plant that is widely grown in Indonesia and commonly used for greening purpose. This plant produces lots of leaves, but this plant leaves have not been optimally utilized. In fact, lead tree leaves contain high nitrogen, phosphorus, potassium, carbon, and oxygen elements, which are necessary for supporting plant growth. ter Meulen et al. (1979) reported that lead tree leaves (LTL) contain 19.0 g/kg calcium, 2.16 g/kg phosphorus, and 17.0 g/kg potassium. It was also described that fermented LTL can be applied as liquid organic fertilizer (ter Meulen et al. 1979). The other agricultural waste employed for LOF formulation in this work was banana peels. Banana peel is potential to be used for organic fertilizer since it contains nutrients for plant. Kalemelawa (2012) stated that solid organic fertilizer made of banana peel was a potential source of K and N (Kalemelawa et al. 2012).

10.2 Vinasse

The bioethanol industry is growing because people are focusing on finding renewable energy sources. The most developed bioethanol industry in Indonesia is molasses-based bioethanol. Molasses is sugar syrup which does not crystallize after the crystallization process. Molasses which is usually reddish brown is a by-product of the sugar industry which still contains sucrose. Although there are other raw materials, generally bioethanol manufacturers prefer using molasses because molasses is cheaper and easier to obtain, the process of making molasses-based bioethanol is simpler, it contains high sucrose and nitrogen, phosphorus, sulfur, minerals, and vitamins required by yeast. However, the molasses-based bioethanol industry creates a problem of producing liquid waste called vinasse in very large quantities and it is polluting the environment.

Vinasse is the liquid waste resulting from the bottom product of distillation in the maisch column during the production of bioethanol from molasses. Vinasse contains a chemical compound that causes Chemical Oxygen Demand (COD) to increase more than 50,000 ppm and Biological Oxygen Demand (BOD) to increase more than 30,000 ppm. This waste cannot be directly discharged into the water environment or river, because it will eliminate the dissolved oxygen in it which ultimately damages the ecosystem of the biota. The main impacts caused by vinasse waste in soil and groundwater resources are salinity and increased concentrations of nitrates, nitrite, ammonia, magnesium, phosphate, aluminum, iron, manganese, chloride, and organic carbon. Metal mobilization/dissolution such as iron, copper, cadmium, chromium, cobalt, nickel, tin, and zinc can occur, as well as changes in pH in soil and ground water. The bioethanol plant produces vinasse as its liquid waste of 12 times more than the volume of bioethanol production (Leme and Seabra 2016). The characteristics of vinasse waste can be seen in Table 10.1.

Vinasse waste has high organic matter and low acidity with pH around 3.9–4.3 (Parnaudeau et al. 2008). The problem caused by the low acidity of vinasse waste is the difficulty of nutrient adsorption by plants. Nutrients can be easily absorbed by plants at pH 6–7, because at that level of acidity, most nutrients can dissolve easily in water. The level of pH in the soil also indicates the presence of toxic substances for plants. Al (aluminum) elements are found in acid soil which can both poison plants

Table 10.1 Characteristics of vinasse

Character	Information
Debit	±480 m^3 per day
pH	3.9–4.3
Suspended residue	High
NH_3	200 ppm
BOD, 20 °C	Very high
COD	Very high
Color	Dark brown to black

Source: Madubaru (2008)

Fig. 10.1 Untreated vinasse

and bind phosphorus which causes plants to be unable to absorb it. In addition to acidic soil, too many microelements can poison plants. As for alkaline soil, there are many elements of Na (Sodium) and Mo (Molybdenum). The condition of soil acidity also determines the development of microorganisms in the soil. Mushrooms and organic matter-decomposing bacteria can grow well in the environment with pH 5.5–7. In addition, microorganisms which are beneficial to roots of plant can also develop well (Gyaneshwar et al. 2002). Vinasse waste also has a reddish brown color and is malodorous (Fig. 10.1). One of the negative effects of vinasse waste on rice plants is that it causes the color of the grains of rice to become brownish and malodourous, making rice less suitable for consumption. One of the efforts to handle vinasse waste is by using vinasse as organic fertilizer. Vinasse has macro- and micronutrients including N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu which are useful and required for plant to grow (Madubaru 2008). Vinasse has been used as solid organo-mineral fertilizer (Kusumaningtyas et al. 2017). Vinasse can also be processed into liquid organic fertilizer through fermentation and non-fermentation process.

The production of organic fertilizer through fermentation process is usually conducted in the presence of Promi (Promoting Microbes). Promi is active microbes that can stimulate the plant growth. Microbes in promi involve three types of microbes, namely *Aspergillus* sp., *Trichoderma harzianum* DT 38, *Trichoderma harzianum* DT 39, and microbial weathering. *Aspergillus* sp. has an ability to solve phosphate from the insoluble sources. *Trichoderma harzianum* DT 38 has a role in stimulating plant growth. Furthermore, *Trichoderma harzianum* DT 39 is a natural agent to resist infectious soil diseases.

10.3 Organic Fertilizer

Organic fertilizers are plant fertilizers that originate from organic sources, such as animal and plant matters, human excreta, manure, etc. It commonly contains various important nutrients needed by plants, both macro and micro. The macroelements required by plants include nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg). Furthermore, microelements are iron (Fe), copper (Cu), zinc (Zn), chlorine (CI), boron (B), molybdenum (Mo), and aluminum (AI) (Chang et al. 2007). Vinasse waste, which is derived from agro-industrial process, is among the potential feedstock for composing organic fertilizer. To improve its properties as organic fertilizer, vinasse can be combined with other organic fertilizer sources, for example filter cake and agricultural wastes.

Filter cake is one of the wastes produced by sugar mills in the process of manufacturing sugar. This waste comes out of the process in solid form containing water, still has high temperature, and in the form of soil. The filter cake is actually sugarcane fiber mixed with dirt and separated from the sap. The filter cake composition consists of coir, wax and crude fat, crude protein, sugar, total ash, SiO_2, CaO, P_2O_5, and MgO. The composition of the filter cake has different percentage of content from one sugar mill to another, depending on the production pattern and origin of the sugarcane (Prado et al. 2013).

The filter cake is generally used as an organic fertilizer. The filter cake of some sugar mills are recycled as fertilizer, which is then used for fertilizing sugarcane in sugarcane plantation areas. The process of using organic fertilizer is not complicated. The fertilizer undergoes drying process for a few weeks/months for aeration in an open area. The drying process is aimed to reduce the temperature and the excessive content of nitrogen in the filter cake. By continuing to use inorganic fertilizers as a starter, the use of filter cake organic fertilizer can still be accepted by the community (Prado et al. 2013). The filter cake can be used directly as fertilizer, because it contains nutrients required by the soil. To enrich the N element, it is composted with bagasse and kettle ash. Administering 100 tons of filter cake or its compost per hectare to sugarcane can significantly increase the weight and yield of sugarcane. Besides, combining filter cake with vinasse to produce organic fertilizer is considered attractive as well.

Beside filter cake, the other potential organic substance which can be formulated with vinasse to prepare organic fertilizer is agricultural wastes. As an agricultural country, Indonesia has various and abundant sources of organic fertilizer from crops, among the others are banana peels and lead tree (*Leucaena leucocephala*) leaves. Bananas and banana peels are rich in minerals such as potassium, magnesium, phosphorus, chloride, and iron. The contents of banana peel can be seen from Table 10.2.

Lead tree leaves are widely available in Indonesia and are used for reforestation. This plant contains high levels of nutrients required by plants. Lead tree leaves contain 19.0 g/kg calcium, 2.16 g/kg phosphorus, and 17.0 g/kg potassium (ter Meulen et al. 1979). According to Kang (1981), the organic fertilizer in the form of lead tree leaves can improve soil fertility and affect the growth of plants (Kang et al. 1981).

Table 10.2 Mineral and nutrient contents in banana peel

Mineral and nutrient contents of banana peel	Total
Water (%)	68.90
Carbohydrates (%)	18.50
Fat (%)	2.11
Protein (%)	0.32
Potassium (mg/100 g)	71.5
Phosphorus (mg/100 g)	11.7
Iron (mg/100 g)	1.6
Vitamins:	
B (mg/100 g)	0.12
C (mg/100 g)	17.5

Source: Essien (2005)

10.4 Case Study: Formulation of Vinasse, Filter Cake, and Agricultural Wastes to Liquid Organic Fertilizer

One case study of vinasse valorization is the formulation of vinasse, filter cake, and local agricultural wastes (banana peels and lead tree leaves) to produce liquid organic fertilizer (LOF). In this case, vinasse was obtained from Micro, Small, and Medium Enterprises (MSME) of bioethanol production in Sukoharjo, Central Java, Indonesia. Meanwhile the filter cake was supplied by PT Madubaru Sugar Factory, Yogyakarta, Indonesia.

10.4.1 Methods

The main raw materials of the process were vinasse, filter cake (FC), banana peels (BP), and lead tree leaves (LTL). The raw materials were formulated with and without fermentation process. The fermentation was performed in the presence of promoting microbes (promi). The formulations were conducted at various compositions of feedstocks and operation condition, as shown in Table 10.3.

The conversion of vinasse into LOF was conducted through the following procedure. Vinasse (100 mL) was prepared in the beaker glass. The acidity (pH) of vinasse was adjusted by the addition of 40% NaOH solution until it reached pH 7 (neutral). Subsequently, vinasse was evaporated at the temperature of 100 °C for 1 h to remove the water and volatile compounds content until the volume of vinasse reached 80 mL (Kusumaningtyas et al. 2017). After being cooled, vinasse was then mixed with the other feedstocks as depicted in Table 10.3. Promi as much as 2 g was added for the fermentation process. The fermentation was conducted as set in Table 10.1 and in the anaerobic condition. The LOFs resulted from this process were then characterized to determine the values of N, P, and K contents, organic C content, and C/N ratio. The values were compared to the Indonesian Standard quality

Table 10.3 Formulation of liquid organic fertilizer (LOF) from vinasse

Types of LOF	Composition of raw materials				Texture	Length of fermentation, days
	V, mL	FC, g	BP, g	LTL, g		
LOF 1	100	–	–	–	Smooth	–
LOF 2	100	–	–	–	Smooth	7
LOF 3	100	12	6	12	Rough	7
LOF 4	100	12	6	12	Rough	14
LOF 5	100	12	6	12	Smooth	7
LOF 6	100	–	30	–	Smooth	7
LOF 7	100	–	–	30	Smooth	7
LOF 8	100	30	–	–	Smooth	7
LOF 9	Blending of LOF 6 + LOF 7 + LOF 8					–

Table 10.4 Indonesian Standard Quality of organic fertilizer (SNI 19-7030-2004)

No	Parameter	Unit	Minimum	Maximum
1	Water content	%	–	50
2	Temperature	°C	–	Water temperature
3	Color		–	Black brownish
4	pH		6.8	7.49
5	Impurities	%	–	1.5
	Macro element			
6	Organic compounds	%	27	58
7	Nitrogen	%	0.40	–
8	Organic C (carbon)	%	9.80	31
9	Phosphorus	%	0.10	–
10	C/N—Ratio	%	10	20
11	Kalium	%	0.20	–

of organic fertilizer (Table 10.4) in terms of the tested parameters. Next, the LOFs were applied to fertilize the tomato plant.

To examine the effectiveness of LOFs in fertilizing tomato plant, firstly, 22 pots were filled with soil and prepared as plant growing media. Seeding of tomato plant seedlings were carried out on the growing media. After 14 days, 15 mL each type of LOF was added on two pots of tomato plants for the first time. It was called as Period 1 of Fertilization. The next fertilizing periods were conducted every 10 days until it reached the sixth period of fertilization (60 days after the first period of fertilization). On each period, the growth of tomato plants was observed in terms of the height of tomato plant, diameter of stem, number of the leaves, first time of flowering, first time of fruiting, as well as the number and diameter of fruit on the last period of fertilization. An identical procedure was also performed for the tomato plant fertilized by commercial NPK fertilizer and the plant without fertilization for comparison.

10.4.2 Characterization of Liquid Organic Fertilizers (LOFs)

The physical properties of LOFs in terms of main N, P, and K contents, organic C content, and C/N ratio are demonstrated in Table 10.5.

Production of liquid organic fertilizer (LOF) can be conducted with or without fermentation. However, anaerobic fermentation in the presence of promi could accelerate the formation of fertilizer and enhance the quality of LOF (Pangnakorn et al. 2009). In the anaerobic vinasse fermentation, specific gasses, i.e., methane (CH_4) and carbon dioxide (CO_2), were released (Sydney and Sydney 2013).

The first experiment was the preparation of LOF without fermentation process (LOF 1). In this case, the solely vinasse was simply treated with 40% NaOH solution to adjust the pH and evaporated to reduce the water content. It was found that LOF 1 has fulfilled the standard parameter of N, P, K, organic C, and C/N ratio. However, when the anaerobic fermentation process was carried out on the vinasse (LOF 2) for 7 days, the NPK and organic C content were significantly improved as it is presented in Table 10.5. Besides, the C/N ratio also increased. C/N ratio is among the important parameters to control the quality of organic fertilizer (Chanyasak and Kubota 1981).

To enhance the properties of organic fertilizer, especially the content of NPK and organic C, vinasse was combined with filter cake (FC) and agricultural wastes, i.e., banana peel (BP) and lead tree leaves (LTL), with the specific formulation of LOF 3, LOF 4, and LOF 4 as depicted in Table 10.3. In LOF 3, agricultural wastes were blended with the other feedstocks in a rough texture and fermented for 7 days. A similar composition was also applied for LOF 4, but it was fermented for a longer period (14 days). It was revealed that the simultaneous blend of vinasse with FC, BP, and LTL could not provide a higher NPK and organic C content compared to the pure vinasse and fermented vinasse fertilizer (LOF 1 and LOF 2). Moreover, the C/N ratio value of LOF 3 and LOF 4 are too high (excess carbon). If the C/N ratio is too high, the decomposition will slow down. It was also found that the longer fermentation time than 7 days did not improve the quality of LOF. Hence, 7 days of fermentation is considered efficient.

Table 10.5 Properties of various types of liquid organic fertilizer (LOF)

No	Types of LOF	NPK content, %			Organic C content, %	C/N ratio
		N	P	K		
1	LOF 1	0.63	0.42	0.38	12.35	19.60
2	LOF 2	0.68	0.48	0.43	14.20	20.88
3	LOF 3	0.65	0.46	0.36	13.30	21.45
4	LOF 4	0.59	0.56	0.29	13.13	22.55
5	LOF 5	0.56	0.52	0.35	12.63	22.55
6	LOF 6	0.68	0.63	0.61	13.25	19.48
7	LOF 7	0.71	0.54	0.59	12.30	17.32
8	LOF 8	0.66	0.56	0.59	12.90	19.55
9	LOF 9	0.75	0.67	0.70	14.28	19.05

To examine the effect of agricultural wastes texture, the identical formulation of vinasse, FC, BP, and LTL was blended together, but the agricultural wastes were added in a smooth texture (LOF 5). The smooth form of material was expected to result in a more homogeneous mixture. The mixture was then fermented for 7 days. However, the changing of the texture yet did not enhance the NPK content. The value of C/N ratio was also higher than the Indonesian National Standard of organic fertilizer product.

To explore the better quality of organic fertilizer, vinasse was mixed with the BP, LTL, and FC separately as demonstrated in the LOF 6, LOF 7, and LOF 8 formulation, respectively (Table 10.3). Each mixture was fermented for 7 days. It was shown that a single blending of vinasse with BP, TLT, and FC resulted in the significant improvement of NPK and organic C contents. The value of C/N ratio also met the Indonesian standard of organic fertilizer. However, the best enhancement was given by blending LOF 6, LOF 7, and LOF 8 with ratio of 1:1:1 to obtain LOF 9. LOF 9 demonstrated the highest NPK and organic C content. The C/N ratio of LOF 9 satisfied the standard as well. Among all the types of LOF, LOF 9 has shown the best properties in terms of NPK content, C organic content, and C/N ratio. To investigate the effectiveness of vinasse-based organic fertilizer, all types of LOFs were applied for fertilizing tomato plant.

10.4.3 Application of Liquid Organic Fertilizers (LOFs) on Tomato Plants

LOFs produced in this work were applied to tomato plants. The effects of fertilization using LOFs on the tomato plants in terms of the height of tomato plant, diameter of stem, number of the leaves, first time of flowering, first time of fruiting, as well as the number and diameter of fruit on the last period of fertilization were observed. Tomato plant is selected as the medium for applying LOFs because tomato plant has a fast growth period. LOFs were primarily applied to the tomato plants which were approximately 14 days old. At this age, tomato plants already had complete growth organs. The fertilizing period was conducted every 10 days with the LOFs dose of 15 mL. There were totally six periods of fertilization during this observation, meaning that the observation was conducted for 60 days. As control, observation was also performed for the plants fertilized by commercial NPK fertilizer and plants without fertilization.

Plant height is an important parameter in plant ecological aspect. It is generally related to the life span, seed mass, and time to maturity, and is an essential factor of plant's capability to struggle for light (Moles et al. 2009). The height of tomato plants fertilized by LOFs, commercial NPK fertilizer, and without fertilization for each period of fertilization is shown in Fig. 10.2. It was disclosed that all types of LOFs provide the higher stem height of tomato plants. However, LOF 9 has shown the best enhancement on the stem height growth compared to that of the other types

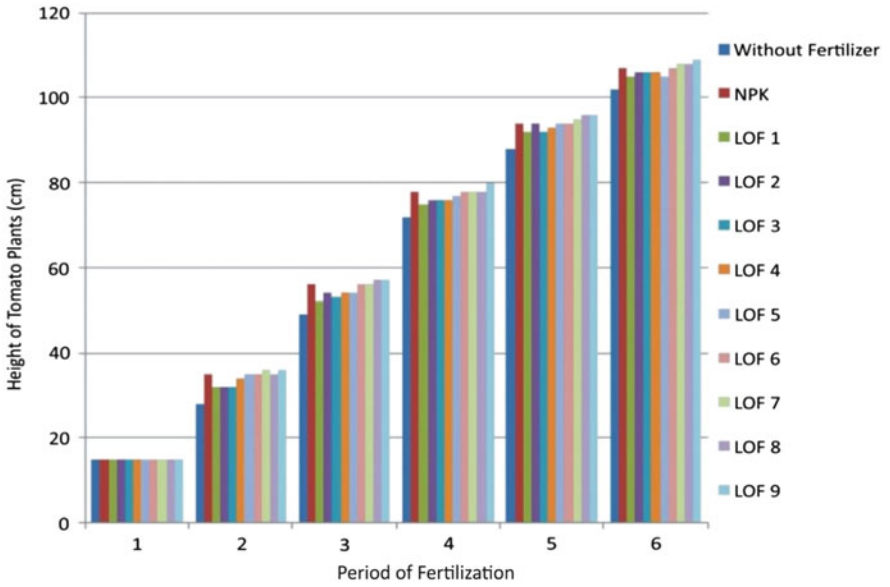

Fig. 10.2 The effect of LOFs fertilization on the height of tomato plants for each period of fertilization

of LOFs and commercial NPK fertilizer. It is due to the fact that LOF 9 contained higher organic C, N, P, and K elements than the other types of LOFs. Meanwhile, commercial NPK fertilizer has high element of N, P, K, but it does not contain organic C. Tomato plant needs nutrients such as N, P, K, and C for growing, flowering, and fruiting.

Organic C has an important function to support the photosynthesis process (Xu et al. 2015). C element can be absorbed by the plant from the air (CO_2) or from the soil (HCO_3^-). Deficiency of C element could interrupt photosynthesis process. On the other hand, the N element is important as the precursor of protein in the plant. It is also essential in the chlorophyll formation. Thus, N has a vital role to create the green color of the plant's organs. Besides, N element has a function to enhance the plant's growth, such as the growth of stem, branch, and leaves, and improve the quality of the plant (Dobermann and Fairhurst 2001). N element is absorbed by the plant in the form of NH_4^+ or NO_3^-. The lack of nitrogen brings about the symptoms of slow or dwarf growth, withering of plants, and the lack of chlorophyll, which cause the old leaves to quickly turn into yellow and die.

The effect of tomato plant fertilizing using LOFs on the growth of stem diameter is shown in Fig. 10.3. It was revealed that the addition of fertilizer increased the rate of stem growth. However, the best rate of stem growth was provided by the fertilization using NPK and LOF 9. It is not surprising since NPK and LOF 9 comprised the highest content of N, P, and K elements. In addition, LOF 9 contained the highest substance of C, compared to the other types of LOFs. Beside N, plants also need a considerable amount of P and K elements to support

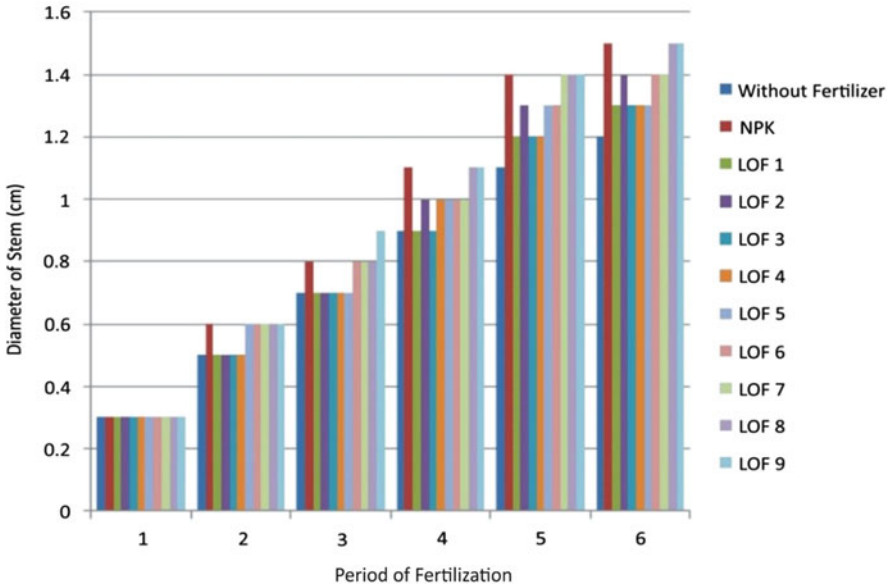

Fig. 10.3 The effect of LOFs fertilization on the stem diameter of tomato plants for each period of fertilization

their growth. The P element is principal to maintain the development of carbohydrate and to ensure the efficient mechanism of the chloroplast activity. It also plays an important role in the metabolism activity (Dobermann and Fairhurst 2001). P is beneficial to stimulate root growth and plant growth as well as supports the formation of flowers and seeds. Moreover, it accelerates fruit ripening so that it speeds up the harvest period. The lack of P causes the incomplete plant roots, dwarf, and thin plants. It also makes the leaves to become dry, and the color becomes reddish and brown.

Figure 10.4 demonstrates the influences of fertilization using LOFs on the number of leaves of tomato plants. The highest number of leaves was provided by the tomato plants fertilized by commercial NPK and LOF 9. NPK and LOF 9 had the highest content of potassium (K) element. Potassium elements are usually available for plants nutrient in the form of K^+ cations. The existence of adequate K is crucial since this element plays an important role in the carbohydrate formation, promoting the production of chlorophylls and flower, increasing root absorption to nutrients capability, and improving the plant's resistance to diseases. Inadequate K supply to the plant will bring about several indications, for instance, slow stem formation and dwarf plants, yellowing shoots like burning at the edges, the death of roots and hair roots, and the disruption on nutrient absorption (Xu et al. 2015).

Fertilization using LOF also affected the flowering and fruiting time of the tomato plants. The addition of fertilizer enhances the flowering and fruiting speed. It can be seen in Table 10.6 and Fig. 10.5 that the tomato plants which had been given LOFs

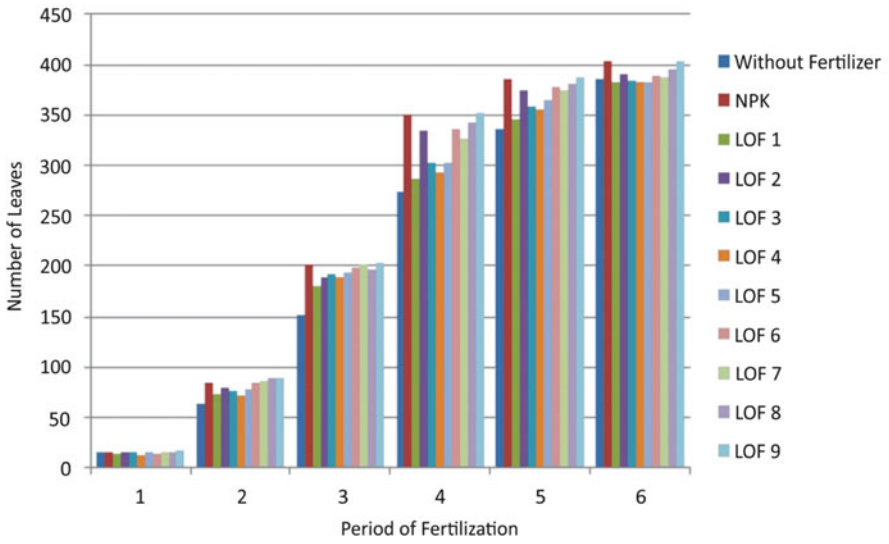

Fig. 10.4 The effect of LOFs fertilization on the number of leaves of tomato plants for each period of fertilization

Table 10.6 Flowering and fruiting time of tomato plant fertilized by LOF

Types of fertilizer	Flowering time (days)
Without fertilizer (control 1)	50
NPK (control 2)	46
LOF 1	48
LOF 2	47
LOF 3	48
LOF 4	47
LOF 5	48
LOF 6	47
LOF 7	47
LOF 8	48
LOF 9	46

needed flowering time less than 50 days. At this time, very small fruits were also found on the tomato plants. Among the others, LOF 9 has shown the earliest time of flowering and fruiting (46 days). It was due to the fact that LOF 9 had the highest content of N, P, K, and C elements, which are important for supporting the plant growth.

The flowering and fruiting speed of the tomato plants has a significant impact on the fruits produced by the plant, especially in terms of the number and diameter of the fruit as indicated in Table 10.7 and Fig. 10.6. It was demonstrated that LOF 9 produced the highest number of fruits (9) with a big diameter of fruit (4.1 cm). For comparison, tomato plants fertilized by commercial NPK could produce a slightly

Fig. 10.5 The flower and small fruit on the tomato plant fertilized by LOF at the age less than 50 days

Table 10.7 Number and diameter of tomato fruit

Types of fertilizer	Number of fruit	Diameter (cm)
Without fertilizer (control 1)	3	3.5
NPK (control 2)	8	4.2
LOF 1	5	3.7
LOF 2	7	4
LOF 3	5	3.6
LOF 4	6	3.6
LOF 5	4	3.7
LOF 6	6	3.7
LOF 7	7	3.9
LOF 8	7	3.8
LOF 9	9	4.1

higher diameter of fruit (4.2 cm), but the number of fruits was only eight. As control, tomato plant without any fertilization barely produced three fruits having a small diameter (3.5 cm).

The experimental result has denoted that vinasse-based organic fertilizers could promote the growth of the tomato plant in terms of height, stems, leaves, the speed of flowering and fruiting time of tomato plants, as well as the quality and quantity of the fruits. This innovation is a prospective alternative to give added value to vinasse waste. It is also an appropriate option to provide an economical organic fertilizer for farmers, which will support the government in increasing food security.

10.5 Future Trend: Slow Release Solid Organic Fertilizer

10.5.1 Vinasse-Based Slow Release Organo-Mineral Fertilizer

Upgrading vinasse to organic fertilizer has a high economic potency. Vinasse can be converted to liquid or solid organic fertilizer. Solid organic fertilizer provides advantages compared to liquid organic fertilizer to some extent, such as: it is easy in handling and transportation, it is more stable, and has a longer life time than LOF. Vinasse-based solid organic fertilizer, which was called organo-mineral fertilizer (OMF), has been developed by (Kusumaningtyas et al. 2017). It has been proved that OMF contained high N, P, K, and organic C and it was effective in improving the tomato and watermelon plants. However, there are some issues related to the losses of N during the application of OMF in soil for plant fertilization.

N element is important since one of the essential and limit nutrient required in agriculture production is nitrogen (Qiu et al. 2018). N fertilizer is commonly used to improve crop production. Therefore, it is produced in a huge amount to fulfill the demand of the agricultural production worldwide. The conventional use of N fertilizer becomes inefficient because more than 42–47% of N fertilizer will be leached, denitrified, or volatile (Zhang et al. 2015; Bouwman et al. 2017; Zhu et al. 2018; Siva et al. 1999). The loss of N causes some environmental pollution such as the contamination of groundwater by nitrate leaching and even causes greenhouse gas emission (Zhang 2017). It also can increase the cost in agriculture production but give less effect to the yield. Therefore, it is important to prevent the N loss through some pathways to increase the use efficiency of N fertilizer.

Some research has been conducted to develop the strategies in N fertilizer loss prevention. The biochar addition with urea can reduce the loss of N through ammonia volatilization (Zhu et al. 2018). Biochar combined with water treatment has high capabilities to decrease N loss through leaching (Zhu et al. 2018). Slow release of fertilizer from urea can also be used as a treatment to decrease the loss of nitrogen through N leaching and ammonia emission with a high yield of the crop (Yang et al. 2017; Tian et al. 2018).

Organo-mineral fertilizer (OMF) is becoming a popular strategy to enhance the efficiency of the use of fertilizer and increase the crop yields and soil health compared to the use of organic or inorganic fertilizer because of its slow release properties (Buss et al. 2019). OMF provides nutrients simultaneously for the crops through controlling the nutrient release to the soil, surface water, groundwater, and atmosphere. OMF which was made of biochar-ash composite increased the crop yields and P uptake compared with the conventional phosphate fertilizer (Buss et al. 2019). It was also used to enhance the use efficiency of N, N uptake, plant photosynthesis, and N availability during plant cycle compared to commercial organic fertilizer (Nguyen et al. 2017). Brown coal mixed with urea as a slow release organo-mineral fertilizer reduced the loss of gaseous N and increased crop yields and N uptake (Saha et al. 2019). Fly ash in a coal power plant also has potential as a slow-release fertilizer even

in calcareous soils (Hermassi et al. 2017). Slow-release fertilizer is also applied in a mixed of mono-ammonium phosphate, triple superphosphate, and phosphoric acid, with and without the addition of magnesium oxide with poultry litter yielding the slow release of P which makes potential in tropical soils (Lustosa Filho et al. 2017).

The technique of slow-release fertilizer for improving the quality of vinasse-based OMF has not been previously developed. On the other hand, the use of vinasse with the controlled-release characteristic becomes critical to enable the effective use of the nutrient as soil fertilizer. In this study, novel slow-release organo-mineral fertilizer from vinasse or vinasse-based slow release solid organo-mineral fertilizer (SR-OMF) with the chitosan–bentonite matrix was developed to investigate the nitrogen release performance in soil fertilized.

10.5.2 Methods

The first step of SR-OMF production is preparation of vinasse organo-mineral fertilizer (OMF). Initially, vinasse of 100 g was dissolved in NaOH to adjust the pH of vinasse to 7. The solution was evaporated in the temperature range of 80–90 °C for about 30 min to remove 80% water from the solution. Then, NPK was added to the vinasse-rich solution and stirred at a constant speed until NPK completely dissolved. The solution was heated using the oven in a constant temperature of 110 °C and stopped when the weight remains constant.

The second step is chitosan–bentonite matrix composite production. Primarily, chitosan solution (1% w/v) was prepared by dissolving 1 g of chitosan in 100 mL of acetic acid (2% w/v) and stirred at a constant speed. Subsequently, chitosan–bentonite matrix composite was prepared by dissolving bentonite in a chitosan solution to obtain the various concentrations of 2%, 3%, and 5%. The solution was stirred for about 5 h and then 2 drops of Tween 80 surfactant were added to the solution followed by stirring for about 1 h.

The third step, the final step, is SR-OMF production. SR-OMF was prepared by mixing OMF which consists of 9% NPK with the chitosan–bentonite matrix in a ratio of 7:3. The solution is stirred using magnetic stirrer for about 2 h at a temperature of 25 °C and then heated in a temperature of 50 °C until the mass of the solution remains constant. The SR-OMF was then obtained. It was then characterized to determine the content of N element and the N release rate.

The nitrogen release rate was conducted using incubation method in an open space in the laboratory. Dry soil of 113.79 g was undertaken in low-density polyethylene tubes of 6.0 cm diameter and 6.70 cm height. SR-OMF 1 g was mixed with dry soil of 200 g and then added to each tube. During the incubation, the leaching of nitrogen for each tube will be analyzed at days 0, 7, 14, 21, 28, and 60 through watered method. The nitrogen compositions dissolved in water was analyzed through Kjeldahl method. The carbon compositions was analyzed using a UV/Vis spectrophotometer.

10.5.3 Nitrogen Release Pattern of SR-OMF

The nitrogen concentrations were initially analyzed for the vinasse SR-OMF which consists of the mixture of the vinasse OMF and chitosan–bentonite matrix. The ratio of chitosan–bentonite was 7:3. The effect of chitosan–bentonite matrix concentration in a vinasse OMF is shown in Fig. 10.6. The concentration of chitosan–bentonite matrix added in a vinasse OMF were 0, 1, 2, 3, and 5%w/v which produced nitrogen of 0.5, 1.51, 1.66, 1.91, and 2.10%w/w, respectively. Figure 10.7 shows that the increase of the chitosan–bentonite matrix concentration can increase the total nitrogen. The maximum total nitrogen achieved in a chitosan–bentonite matrix concentration was 5%w/v.

The nitrogen release rate was analyzed through the incubation method to obtain the amount of nitrogen released to the soil. The nitrogen release rate of SR-OMF was compared to the OMF. The comparison between two fertilizers for the nitrogen release and the interval comparison before and after incubation is listed in Table 10.1 which shows that the nitrogen dissolved in water for the OMF are 0.011, 0.01, 0.008, and 0.006%w/w in 3, 6, 9, and 12 days, respectively. The total nitrogen dissolved is 0.035%w/w which is about 6.36% compared to the nitrogen concentration before the incubation. In a SR-OMF, the nitrogen dissolved is 0.01, 0.08, 0.004, and 0.003%w/w in 3, 6, 9, and 12 days, respectively. The total nitrogen dissolved in water is 0.025 which is smaller compared to the total dissolved nitrogen in OMF. This number is about 1.19% from the nitrogen concentration before the incubation. Thus, the addition of the chitosan–bentonite matrix to the fertilizer causes the decrease of the nitrogen release rate to the water because the nitrogen release is controlled by the matrix.

The nitrogen release profile for both fertilizers is shown in Fig. 10.8 which indicates the decrease of nitrogen as the day increases. The decrease in nitrogen happened because of the less nitrogen dissolved in water. This condition occurred due to the volatilization of nitrogen to the air. Nitrogen is a volatile compound which is easy to be released from the soil to the air.

Fig. 10.6 The fruit produced by the tomato plant fertilized by LOF

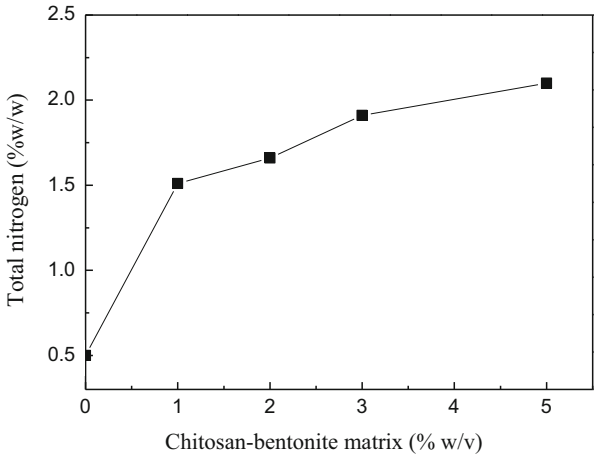

Fig. 10.7 Total N in a vinasse SR-OMF mixed with chitosan-bentonite matrix

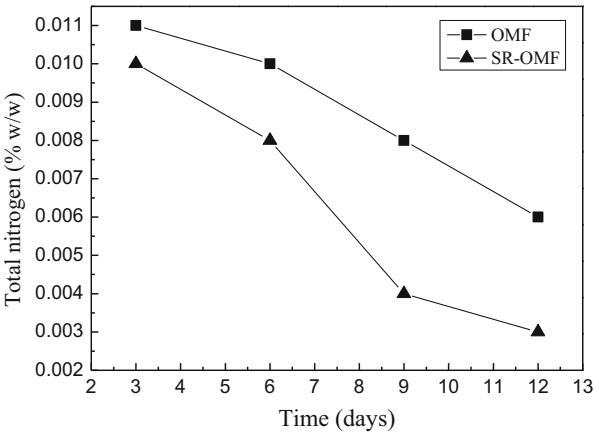

Fig. 10.8 The release rate of nitrogen for a vinasse SR-OMF and OMF

10.6 Conclusion

Vinasse waste of bioethanol industry is potential to be upgraded into liquid organic fertilizer as well as solid organic fertilizer. The ability of vinasse-based organic fertilizer to promote the growth of the tomato plant in terms of height, stems, leaves, the speed of flowering and fruiting time of tomato plants, as well as the quality and quantity of the fruits is evidence. Combination of vinasse with co-feedstocks (filter cake, banana peels, and lead tree leaves) could improve the properties of vinasse-based liquid organic fertilizer. The best way of LOF formulation vinasse, banana peels, and lead tree leaves is through the blending and fermentation process of vinasse with each feedstock separately, resulting in LOF 6, LOF 7, and LOF 8. Subsequently, the three types of LOFs (LOF 6, LOF 7, and LOF 8) were mixed together to produce LOF 9, which becomes the best formulation of vinasse and filter

cake–banana peels–lead tree leaves. On the other hand, the conversion of vinasse into solid fertilizer (OMF) is also an attractive option. To improve the effectiveness of OMF in fertilization, development of the slow-release organo-mineral fertilizer from vinasse becomes of increasing interest in research. In this work, the soil fertilized is used and analyzed to understand the nitrogen release behavior. The increase of the chitosan–bentonite matrix concentration causes the increase of the nitrogen with the maximum concentration of the matrix is 5%w/v yielding 2.1%w/w of nitrogen. The nitrogen release rate of SR-OMF is slower compared to OMF. It shows that SR-OMF from vinasse has a promising potential as a fertilizer which allows nitrogen release control to optimize the nutrient use efficiency in agricultural crops.

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Chapter 11
Pyrolytic Products from Oil Palm Biomass and Its Potential Applications

Khoirun Nisa Mahmud and Zainul Akmar Zakaria

Abstract Lignocellulosic biomass has been recognized as a sustainable feedstock for the production of renewable energy and bio-products. Various technologies including biochemical and thermochemical have been developed and applied for the conversion of the oil palm biomass. Thermochemical processes (i.e., combustion, pyrolysis, gasification, and liquefaction) could be the more economically feasible option to convert the lignocellulosic biomass quickly with lower cost compared to biochemical process due to high recalcitrant level of lignocellulosic biomass toward microbial degradation. Pyrolysis is one of the predominant technologies for lignocellulosic biomass conversion into valuable end products. This chapter provides an overview on the palm oil industry, oil palm biomass and current management scenario, pyrolysis process, parameters that influence the pyrolysis process, and the effect of these parameters on the pyrolysis product yield. The potential applications of pyrolytic products from oil palm biomass were also comprehensively addressed.

Keywords Oil palm · Biomass · Pyrolysis · Biochar · Bio-oil · Pyroligneous acid

11.1 Introduction

Agriculture is an important sector for nearly all major economies in the world. This has also resulted in huge generation of lignocellulosic biomass waste which would lead to severe environmental pollutions if not properly treated. Conversion of lignocellulosic biomass into various high-value bio-products and renewable energy through thermochemical and biochemical processes has become an interesting alternative lately (Mu et al. 2010). Thermochemical conversion process includes

K. N. Mahmud · Z. A. Zakaria (✉)
School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia
e-mail: zainulakmar@utm.my

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combustion, gasification, liquefaction, and pyrolysis (Goyal et al. 2008) while for biochemical conversion, the process includes digestion and fermentation (Balat 2006). Compared to the biochemical process, thermochemical process requires shorter period to complete, i.e., few seconds to hours. Additionally, thermochemical process utilizes entire feedstock to generate value-added end products, in contrast to biochemical process which produces lignin as a by-product that would be difficult for further conversion to C5 sugars. Thermochemical process is not feedstock specific and can even consist of various processes of feedstock (Dhyani and Bhaskar 2018).

Pyrolysis is one of the predominant techniques available to transform the lignocellulosic biomass into different value-added bio-products, biochemical compounds, and renewable energy. It is a heating process in the absence of oxygen at high temperature (Bhatia et al. 2012). The final pyrolysis product consists of solid (char), liquid (bio-oil, tar, and pyroligneous acid), and gaseous product (Demirbas 2004). Final temperature, heating rate, holding time, gas flow rate, and particle size are among the factors that would significantly influence the production and composition of pyrolytic products (Mimmo et al. 2014; Paethanom and Yoshikawa 2012). Pyrolysis process can be classified into three categories, namely slow, fast, and flash pyrolysis. Slow pyrolysis generally occurs at high temperature and slow heating rate which mainly generates high char product accompanied by liquid pyroligneous acid. Meanwhile, both fast and flash pyrolysis occur at higher temperature compared to slow pyrolysis with fast heating rate which mainly generates high liquid bio-oil and gaseous matters, respectively. This work highlights the overview on the oil palm industry and current management scenarios of the biomass generated. Potential applications of pyrolysis to convert the biomass into various end products are also discussed.

11.2 Oil Palm and Palm Oil Industry

Oil palm (*Elaeis guineensis*) is the most important species in Elaeis genus which belongs to the family of Palmae (Mekhilef et al. 2011). Oil palm tree is a large, pinnate-leaved palm having a solitary columnar stem with short internodes (Corley and Tinker 2008). Malaysia is currently the second largest producer (behind Indonesia) and exporter of palm oil in the world (Gan and Li 2014). The Malaysian palm oil industry is the fourth largest contributor to the Malaysian Gross National Income (GNI) with a value of RM 52.7 billion and is set to increase to RM 178.0 billion in 2020 (Dompok 2013). In 2018, a total of 5.85 million hectares (Malaysian Palm Oil Board 2018a) of land was planted with oil palm that has yielded 19.5 million tons of crude palm oil (Malaysian Palm Oil Board 2018b). Of these, 16.5 million tons were exported to countries worldwide with total value of RM 41.0 billion (Malaysian Palm Oil Board 2018c). Currently, the state of Sarawak has the largest area of oil palm cultivation at 1.57 million ha followed by Sabah (1.55 million ha), Pahang (0.76 million ha) and Johor, 0.75 million ha (Malaysian Palm Oil Board 2018a).

11.3 Oil Palm Biomass and Current Management Scenario

Malaysia has approximately 446 palm oil mills in February 2019, processing about 112 million tons of fresh fruit bunch (Malaysian Palm Oil Board 2019). With every kg of palm oil production, about 4 kg of dry biomass is produced (Ng et al. 2012). The reported production of oil palm biomass in 2014 is as follows: EFB—22,100,614 tons, total OPF & OPT—16,176,705 tons, MF—13,561,741 tons, PKS—5,525,154 tons, and POME—72,767,726 tons (Aditiya et al. 2016). Typical characteristics of oil palm biomass are shown in Tables 11.1 and 11.2. The varying proportion of cellulose, hemicelluloses, and lignin depends on the types of biomass. Cellulose and hemicelluloses are the main compositions present in all types of solid oil palm biomass except palm kernel shell which has high lignin content. According to Loh (2017), the high amount of cellulose and hemicellulose in EFB, MF, OPT, and OPF can be a good source for bioprocessing, while the high lignin content particularly in PKS can be a good source of biofuel for thermal combustion.

Normally, most of the biomass generated is either disposed via landfill, converted to fertilizers, or used as animal feed. Land application of palm oil mill biomass is very common practice as biomass contains numbers of beneficial nutrients by recycling it to the plant in the plantation. Composting is another conventional recycling method that has been applied for oil palm biomass. Baharuddin et al. (2009) carried out co-composting study of EFB and partially treated POME in pilot scale and results gave acceptable quality of compost with very low levels of heavy metals detected. Mixing of biomass with livestock wastes also can be a promising method to produce good compost. Study by Kolade et al. (2006) has successfully

Table 11.1 Lignocellulosic content in solid oil palm biomass

Biomass type	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Empty fruit bunch	38.30	35.30	22.10	Kelly-Yong et al. (2007)
	23.70	21.60	29.20	Samiran et al. (2015)
	38.52	33.52	20.36	Khor et al. (2009)
Mesocarp fibre	33.90	26.10	27.70	Kelly-Yong et al. (2007)
	32.40	38.20	20.50	Aziz et al. (2002)
	23.70	30.50	27.30	Abnisa et al. (2011)
	40.12	20.12	30.33	Kabir et al. (2017)
Palm kernel shell	20.80	22.70	50.70	Kelly-Yong et al. (2007)
	27.70	21.60	44.00	Abnisa et al. (2011)
	35.64	21.39	42.97	Mahmud (2017)
Oil palm frond	40.01	30.78	29.50	Kristiani (2013)
	50.33	23.18	21.70	Abnisa et al. (2013a, b)
	45.22	19.22	31.24	Kabir et al. (2017)
Oil palm trunk	34.50	31.80	25.70	Kelly-Yong et al. (2007)
	34.44	23.94	35.89	Abnisa et al. (2013a, b)

Table 11.2 Proximate analysis of solid oil palm biomass

Biomass type	Moisture content (%)	Ash (%)	Volatile matter (%)	Fixed carbon (%)	References
Empty fruit bunch	8.75	3.02	79.67	8.65	Yang et al. (2006)
	8.65	3.92	75.09	12.34	Khor et al. (2009)
	–	6.1	77.1	16.8	Idris et al. (2012)
	–	7.3	75.7	17	Husain et al. (2002)
Mesocarp fibre	9.02	7.13	71.34	12.51	Hooi et al. (2009)
	6.56	5.33	75.99	12.39	Yang et al. (2006)
	4.75	1.4	66.84	27.01	Kabir et al. (2017)
	–	10.83	73.03	16.13	Idris et al. (2012)
	–	8.4	72.8	18.8	Husain et al. (2002)
Palm kernel shell	7.96	1.1	72.47	18.7	Daud et al. (2000)
	–	11.08	73.77	15.15	Idris et al. (2012)
	–	3.2	76.3	20.5	Husain et al. (2002)
	5.73	2.21	73.74	18.37	Yang et al. (2006)
	9.5	2.5	84.5	3.5	Mahmud (2017)
Oil palm frond	13.84	0.24	82.7	3.22	Abnisa (2013b)
	–	1.3	83.5	15.2	Guangul et al. (2012)
	4.83	5.87	70.33	18.97	Kabir et al. (2017)
Oil palm trunk	9.97	4.79	75.2	10.04	Khor et al. (2009)
	7.16	5.27	82.6	4.97	Abnisa (2013b)
	2.1	4.3	76.7	16.9	Alias et al. (2015)

converted the palm kernel cake, mixed with goat or poultry manure, as environmentally friendly fertilizer to improve plant growth.

Nasrin et al. (2008) found that milled empty fruit bunch (EFB) and EFB mixed with sawdust was a potential raw material for briquettes production and it showed good burning properties. Oil palm biomass also has been utilized in paper making industry as an alternative to reduce the dependency on current trees (i.e., coniferous and deciduous trees). Two types of oil palm biomass namely OPT and EFB are potential raw materials for pulp and paper making industry. According to Singh et al. (2013), paper from EFB would have good printing properties and a good formation within paper making due to the high number of fibers/unit weight. Review by Sumathi et al. (2008) discussed the application of OPT to produce plywood, particle board, laminated board, fiber board, furniture, and paper. The plywood made from OPT is higher in strength compared to commercial plywood. The OPT also could be used as an alternative source for compressed wood for industrial application (Sulaiman et al. 2012).

11.4 Pyrolysis Process

Pyrolysis is generally described as thermal degradation of the organic components in biomass wastes at high temperature in the absence of oxygen (Demirbas and Arin 2002). Three categories of pyrolysis (i.e., slow, fast, and flash) can be differentiated in terms of chemistry, yields, and product quality (Karaosmanoğlu et al. 1999). Slow pyrolysis can be classified into conventional charcoal making and modern processes. Slow pyrolysis is characterized by a low to intermediate temperature with slow heating rate and long residence time. The biomass feed size is generally larger than biomass feed for fast or flash pyrolysis. Fast pyrolysis is applied to produce high yields of liquid fuels that can be used as substitute for conventional fuels or as chemical source (Kan et al. 2016). Fast pyrolysis is described by high reaction temperature, fast heating rate, short residence time, and small size of biomass feed. Fast or flash pyrolysis is the process of choice when the primary product of interest is bio-oil (Kan et al. 2016).

11.4.1 Pyrolysis Product

Pyrolysis offers efficient utilization of biomass to produce solid (biochar), liquid (bio-oil, tar and pyroligneous acid), and gaseous product. The first main product of the pyrolysis is biochar. For high biochar production, a low temperature with low heating rate and longer vapor residence time would be required (Demirbas 2004). The properties of biochar such as macroscopic morphology and microscopic porosity are affected by the properties of biomass feedstock, heating rate, final temperature, and residence time (Onay 2007; Demirbas 2004). For char, relevant properties relative to its effect on slow pyrolysis are calorific value, elemental composition, and surface area property. The surface area is important for reactivity and combustion behavior of char. Highest amount of biochar can be generated from the pyrolysis of PKS with a value of 32.26% followed by MF (29.8 wt.%) and EFB (29.05 wt.%). The second main product of pyrolysis is liquid product. The yield of liquid product could be maximized by applying moderate temperature with high heating rate and short vapor residence time during pyrolysis (Demirbas 2004). The liquid fraction of the pyrolysis products consists of two phases namely nonaqueous phase (wood tar, bio-oil) and aqueous phase (pyroligneous acid, wood spirit) (Beis et al. 2002; Karaosmanoğlu et al. 1999). The nonaqueous phase contains high molecular weight insoluble polar organics, mostly aromatics and water. Bio-oil is nonaqueous phase of liquid pyrolysis product and its production commonly occurs via fast pyrolysis (Demirbas and Arin 2002). Bio-oil is composed of complex mixtures of organic compounds such as anhydro-sugars, alcohols, ketones, aldehydes, carboxylic acids, phenols, alkanes, and aromatic compounds (Choi et al. 2015; Abnisa et al. 2013a, b). Pyroligneous acid is an aqueous phase fraction generated from condensation of vapor released during charcoal production or pyrolysis. It has smoky and sour

odor with acidic pH (Wei et al. 2010). Pyroligneous acid contains a complex mixture of water (80–90%) and organic compounds (10–20%).

The third main product of pyrolysis process is the gaseous product. For a maximum fuel gas yield, a high temperature with low heating rate and long gas residence time was chosen during pyrolysis process (Demirbas 2004). The main gas compounds present are of H_2, C_2, CO_2, CH_4, and traces of C_2H_4 and C_6H_6. The synthesized gas can be burned directly or processed to create higher energy fuels or chemical products.

11.5 Pyrolytic Product of Oil Palm Biomass

11.5.1 Biochar

Pyrolysis of solid oil palm biomass mixtures produces biochar with high chemical resistance, surface area, porosity, carbon, and low ash and moisture, thus showing desirable characteristics for potential use as pollutant adsorbent in wastewater treatment, or bio-fertilizer to absorb nutrients and promote plant growth (Liew et al. 2018). Study by Kong et al. (2019) reported that the biochar produced from palm kernel shell prove to be a good adsorbent of methylene blue dye because of high fixed carbon content, porosity, BET surface area, and pore volume of the biochar. Lee et al. (2017) showed the potential of palm kernel shell and empty fruit bunch were very promising sources for biofuel. The hydrogen released as flue-gas during microwave pyrolysis of oil palm fiber is also a promising alternative and sustainable energy resource. Microwave pyrolysis of palm kernel shell produced a biochar with zero sulfur content and high heating value (23–26 MJ/kg) that is comparable to conventional coal, thus indicating its potential as an eco-friendly solid fuel (Liew et al. 2018). Biochar porosity is an important character which is significant for soil fertilization. Biochar with high porosity can be used as soil conditioner material because it allows microbials to stay alive in drought periods and enhances the soil ability to retain water, nutrients and agricultural chemicals, thus preventing water contamination and soil erosion (Mullen et al. 2010). The pyrolysis of palm kernel shell also produced biochar containing a highly porous structure with a high BET surface area, thus exhibiting desirable adsorption properties to be used as bio-fertilizer since it can adsorb nutrient and water (Nam et al. 2018).

The biochar from PKS which has high fixed carbon and very low ash content makes it suitable as adsorbent agent. Improvement of this biochar as an adsorbent can be done by physical or/and chemical activation in order to produce a high surface area material known as activated carbon. A highly porous activated carbon produced from palm kernel shell via microwave vacuum pyrolysis combined with sodium–potassium hydroxide (NaOH-KOH) mixture and carbonization followed by steam activation (Zainal et al. 2018) successfully reduced the total suspended solid (TSS), oil and grease, biochemical oxygen demand (BOD), and chemical oxygen demand

(COD) in palm oil mill effluent (POME). Activated carbon from empty fruit bunch prepared by pyrolysis followed by physical activation using carbon dioxide (CO_2) also was found able to reduce the organic pollutants and colored component of POME (Wafti et al. 2017). A single-step microwave pyrolysis of palm kernel shell has been explored by Yek et al. (2019) to produce activated carbon and it showed high adsorption efficiency of methylene blue.

11.5.2 Bio-Oil

Bio-oil has a promising potential as fossil energy and fuel substitute due to its great versatility in feedstock and environmental benefits. The high volatile content (73.5–78 wt.%) and low ash content (2.9–8.6 wt.%) of oil palm biomass which consists of PKS, EFB, and MF favors the production of bio-oil (Abnisa et al. 2013a, b). Higher hydrogen content in biomass is also advantageous for production biofuel in terms of its calorific value. The hydrogen content of bio-oil from EFB was approximately 12.03 wt.%, compared to bio-oil from MF (10.18 wt.%) and PKS (9.08 wt.%), thus giving high calorific value of bio-oil (Abnisa et al. 2013a, b). Bio-oil contain more than 70 wt.% of oxygen where high oxygen content contributes to low calorific value of bio-oil. An upgraded of bio-oil is required to increase the calorific value of the oil before can be used in industrial applications. Another problem is the high acidity of bio-oil, which hinders bio-oil storage and processing. The degradation of hemicelluloses and lignin during pyrolysis contributes to the presence of acidic compounds and results in corrosion of storage and fluidization piping system (Abnisa et al. 2013a, b). Study by Chow et al. (2018) reported that an increase in pH value of bio-oil was achieved during co-pyrolysis of empty fruit bunch and palm oil sludge. Nevertheless, it led to a negative synergistic effect in terms of bio-oil yields.

11.5.3 Pyroligneous Acid

Studies showed that phenolic compounds in pyroligneous acid from various sources of biomass are the major compounds responsible for the antioxidant activity (Ma et al. 2013, 2011; Wei et al. 2010). Mahmud et al. (2016) studied the feasibility of pyroligneous acid from palm kernel shell as a potential antioxidant agent. The pyroligneous acid showed higher antioxidant activity as free radical scavengers and ferric reducing power compared to commercial antioxidant agents namely ascorbic acid and butylated hydroxyanisole (BHA). The pyroligneous acid also exhibits superoxide scavenging capability twice lower than ascorbic acid, nevertheless still can be considered as potential alternative antioxidant agent. From their study, phenols and derivatives compounds mainly benzene-1,2-diol (catechol), 1,3-dimethoxy-2-hydroxybenzene (syringol), 3-methoxy-1,2-benzenediol, and

4-methylbenzene-1,2-diol found in the phenolic fraction of pyroligneous acid were presumed to be the major antioxidant compounds.

Abas et al. (2018) reported that pyroligneous acid from oil palm fiber exhibits antibacterial properties against Gram-negative (i.e*Escherichia coli* ATCC 25922) and Gram-positive bacteria (i.e., *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, and *Lactobacillus plantarum* WICC B18) with low minimal inhibition concentration (MIC) value ranging from 0.651 ± 0.13 to 1.563 ± 0.00 mg/mL. Study by Ariffin et al. (2017) reported that pyroligneous acid from pyrolysis of palm kernel shell was able to inhibit the growth of *B. cereus*, *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* with MIC ranging 1.95–3.91 mg/mL. Their study also reported the reduced formation and metabolite activities of biofilm by these bacteria up to 80–93% at 64-MIC and 77–93%, respectively, within 24 h. Pyroligneous acid from oil palm trunk has been evaluated as antifungal by Oramahi et al. (2018) where the growth of white-rot fungus, *Trametes versicolor*, and a brown-rot fungus, *Fomitopsis palustris*, was inhibited at different specific concentrations. Their study on antitermitic activity of pyroligneous acid from oil palm trunk toward *Coptotermes formosanus* also was successfully evaluated. Study by Mahmud et al. (2016) reported the effectiveness of pyroligneous acid from palm kernel shell as antifungal toward *Aspergillus niger* and *Botryodiplodia theobromae*. The effects of pyroligneous acid from palm kernel shell as anti-inflammatory agent which was evaluated by Mahmud (2017) in a RAW 264.7 macrophage cell induced with lipopolysaccharides (LPS) from *E.coli* 0111:B4 strain revealed that the pyroligneous acid possessed anti-inflammatory properties by suppressing the inflammatory mediator nitric oxide (NO) generation better compared to steroidal drug prednisolone at concentration of 12.5 μg/mL. Furthermore, there was no toxicity sign showed toward RAW 264.7 cell at this indicated concentration.

11.6 Future Prospects of Oil Palm Biomass Pyrolysis

In recent years, studies have been extensively carried out on oil palm biomass conversion through pyrolysis process for production of biofuel, bioenergy, and bio-products. However, the use of these pyrolytic products at commercial scale is still challenging in particular the application of bio-oil as biofuel due to its unfavorable fuel properties (high oxygen content and low calorific value) and instability (high acidity). Researchers have developed various processes, reactors, and catalysts for pyrolysis process to overcome these issues. Development of new bio-products for nutraceutical and pharmaceutical application using pyroligneous acid also requires further investigation through in vivo studies for humans and clinical assays.

11.7 Conclusion

Malaysia has the potential to be one of the major contributors of bio-based products generated from oil palm biomass. This was based on the availability of feasible technologies and the abundance of oil palm biomass throughout Malaysia. Development of newer technologies and technical knowledge that will enhance production of high-quality bio-based products (i.e., biofuel, bioenergy, and bio-products) from pyrolysis of oil palm biomass is crucial to make it attractive for commercialization and able to compete with existing products in the market. Furthermore, appropriate infrastructure and sufficient financial support by the government are also much needed for the expansion and advancement of oil palm biomass industry.

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