Guanqun Chen · Randall J. Weselake
Stacy D. Singer *Editors*

Plant Bioproducts

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Editors
Guanqun Chen
Department of Agricultural, Food and Nutritional Science
University of Alberta
Edmonton, AB, Canada

Randall J. Weselake
Department of Agricultural, Food and Nutritional Science
University of Alberta
Edmonton, AB, Canada

Stacy D. Singer
Agriculture and Agri-Food Canada
Lethbridge, AB, Canada

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Preface

As a society on a global level, we have become increasingly dependent on energy, industrial products and consumer goods derived from petroleum. Unfortunately, such heavy use of petroleum results in the production of more carbon than can be offset by its capture and sequestration by living or recently living organisms, most often in the form of plants that are not used for food or feed. One possible way to reduce carbon emissions is through the increased use of sustainable, bio-based alternatives to petrochemicals. Bioproducts are materials, chemicals, fuels and energy derived from various renewable biological sources including plants, animals and microorganisms. In the strictest sense, bioproducts do not include food and feed. They may, however, have applications such as materials used in automobiles, adhesives, packaging, coatings for food products, components of pharmaceuticals, materials used in medical products and industrial enzymes. In terms of plant bioproducts, the major components used for their production include storage lipids, complex carbohydrates and proteins. Increasing our use of plant components for the generation of fuel and other bioproducts will contribute to a more sustainable and environmentally acceptable approach than a system which is heavily reliant on fossil fuels.

Plant Bioproducts presents an overview of how various plant-derived components can be used to generate bioproducts. The impetus for this book stems from our involvement in delivering third-year courses in plant bioproducts at the University of Alberta and University of Manitoba. We anticipate that this book will be particularly useful to the senior undergraduate student in plant sciences who may have a limited understanding of chemistry, biochemistry and molecular biology. It could also be valuable to those from the general public who are interested in learning about plant bioproducts, non-biological engineers who are interested in moving into the realm of biological engineering, and agricultural industrialists and other stakeholders who wish to increase their understanding of biology in relation to the generation of bioproducts.

Plant Bioproducts begins with a brief discussion of environmental problems associated with increasing carbon emissions and how plant bioproducts can contribute to help close the carbon cycle (Chap. 1). Chapter 2 presents a brief and

simplified review of how atoms can be united to form biomolecules of increasing complexity in plants and insight into the chemical diversity of plants as it relates to providing starting material for bioproducts. Depending on the reader's background knowledge, this chapter may serve as a minimal prerequisite for appreciating the ensuing discussions concerning the production of plant bioproducts from various plant components. Chapter 3 provides insight into how bioproduct production can benefit from the integration of various disciplines. Indeed, the area of plant bioproducts is based on several interdependent and allied disciplines including chemistry, plant biochemistry and physiology, plant breeding, agronomy, microbiology, molecular biology, chemical and biochemical engineering, biotechnology and the social sciences. Chapters 4, 5, 6, 7, 8 and 9 introduce plant lipids, carbohydrates and proteins, and demonstrate how these plant components can be used to produce various bioproducts. Chapters 4 and 5, in particular, place a strong emphasis on the potential of genetic engineering as a means of altering the lipid components of plants so as to provide a starting material that is more amenable to bioproduct production. To make plant bioproducts commercially attractive, it is important to integrate the production of bioproducts into existing well-developed chemical industries. The chemical industry is currently based on relatively small building block chemicals derived from petroleum; however, these molecules can also be produced from plant biomass. With the help of the background information provided in previous chapters, Chap. 10 introduces these molecules and describes how they can be used to produce a wide range of intermediates or end-products, with an emphasis on the production and use of these building block molecules from plants. Moreover, the full and effective use of all plant parts is important, in which the biorefinery plays a crucial role. Therefore, in Chap. 11, the biorefinery process of plant-based products is described, along with current research and development in this field. Although the production of bioproducts from plants is attractive, there have been concerns regarding the increasing use of plants for biofuel production instead of food. In the final chapter (Chap. 12), a background to biofuel policies in the leading biofuel producing nations and a contextual overview of what has occurred regarding commodity prices are presented. Subsequently, a discourse of the price spike assessments is offered, providing insight into just how complicated an issue this has turned out to be.

We thank our various colleagues who contributed critical chapters to making this book possible. We would also like to acknowledge Dr. Xiao Qiu of the University of Saskatchewan for stimulating discussions on plant bioproducts. In addition, we express our gratitude to Crystal Snyder for her outstanding contributions in the early stages of this project and to Lucas Falarz and Trinh Nguyen for their contributions to figures, tables and editorial aspects related to overall development of the book. We are deeply grateful to Susan Safren and Sabina Ashbough of Springer. Susan was incredibly patient and provided solid support from the early stages of this book's development. Finally, we acknowledge the support of the University of Alberta, Agriculture and Agri-Food Canada, Alberta Innovates

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Edmonton, AB, Canada — Guanqun Chen
Edmonton, AB, Canada — Randall J. Weselake
Lethbridge, AB, Canada — Stacy D. Singer

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Contributors

Muhammad Arif Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada

Guanqun Chen Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Loo-Sar Chia Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada

Michael K. Deyholos Department of Biology, University of British Columbia, Kelowna, BC, Canada

Shahram Emami Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Lucas J. Falarz Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Simona Lubieniechi Department of Agricultural and Resource Economics, University of Saskatchewan, Saskatoon, SK, Canada

K. Peter Pauls Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada

Kornsulee Ratanapariyanuch Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Martin J.T. Reaney Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Prairie Tide Chemicals Inc., Saskatoon, SK, Canada

Guangdong Saskatchewan Oilseed Joint Laboratory, Department of Food Science and Engineering, Jinan University, Guangzhou, Guangdong, China

Youn Young Shim Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Prairie Tide Chemicals Inc., Saskatoon, SK, Canada

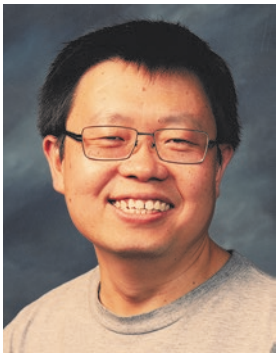
Guangdong Saskatchewan Oilseed Joint Laboratory, Department of Food Science and Engineering, Jinan University, Guangzhou, Guangdong, China

Stacy D. Singer Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

Stuart J. Smyth Department of Agricultural and Resource Economics, University of Saskatchewan, Saskatoon, SK, Canada

Randall J. Weselake Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Editor Biographies



Guanqun (Gavin) Chen is an Assistant Professor and Canada Research Chair (Tier II) in Plant Lipid Biotechnology with the Department of Agricultural, Food and Nutritional Science at the University of Alberta (Edmonton, Alberta, Canada) and Adjunct Professor at the University of Manitoba (Winnipeg, Alberta, Canada). He is well recognized for his contributions to the development of biotechnological strategies for producing specialty fatty acids and functional oils in microorganisms and plants. He received his Bachelor of Biochemical Engineering and Master of Fermentation Engineering at Tianjin University of Science and Technology (Tianjin, China) and PhD in Plant Biotechnology at The University of Hong Kong (Hong Kong, China), and served as a postdoctoral researcher at the University of Alberta before he started his career at the University of Manitoba as an assistant professor (2015–2017), where he developed and taught a senior level course *Plants for Industrial Uses*. Gavin has rich research experience in plant biotechnology, applied microbiology, molecular biology and lipid biotechnology, and his work has been published in dozens of peer-reviewed papers.

Randall J. Weselake is Professor Emeritus with the Department of Agricultural, Food and Nutritional Science at the University of Alberta. Randall has over 30 years of experience in the biochemistry and molecular biology of storage lipid metabolism in oil crops and other oleaginous plant species. In addition, he has conducted research on lipid metabolism in yeast and livestock. Randall held a Tier I Canada Research Chair in Agricultural Lipid Biotechnology at the University of Alberta from 2005 to 2016. During his tenure there, Randall developed and taught a senior level course

dealing with plant bioproducts. From 2010 to 2016, Randall served as Scientific Director of the Alberta Innovates Phytola Centre which specialized in oilseed innovations, including research and development of industrial oil crops. In addition, Randall was leader (2007–2013) of the "Bioactive Oils Program", funded by AVAC Ltd., and co-leader (2006–2011) of the large-scale Genome Canada/Genome Alberta project "Designing Oilseeds for Tomorrow's Markets". From 1989 to 2004, Randall was with the Department of Chemistry and Biochemistry at the University of Lethbridge (Alberta, Canada) where he served as Chair from 1996 to 1999. His doctoral research in plant biochemistry was conducted at the University of Manitoba and Grain Research Laboratory of the Canadian Grain Commission (Winnipeg, Manitoba, Canada). Randall previously served as Joint Editor-in-Chief of the *American Oil Chemists' Society* (*AOCS*) *Lipid Library*, Associate Editor for *Lipids* and Editor for *Biocatalysis and Agricultural Biotechnology*. He has published extensively in refereed journals and books and is editor of the book *Teaching Innovations in Lipid Science* which was published in 2007 by the Taylor & Francis Group of the CRC Press and the AOCS Press. He is also one of the co-editors of the book *Industrial Oil Crops* which was published by Elsevier/AOCS in 2016. Randall is a Fellow of both the *AOCS* and the *International Society of Biocatalysis and Agricultural Biotechnology*.

Stacy D. Singer is a lead scientist with Agriculture and Agri-Food Canada at the Lethbridge Research and Development Centre (Lethbridage, Alberta, Canada) where her research focuses on the biotechnological improvement of forage crops using advanced molecular technologies. She received her bachelor's and PhD degrees from the University of Regina, Canada. She was a postdoctoral research in plant-related research in the USDA, Cornell University, and the University of Alberta. She has published dozens of peer-reviewed research articles in such high impact journals as *Plant Physiology*; *Plant Journal*; *Plant, Cell & Environment*; and *Journal of Experimental Botany*. She has rich experience in plant molecular biology and crop improvement, and her research has contributed significantly to various areas of plant molecular biotechnology.

Chapter 1
Building a Case for Plant Bioproducts

Randall J. Weselake, Guanqun Chen, and Stacy D. Singer

Chapter Highlights

- Global carbon emissions associated with fossil fuel dependence are increasing steadily and are a major root cause of climate change.
- In order to reduce our reliance on fossil fuels, there is an imminent need to find sustainable alternatives to petrochemicals.
- Bioproducts are produced from renewable biomass and include biochemicals, bioenergy, biofuels and biomaterials.
- Increased use of bioproducts will assist in curtailing our dependence on fossil fuels and reduce greenhouse gas emissions.
- Ultimately, our goal should be for all carbon dioxide produced to be recaptured into renewable biomass, thus closing the carbon cycle.

1.1 Introduction

Until about 200 years ago, humans relied almost exclusively on bioproducts to fulfil their material and energy needs (Primer on Bioproducts 2004). Since the Industrial Revolution, however, the global community has become increasingly dependent on energy derived from petroleum for heat, generation of electricity, transportation fuel and the manufacture of industrial products and consumer goods (Fig. 1.1). Indeed,

R. J. Weselake (✉) · G. Chen
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
e-mail: randall.weselake@ales.ualberta.ca

S. D. Singer
Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

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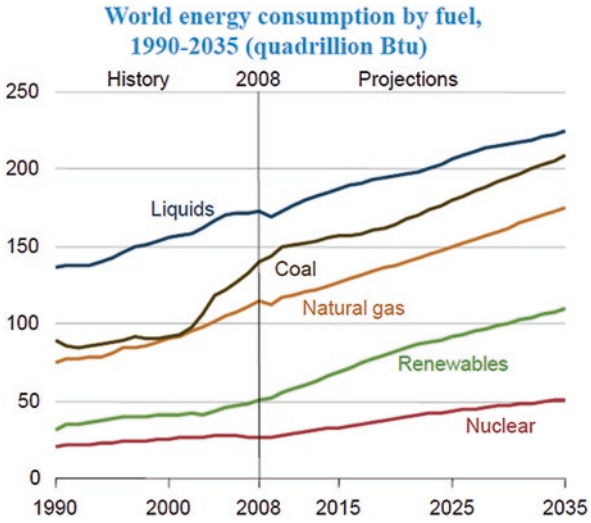

Fig. 1.1 World energy consumption by energy source, 1990–2040 (quadrillion Btu). (From: International Energy Outlook (2017))

today's petroleum provides about 85% of the world's energy requirements. Although there is currently a glut of oil on the market, fossil fuels are still a finite resource. Experts debate on when fossil fuels will run out; however, a supply crisis is sure to eventually occur given our insatiable appetite for fossil fuel-derived energy. Furthermore, political instability and war in some oil-producing areas can also impact supply. In March 2002, a white paper prepared by the Colorado River Commission of Nevada (2002) indicated world petroleum, natural gas and coal reserves were estimated to last 98, 166 and 230 years, respectively. The recent identification of new fossil fuel deposits, along with improved extraction procedures, however, will likely increase the amount of time we have left before reserves run out (Kerr 2010, 2011).

Carbon emissions associated with fossil fuel dependence are a main driver of global **climate change** and are thus a major concern, especially given the rate at which their production has been escalating in the past few decades. These emissions contain **greenhouse gases** (GHGs), which are gases that absorb and emit radiation within the thermal infrared range and result in atmospheric warming. Indeed, escalating fossil fuel emissions have been linked to rising global temperatures, increasing sea level and reduced snow cover and glacial thickness (Intergovernment Panel on Climate Change [IPCC] 2007). As an example of this, in early August 2016, arctic conditions allowed the 280-metre-long cruise ship, Crystal Serenity, to travel through the Northwest Passage and stop at the hamlet of Cambridge Bay, Nunavut (Canadian Broadcasting Corporation [CBC] News 2016). Climate change has also been implicated in an increase in severe weather and flooding (Smith and Katz 2013; Estrada et al. 2015), with other well-known environmental costs including the destruction of wildlife habitat and accidental exposure to chemicals. The BP oil spill in the Gulf of Mexico, which commenced on April 20, 2010, and continued until July 15, 2010, spilling an estimated 650 million litres of oil (CBC News 2015) is one example of this. This was one of the largest oil spills in history, affecting up to 400 animal species.

Table 1.1 Greenhouse gas emissions based on type of gas from 2010

Type of gas	Global contribution (%)
Carbon dioxide from fossil fuel and industrial processes	65
Methane	16
Carbon dioxide from forestry and land use	11
Nitrous oxide	6
Fluorinated gases	2

Source: IPCC (2014)

Table 1.2 Contributions of various countries to carbon dioxide emissions from fossil fuel consumption and some industrial processes

Country	Global contribution for carbon dioxide emission (%)
China	30
USA	15
India	7
Russian Federation	5
Japan	4
Other countries	39

Source: Data of 2014, from Boden et al. (2015)

GHG emissions come in many forms having different contributions (Table 1.1). Interestingly, while water is actually the most abundant GHG, it is not as sensitive to the impact of human activities as other gases and, as such, does not have much of an effect on the environment. Conversely, carbon dioxide is the worst offender in terms of GHGs, followed by methane and nitrous oxide (IPCC 2014). Global contributors to carbon dioxide emissions stemming from fossil fuel combustion and some industrial processes are shown in Table 1.2 (Boden et al. 2015), with China and the USA being the leading contributors.

GHG emissions come from various sources (IPCC 2014; Table 1.3), with fossil fuels and industrial processes being the greatest contributors to carbon dioxide emissions. GHG emissions from agricultural activity account for about 24–26% of total global **anthropogenic emissions** (Sejian et al. 2011; Natural Environment Research Council [NERC] 2016), which refers to pollutants originating from human activity. Similarly, livestock account for about 18% of the world's anthropogenic GHG emissions, which includes the effect of deforestation to generate grazing land along with methane gas emissions (Gill et al. 2010; NERC 2016). Since plants actively take up carbon dioxide during photosynthesis, deforestation removes critical "carbon sinks" from the environment, which causes changes in the amount of solar radiation reflected back into the atmosphere and is referred to as **albedo**.

Furthermore, the "concrete jungles" of the world are dependent on enormous amounts of cement, which also contributes to GHG emissions. Cement production

Table 1.3 Global greenhouse gas (GHG) emissions by economic sector

Economic sector	Contribution to GHG emissions (%)
Electricity and heat production	25
Agriculture, forestry and other land uses	24
Industry	21
Transportation	14
Buildings	6.4
Other energy	9.6

Source: IPCC (2014)
GHG greenhouse gas

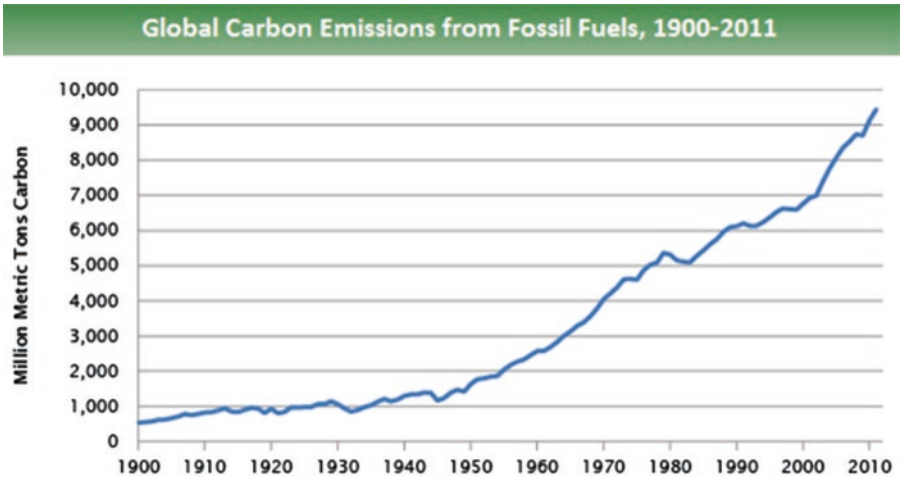

Fig. 1.2 Carbon emissions over time. (From: Boden et al. (2015))

accounts for about 5% of anthropogenic carbon dioxide emissions (Hendricks et al. 2004; NERC 2016), with about half arising from the chemical process itself since cement is mostly made of calcium carbonate. Another 40% of carbon dioxide emissions related to cement production come from burning fuel associated with the process. Indeed, approximately 900 kg of carbon dioxide is emitted for every 1000 kg of cement produced.

Between 1950 and 2010, our global carbon emissions have increased by about sixfold (Boden et al. 2015; Fig. 1.2). If this rate of GHG emissions continues, it has been estimated that we could exceed the 2 °C global warming threshold set by the IPCC as early as 2036 (Mann 2014). Global warming above this threshold value has been predicted to have serious, and potentially irreversible, consequences to both the environment and human livelihood. In order to limit global warming to 2 °C or less between now and the year 2100, we will need to reduce our GHG emissions by 40–70% by 2050 and reach a point of zero GHG emissions by the end of the century (IPCC 2014). Given that our global demand for energy, fuel and industrial chemi-

cals is projected to grow at a very fast rate over the next few decades, it is essential that we reduce our reliance on fossil fuels and switch to cleaner and more sustainable forms of energy.

1.2 Towards Closing the Carbon Cycle

In order to reduce our reliance on fossil fuels and reduce GHG emissions, there is a need to use them more efficiently and to diversify our energy usage to include hydroelectric, hydrogen fuel cell, nuclear, wave action, wind, solar, geothermal and **biofuel**-derived power. Currently, the burning of fossil fuels results in the production of more carbon than can be offset by **biomass** (Fig. 1.3a), which can be generally defined as biological material from living or recently living organisms and most often refers to plants or plant-based materials that are not used for food or feed (https://en.wikipedia.org/wiki/Biomass). If our fossil fuel dependence is eliminated, however, we will then have a situation where the carbon dioxide produced from burning biomass is recaptured into renewable biomass, thus closing the carbon cycle (Fig. 1.3b).

In 2005, 192 countries signed the Kyoto Protocol, which was aimed at "stabilizing GHG concentrations in the atmosphere to a level that would prevent dangerous anthropogenic interference in the climate system" (United Nations Framework Convention on Climate Change). This document established legally binding commitments to reduce major GHGs. The USA, however, did not end up ratifying and Canada withdrew in 2011 (CBC News 2011). That being said, Canada remains a member of the Copenhagen Accord (2009), which represents a nonbinding agree-

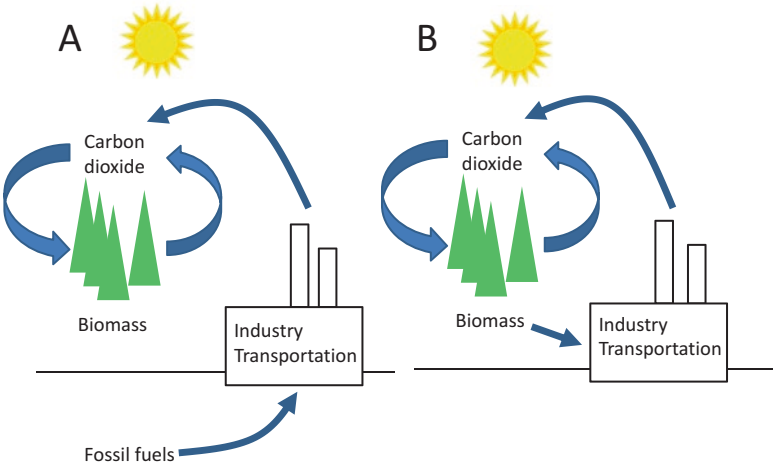

Fig. 1.3 Using biomass for energy can close the carbon cycle. (**a**) Burning fossil fuels results in the release of more carbon than can be utilized. (**b**) Using only biomass for energy closes the cycle. (Adapted from Primer on Bioproducts (2004))

ment involving a subset of Kyoto signatories. As such, Canada is still committed to reducing emission levels, but the target is more realistic, with updated commitments reflected in the Paris Agreement (World Economic Forum 2016).

There is a lot that we can do as citizens of the globe to reduce our own carbon footprint. Strategies to conserve energy include the use of energy-efficient appliances and vehicles, improved building practices, increased use of public transport and carpooling and purchasing carbon offsets. In this regard, it can be an interesting exercise to calculate one's own carbon footprint (see www.carbonfootprint.com/calculator.aspx).

There has also been recent interest in generating large-scale carbon sinks for capturing carbon dioxide from the atmosphere. For example, in the case of **carbon capture and storage**, carbon dioxide is captured, compressed into a liquid and injected into deep reservoirs for permanent storage (Haszeldine 2009; Alberta Energy 2016). Intriguingly, Mayumi et al. (2013) have shown that captured and stored carbon dioxide can lead to methane production via the action of microbial methanogens. These investigators suggested that this represented a new opportunity for the production of energy from methane derived from the stored carbon dioxide. Unfortunately, carbon capture and storage also has some potential disadvantages. This includes the fact that the separation of carbon dioxide from other gases is energy intensive and there are technical challenges associated with large-scale capture that still need to be overcome.

The potential use of microalgae to capture carbon dioxide derived from industrial processes is also under extensive investigation (Sayre 2010). Some types of microalgae produce very high levels of vegetable oil, which accounts for as much as 60% of its dry weight. Since carbon makes up about 75% of this oil, it can be extracted from the microalgae and injected into geological formations to store the carbon. Alternatively, microalgae can be converted into **biochar** by **pyrolysis** under oxygen-free conditions in the presence of catalysts (Hielmann et al. 2010). Biochar contains more than 90% carbon and can remain unaltered in the soil for millions of years.

1.3 Why Plant Bioproducts?

Another way in which we can reduce GHG emissions is through increased usage of sustainable, bio-based alternatives to petrochemicals. **Bioproducts** are industrial and consumer goods manufactured wholly or in part from renewable biomass and may be derived from crops, trees, marine plants, microorganisms and some animals (Spellman 2014). In terms of plant bioproducts, the major plant components used for their production include storage lipids, complex carbohydrates and proteins.

In the strictest sense, bioproducts have more to do with industrial applications than representing a source of human food or animal feed and may include biochemicals, bioenergy, biofuels and biomaterials. For example, many fatty acids produced by certain plants are highly valued for their use in the generation of various industrial products that are currently derived mainly from petroleum, such as lubricants and solvents. In addition, various other biochemical components of plants have also

been found to be useful in the development of a plethora of bioproducts such as natural rubber and bioplastics.

The use of bioproducts as renewable replacements for petrochemical-based materials has far-reaching benefits. This includes not only a decreased dependence on fossil fuels, along with an associated reduction in GHG emissions and enhancement in environmental safety, but also the generation of additional markets for commodities and by-products that were considered waste materials in the past for growers and food processors.

1.4 Closing Comments

Increased use of plant bioproducts, combined with the use of energy derived from hydroelectric, hydrogen fuel cell, nuclear, wave action, wind, solar and geothermal sources, can collectively contribute to decreasing our reliance on petroleum and subsequently lead to environmental benefits such as reduced GHG emissions. Interestingly, **biodiesel**, which can be produced from seed oil, is often considered to be carbon neutral because burned biodiesel ends up as carbon dioxide that plants can take up from the atmosphere and reconvert into new biomass. Unfortunately, this is not entirely true, since growing certain oilseeds, such as canola (mainly *Brassica napus*), requires the application of nitrogen fertilizer, which represents a substantial cost input in crop production (Karmakar et al. 2010). In addition, the production of nitrogen fertilizer generates nitrous oxide, which we have already identified as powerful GHG. In contrast, soybean (*Glycine max*) can be produced with little or no nitrogen, which highlights the fact that crop choice can be extremely important in terms of achieving carbon neutrality.

Although the use of plant-derived products as feedstocks for the generation of biodiesel and biofuel has garnered the majority of interest in terms of non-food applications for crops, a host of additional uses also exist. Indeed, plant bioproducts have the potential to provide a sustainable, renewable, environmentally friendly alternative to many fossil fuel-derived chemicals. Therefore, while the actual closing of the carbon cycle may be considered somewhat of a fantasy by some, it is of the utmost importance that we work towards trying to achieve it, and plant-derived bioproducts will certainly play a role in this endeavour.

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Chapter 2
Introduction to Plant Biomolecules and Cellular Metabolism

Randall J. Weselake, Stacy D. Singer, and Guanqun Chen

Chapter Highlights

- Lipids, carbohydrates, and proteins represent the major starting materials for the production of various bioproducts in plants.
- These organic compounds contain carbon atoms that share electrons with hydrogen and other elements.
- Lipids comprise fatty acids and their derivatives and tend to be either hydrophobic or amphipathic in nature.
- Carbohydrates include sugars and sugar polymers and are made up of carbon, hydrogen, and oxygen atoms.
- Proteins are made up of 20 different amino acids and include enzymes, which act as biological catalysts to speed up the chemical reactions that constitute metabolism.
- Plant metabolites offer an abundance of structural diversity and thus offer enormous potential in terms of bioproduct production.

2.1 Introduction

Some knowledge of elementary chemistry and biochemistry is required to appreciate why certain components of plant biomass are used for producing specific bioproducts with desired functionality. In order to reach a broader audience of readers interested in plant bioproducts, this book only relies on a basic knowledge such as that

R. J. Weselake (✉) · G. Chen
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
e-mail: randall.weselake@ales.ualberta.ca

S. D. Singer
Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

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provided by a junior undergraduate course in biology. This chapter begins with a brief and simplified review of how atoms can be united to form biomolecules of increasing complexity. Most of the discussion in this chapter will focus on lipids, carbohydrates, and proteins, which represent the major components of plants used as starting materials for production of various bioproducts. The goal is to develop an appreciation of the complexity and chemical diversity of plants as it relates to providing starting material for bioproducts rather than becoming overly obsessed with developing an in-depth structural understanding of these biomolecules. The chapter is mainly based on information found in introductory chemistry (e.g., Silberberg and Amateis 2015), biology (e.g., Purves et al. 2001), and biochemistry (e.g., Buchanan et al. 2015; Moran et al. 2012; Nelson and Cox 2005) textbooks.

2.2 Atoms and Molecules

Living organisms are composed of a small number of elements which appear as part of the periodic table. The proportions of elements in plants, animals, and bacteria are depicted in Table 2.1. Oxygen (O) is the most abundant element followed by carbon (C), hydrogen (H), and nitrogen (N). Among the remaining elements, phosphorous (P) and sulfur (S) are also found in living organisms. In their simplest form, elements exist as atoms. An atom consists of a nucleus surrounded by electrons. The outer electrons of an atom tend to be unstable because they are unpaired. Stability can be achieved, however, when electrons from different atoms participate in electron sharing to form molecules. When electrons are shared between two atoms, this is often referred to as a **covalent bond**. Four examples of two-dimensional representations of abundant simple molecules are shown in Fig. 2.1. An **organic molecule** (or compound) contains carbon atoms which share electrons (or form covalent bonds) with hydrogen and possibly other elements. Methane, which is an example of a greenhouse gas, is categorized as an organic molecule. The chemical formula for methane is CH_4. In essence, carbon "shakes hands" four times to form methane. Carbon can also share electrons with oxygen to form carbon dioxide (CO_2), and oxygen can share electrons with two hydrogen atoms to form water (H_2O) (Fig. 2.1). Ammonia (NH_3), which is the result of nitrogen sharing electrons with three

Table 2.1 Proportions of elements found in plants, animals, and bacteria

Element	Composition by weight (%)
Oxygen (O)	65
Carbon (C)	18
Hydrogen (H)	10
Nitrogen (N)	3
Other elements	4

Other elements include phosphorous (P) and sulfur (S)

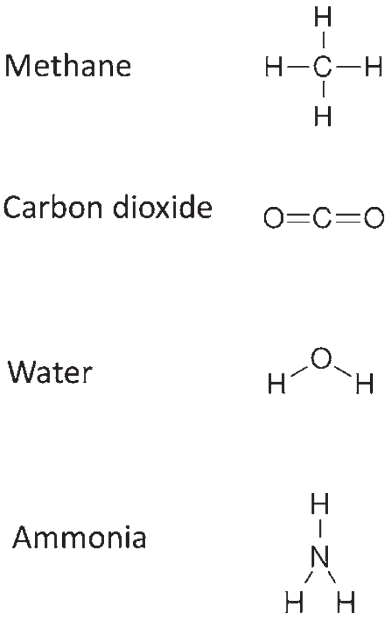


Fig. 2.1 Four examples of two-dimensional representations of simple molecules

hydrogen atoms, is also shown in Fig. 2.1. Unlike methane, carbon dioxide, water, and ammonia are not organic molecules because none of these represent a situation where carbon shares electrons with hydrogen.

2.3 Lipids

Lipids are described as "fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds" (http://lipidlibrary.aocs.org/). Many lipids tend to have **hydrophobic** attributes which are "water-fearing." Hydrophobic groups can associate in a watery environment through hydrophobic interactions. Just imagine two droplets of oil coalescing in a glass of water. The increased disorderliness of water as a result of lipid droplets coming together can be thought of as the driving force for hydrophobic interactions. Other lipids have both hydrophobic and polar ("water-loving") components as part of the same molecule and can be thought of as "schizophrenic" molecules. These types of lipids are often referred to as **amphipathic** molecules. Lipids can serve as an energy store, are critical component of membranes, and can have biological activity within cellular signaling pathways. Compared to other organic molecules, lipids are the most highly reduced yielding a maximum amount of energy when burned (Durrett et al. 2008).

Palmitic acid is an example of a fatty acid. Two different ways of depicting palmitic acid are shown in Fig. 2.2. From Fig. 2.2a, it can be seen that all the bonding requirements are fulfilled (i.e., carbon forms four covalent bonds, hydrogen forms

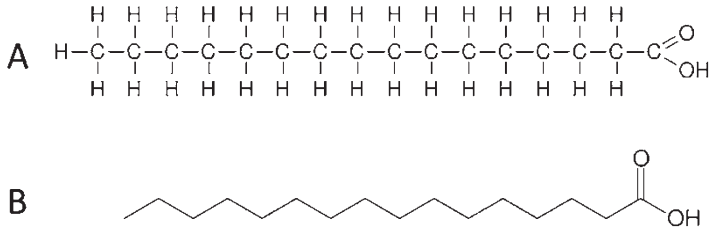


Fig. 2.2 Two representations of palmitic acid (16:0) which is a saturated fatty acid. (**a**) All atoms are shown; (**b**) a more concise representation

one covalent bond, and oxygen forms two covalent bonds). The CH_3- end of palmitic acid is known as the methyl end, whereas the opposite end, containing oxygen, is known as the carboxyl end. The jagged line in Fig. 2.2b implies the tetrahedral nature of the carbon center, and all the H atoms are assumed to be in place. In a watery environment, the carboxyl group can ionize to release a proton (H^+) and become $–COO^–$. This is an attribute of a weak acid, which explains the term fatty acid. Palmitic acid is known as a **saturated fatty acid** because all of the carbons, other than the carbon in the carboxyl group, are saturated with hydrogens. Palmitic acid can be described in shorthand form as 16:0 where "16" means 16 carbon atoms and ":0" refers to the absence of unsaturation or a double bond in the interior of the fatty acid chain.

In contrast, oleic acid has a double bond at the ninth carbon from the carboxyl group (Fig. 2.3). This bond results in a "kink" in the hydrocarbon chain. This introduced bend in the hydrocarbon chain results a compound that has a much lower melting point than palmitic acid or stearic acid (18:0), which is a commonly occurring saturated fatty acid which is two carbons longer than palmitic acid. Oleic acid is an example of a **monounsaturated fatty acid** because it has one point of unsaturation in the fatty acid chain outside of the carboxyl group. In shorthand form, oleic acid can be described as $18{:}1\Delta^{9cis}$ where "18" means 18 carbon atoms with ":1" meaning one point of unsaturation in the interior of the fatty acid chain. "Δ^9" indicates that the double bond occurs at position number 9 from the Δ (delta) end of the fatty acid chain, which is the carboxyl end. "*cis* refers to the configuration of the two hydrogens participating in the double bond. In the *cis* configuration, both hydrogens are on the same side of the double bond as shown in Fig. 2.3. In the *trans* configuration, the hydrogens would be located diagonally from each other around the double bond. Another shorthand form, which tends to be used more by nutritionists, uses the ω (omega) end of the fatty acid chain as a reference point. The ω end is the methyl end of the fatty acid chain. Thus, oleic acid can also be described as 18:1ω9 or 18:1*n*-9. This type of nomenclature is useful in understanding metabolic relationships involving different fatty acids.

Linoleic acid is an example of a **polyunsaturated fatty acid** because it has more than one double bond, or point of unsaturation, in the interior of the fatty acid chain (Fig. 2.3). In shorthand form, linoleic acid can be described as

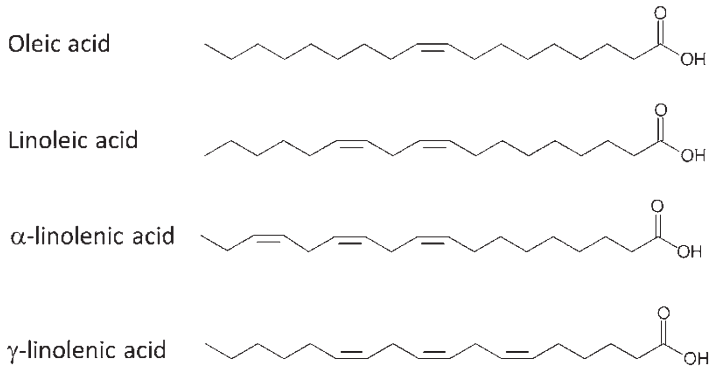


Fig. 2.3 Four examples of unsaturated fatty acids

18:2$\Delta^{9cis,12cis}$ or 18:2*n*-6 (18:2ω6). Note that the omega system only tells you about the bond closest to the methyl end of the fatty acid chain. α-Linolenic acid is an example of a polyunsaturated fatty acid with three double bonds in the interior of the fatty acid chain (Fig. 2.3). In shorthand form, α-linolenic acid can be described as 18:3$\Delta^{9cis,12cis,15cis}$ or 18:3*n*-3 (18:3ω3). γ-Linolenic acid (Fig. 2.3) is another "isomer" of linolenic acid (18:3) which can be described in shorthand form as 18:3$\Delta^{6cis,9cis,12cis}$ or 18:3*n*-6 (18:6ω6).

Fatty acids are typically found as components of more complex lipids such as **triacylglycerol** and **phospholipid** (Fig. 2.4). In triacylglycerol, fatty acids are "esterified" to a three-carbon glycerol backbone. The result is a very hydrophobic molecule since esterification greatly diminishes the polar character of the carboxyl groups. Triacylglycerol is the major component found in plant seed oils and is the main lipid feedstock for producing biodiesel and other bioproducts. A **feedstock** is a starting material for the production of industrial products. Triacylglycerol occurs in micro droplets, known as oil bodies, in the cytoplasm of the oil-forming cells of developing seeds.

Phospholipids, such as phosphatidylcholine (Fig. 2.4), are involved in the formation of cellular membranes which usually consist of two layers of phospholipid molecules (Fig. 2.5).

Phosphatidylcholine is an amphipathic molecule with two fatty acid chains comprising the hydrophobic component and phosphocholine comprising a water-loving polar head group. The fatty acyl chains interact through hydrophobic interactions in the interior of the membrane, while the phosphocholine head group faces a watery environment. The polar head group of a phospholipid can participate in **hydrogen bonding** and ion-dipole interactions with water. In a hydrogen bond, a hydrogen atom with a partial positive charge is shared between two atoms which have tendency to attract a bonding pair of electrons (referred to as electronegative). In a water molecule, the electrons tend to spend more time around the hydrogen atoms than the oxygen atom. This results in a distribution of charge, known as **dipole**, such that one end of a water molecule is positive and the other end is negative.

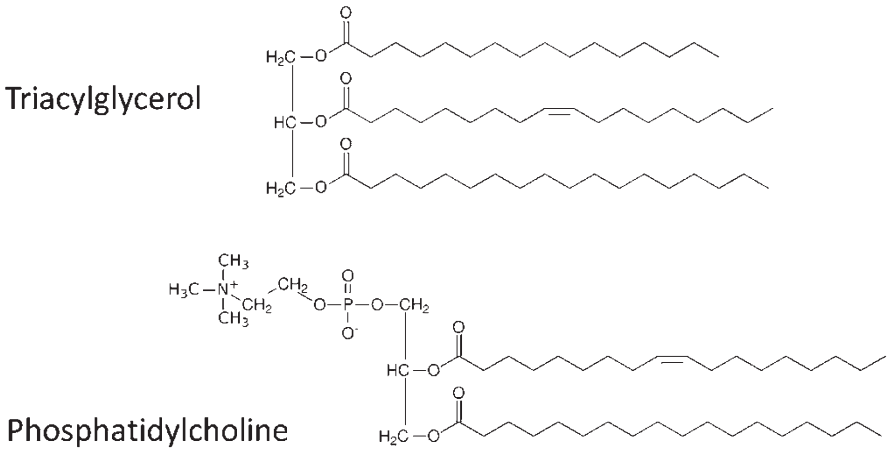


Fig. 2.4 Structures of triacylglycerol and phosphatidylcholine. In the triacylglycerol molecule shown, the glycerol backbone is esterified, from top to bottom, with palmitic acid, oleic acid, and stearic acid, respectively. The phosphatidylcholine molecule is esterified with oleic acid and stearic acid at the middle and bottom position of the glycerol backbone, respectively

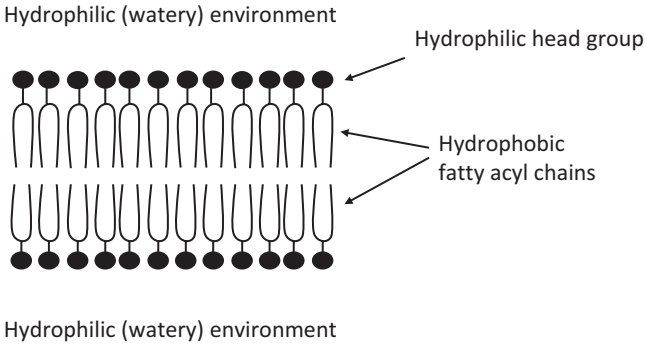


Fig. 2.5 Cross section of a segment of a lipid bilayer. Hydrophobic interactions occur between the fatty acyl chains in the inner region of the bilayer. The polar head groups of phospholipid can participate in hydrogen bonding and ion-dipole interactions with water

Therefore, the positive charge on the choline group can interact with the negative end of a water molecule.

A simplification of a plant cell is shown in Fig. 2.6. Plant cells have a **plasma membrane**, below a **cell wall**, which defines the outer boundary of the cell. In contrast, animal cells do not contain a cell wall. A plant cell contains a nucleus along with various subcellular organelles, which include **mitochondria**, **plastids**, **vacuoles,** and **peroxisomes**. All of these organelles have membranes. In addition, the plant cell contains an extensive network of membrane known as the endoplasmic reticulum. The nucleus houses the genetic material of the cell, which contains the

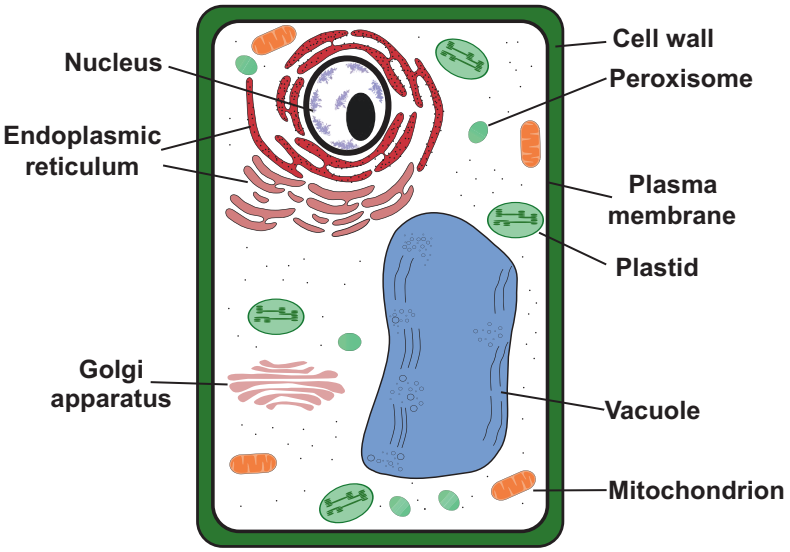


Fig. 2.6 Schematic representation of a plant cell. (Adapted from Alberts et al. 1994)

"blueprint" for the formation of components of the cell and its operation. Mitochondria are known as the "power houses" of the cell because of respiration and the formation of adenosine triphosphate (ATP), the universal energy currency. Fatty acid formation occurs in plastids. The plastids of leaves are known as **chloroplasts** because they contain chlorophyll and are involved in photosynthesis. Fatty acid degradation occurs in both mitochondria and peroxisomes. Vacuoles are multifunctional organelles. In seeds, vacuoles serve as storage sites for reserve proteins and soluble carbohydrates. These various subcellular structures characterize what are known as **eukaryotic cells**. These subcellular organelles are absent in **prokaryotic cells** such as bacteria. In a eukaryotic cell, the content of the cell, minus the nucleus, is known as the **cytoplasm**, whereas the soluble component of the cytoplasm (without subcellular organelles and internal membranes) is known as the **cytosol**.

Bioproducts produced from lipids are dealt with in Chaps. 4 and 5.

2.4 Carbohydrates

Carbohydrates can be thought of as sugars and sugar polymers. Carbohydrates consist of carbon, oxygen, and hydrogen and have the general formula $(CH_2O)n$. Most carbohydrates have cyclic monomers as fundamental structural components. Complex carbohydrates have roles in energy storage and cellular structure, such as contribution to the rigidity of the cell wall. Carbohydrates also serve as a source of

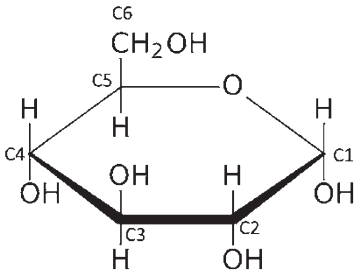


Fig. 2.7 Haworth structure of α-D-glucose. The carbon numbering system is indicated with C1 representing the anomeric carbon. α-D-Glucose is an example of a monosaccharide

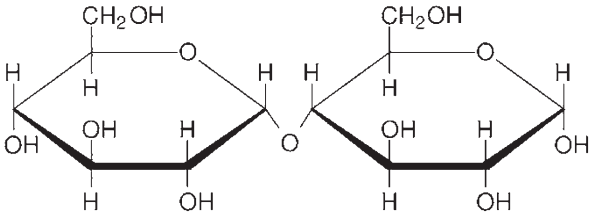


Fig. 2.8 Structure of maltose which is an example of a disaccharide

carbon for various biosynthetic processes. Carbohydrates yield less than half the energy of lipids when burned.

α-D-Glucose is an example of a **monosaccharide** which exists mainly as the ring form in solution. The Haworth projection is shown in Fig. 2.7. Imagine the ring being embedded into the page with the portion in bold projecting out toward you. Hydrogen atoms, hydroxyl groups (–OH), and the –CH_2OH group are above the plane of the ring with some hydrogens and –OH groups below the plane of the ring. Note that all bonding requirements are fulfilled. The carbon (C1) on the right side of the ring is known as the **anomeric carbon**. If the –OH group is downward, then the glucose molecule is known as α-D-glucose. In contrast, if the –OH group is above the plane of the ring, the resulting molecule is β-D-glucose. In reality, the **Haworth projection** is only an approximation of the structure of glucose, and the ring adopts a somewhat different **conformation**. By definition, this has to do with "the spatial arrangements of a molecule that can be obtained by rotation of the atoms about a single bond" (https://www.merriam-webster.com/dictionary/conformation). Glucose, for example, can take on a "boat" or "chair" conformation.

The loss of a water molecule between two α-D-glucose molecules can result in maltose (Fig. 2.8), which is a **disaccharide**. The glucose rings are linked by an α-1-4 glycosidic linkage. The formation of maltose is an example of a condensation reaction. The reverse of the condensation reaction is known as hydrolysis reaction. The addition of a water molecule to maltose can result in the splitting of maltose into two α-D-glucose molecules.

Starch is an example of **polysaccharide** which can vary in molecular weight. Starch can accumulate as an energy reserve in developing cereal grains. Starch also forms transiently in the chloroplasts, accumulating during the day and disappearing at night. Starch consists of long stretches of α-D-glucose molecules linked by α-1-4 glycosidic linkages. On average, α-1-6 glycosidic linkages occur about every 25

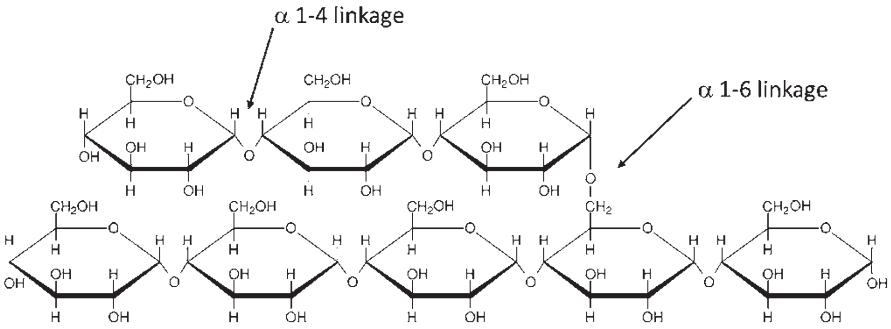


Fig. 2.9 Partial structure of starch

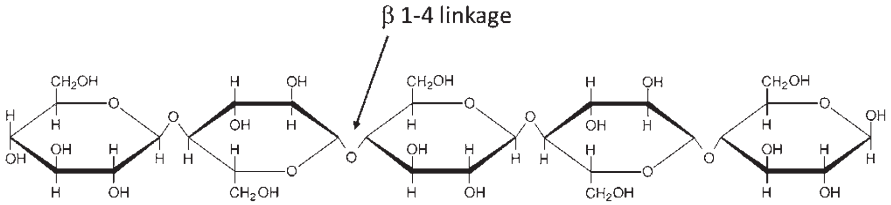


Fig. 2.10 Partial structure of cellulose

glucosyl residues resulting in branching. A partial structure of starch is shown in Fig. 2.9. In contrast, **cellulose** consists of strands of glucose units which are linked by β-1-4 glycosidic linkages (Fig. 2.10). Extensive hydrogen bonding occurs between the glucose chains in cellulose contributing to its strength and insolubility. Cellulose is the most common organic compound on earth. Both starch and cellulose are used as feedstocks in the production of ethanol.

There are numerous other monosaccharides, disaccharides, and polysaccharides. Disaccharides and polysaccharides can consist of mixtures of different monosaccharides, which can also vary in the number of carbon atoms in their rings. Various combinations of linkages can also occur. Additional information on carbohydrates and bioproducts produced from them are discussed in Chaps. 6 and 7.

2.5 Amino Acids and Proteins

A generalized structure of an **amino acid** is shown in Fig. 2.11. The central carbon is known as the α-carbon and to it is bonded a carboxyl group, a hydrogen atom, an amino group, and a variable side group or R group. **Proteins** are made up of 20 different amino acids, which can be generally categorized as nonpolar, polar, and electrically charged (Fig. 2.12). The latter can be further subcategorized as acidic or basic. The pH of the medium can influence the state of ionization of the carboxyl

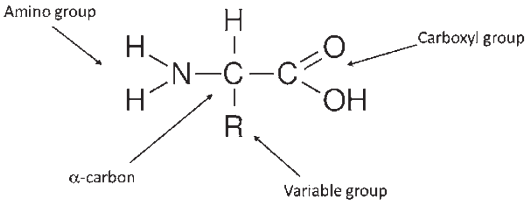


Fig. 2.11 Generalized structure of an amino acid

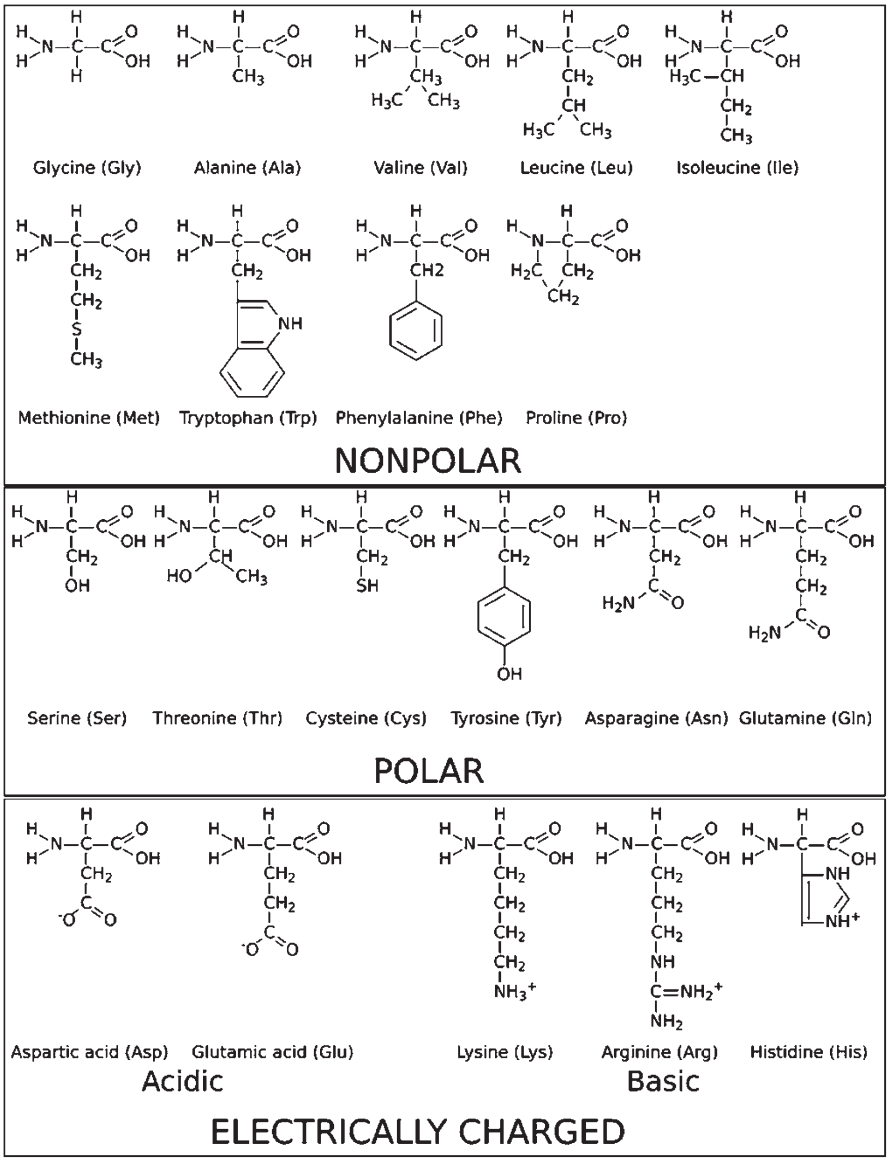


Fig. 2.12 Twenty different amino acids typically found in proteins

and amino groups and some of the variable side chains. For aspartic acid, at pH 7, the main carboxyl group and carboxyl group of the side chain have both lost their protons, whereas the amino group bears a positive charge because of an additional proton (Fig. 2.13). To form polypeptides, amino acid "residues" are connected by **peptide bonds**. A peptide bond connecting two amino acid residues is shown in Fig. 2.14. This linkage also results from the loss of a water molecule.

Different levels of protein structure are depicted in Fig. 2.15. A **monomeric protein** consists of a single polypeptide chain. The sequence of amino acids in the polypeptide chain is known as the **primary structure**. Shorter segments within a polypeptide chain can also exhibit different types of folding including the α-helix and β-strand. This is known as **secondary structure**. Typically a polypeptide collapses upon itself to form the **tertiary structure**. This level of structure is characterized by the formation of β-sheets involving more than one β-strand. Regions of α-helix, β-sheet, and random coil can all exist in one polypeptide. The polypeptide is stabilized by various interactions between the side chains of the amino acid residues. For example, an ionic interaction or salt bridge can occur between the negatively charged carboxyl group of the side chain of an aspartic acid residue and the positive charge on the side chain of a lysine residue. In some cases, disulfide linkages (-S-S-), which are covalent, can occur between cysteine residues that are far apart in the linear sequence of amino acid residues. Disulfide linkages tend to stabilize the tertiary structure. In **quaternary structure**, identical or different subunits, which have their own tertiary structure, can associate through various interactions. A protein consisting of two identical polypeptides, or subunits, is referred to as a homodimer. If the subunits differ, the protein would be a heterodimer. A protein with four identical subunits would be a homotetramer, whereas a protein with a mixture of different subunits would be a heterotetramer. Proteins can be structural, have a role as a storage reserve, or have biological activity such as an enzyme. More detailed information on proteins and bioproducts derived from proteins is the subject of Chaps. 8 and 9.

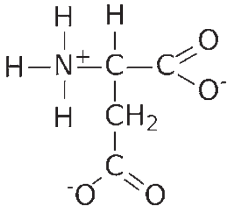


Fig. 2.13 Aspartic acid at pH 7

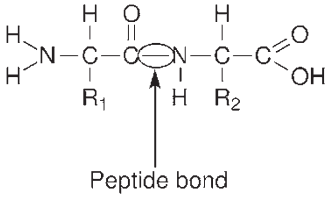


Fig. 2.14 Two amino acid residues connected by a peptide bond

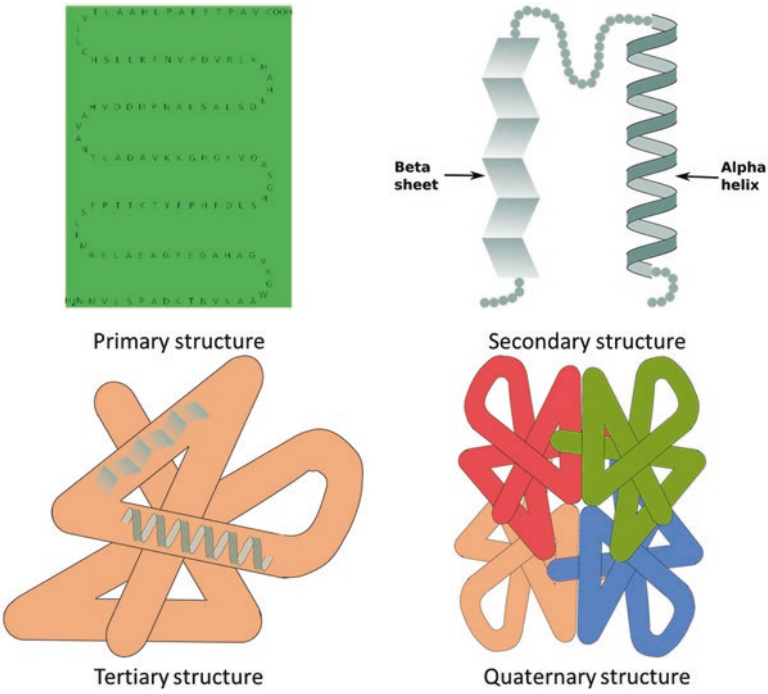


Fig. 2.15 Four different levels of protein structure

2.6 Biochemical Reactions Are Catalyzed by Enzymes

Enzymes are proteins which can act as biological catalysts to speed up chemical reactions such as condensation and hydrolysis. In enzyme-catalyzed reactions, the molecules first interacting with the enzyme are known as **substrates**, and released molecules are **products**. The enzyme has a specialized region or **active site** that can interact with the substrate and can facilitate the reaction much more effectively than without enzyme. The active site has amino acid residues that can temporarily interact with the substrate and other amino acid residues that are involved in the actual process of catalysis. A cartoon showing enzyme action is depicted in Fig. 2.16. Once the product(s) is released from the enzyme, it is ready for another catalytic cycle. Unlike substrates, enzymes are not consumed in the catalytic process. Generally, enzymes are essential for catalyzing biochemical reactions in plants and other organisms under non-extreme temperature conditions. Many of these reactions could be encouraged through a drastic increase in temperature without enzymes, but the consequences on life would be devastating. Later, it will also be shown that enzymes can be useful in the production of bioproducts from plant biomass.

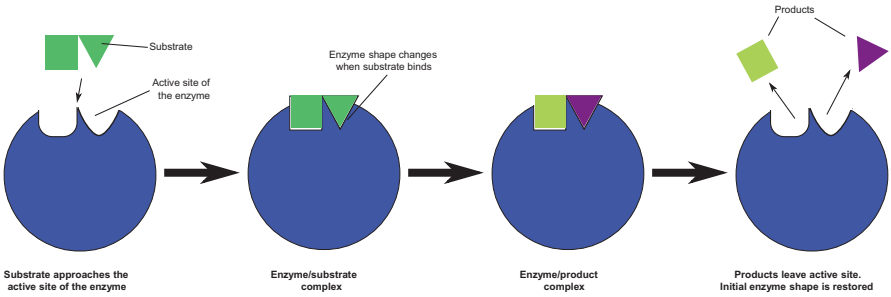


Fig. 2.16 Schematic representation of enzyme action

2.7 Elementary Plant Metabolism

Cellular biochemical pathways involve a plethora of reactions catalyzed by enzymes. All of these reactions constitute metabolism. Metabolism can be divided into **catabolism** and **anabolism**. Catabolism has to do with metabolic processes that break down biomolecules, while anabolism is a building process. Energy production is often associated with catabolic processes, while anabolic processes may require energy input. **Primary metabolism** represents fundamental biochemical processes that are essential to the survival of a cell. These processes include energy production through photosynthesis and respiration and the formation of larger complex biomolecules including lipids, carbohydrates, and proteins. In plants, the products of **secondary metabolism** can aid in the growth and development of plants but are not absolutely required for survival.

Plants are **autotrophs,** which means that they can convert energy from light into primary metabolites. Photosynthesis uses carbon dioxide, water, and light to produce glucose and oxygen. The overall reaction is shown below:

$$6CO_2 + 12H_2O + \text{light} \rightarrow C_6H_{12}O_6\,(\text{glucose}) + 6O_2 + 6H_2O$$

Glucose in turn provides a source of energy and biosynthetic precursors for fatty acid biosynthesis, amino acid biosynthesis, further carbohydrate metabolism, and secondary metabolism. The way in which cellular respiration can yield precursors for various biosynthetic processes is illustrated in Fig. 2.17. This, however, only represents a glimpse of the complexity of the processes. Many of these metabolites and biopolymers can serve as feedstock for the production of bioproducts. In eukaryotes such as plants, various metabolic processes can be compartmentalized. The process of glycolysis wherein glucose is converted to pyruvate occurs in the cytosol, whereas the conversion of pyruvate to **Acetyl-coenzyme (Co)A** and the Krebs cycle occur in the mitochondria. Special transport systems have evolved for moving metabolites across the membranes of organelles.

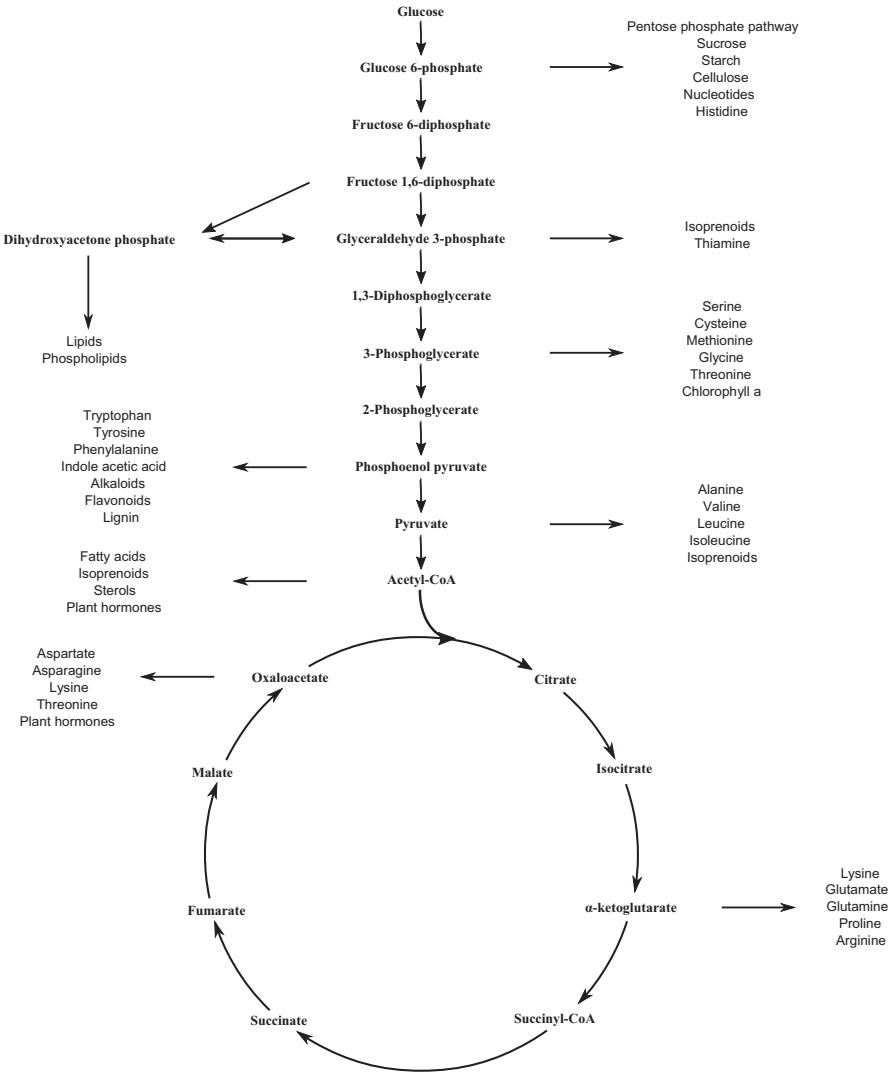


Fig. 2.17 Cellular respiration yields precursors for various biosynthetic processes

Secondary metabolites are usually produced from a product of primary metabolism. Secondary metabolites can have roles as pigments, attractants for pollinators, insect repellents, and antimicrobial or antifungal agents. Examples of some classes of secondary metabolites include alkaloids, terpenoids, polyphenols, and polyketides. Alkaloids contain nitrogen and are often derived from amino acids. Caffeine and nicotine are well-known alkaloids (Fig. 2.18). Terpenoids are derived from the basic five-carbon building block known as isoprene and are often categorized as lipids. The antioxidant lycopene, from the tomato, and the blood thinner, warfarin, are examples of terpenoids (Fig. 2.19). Polyphenols are derived from

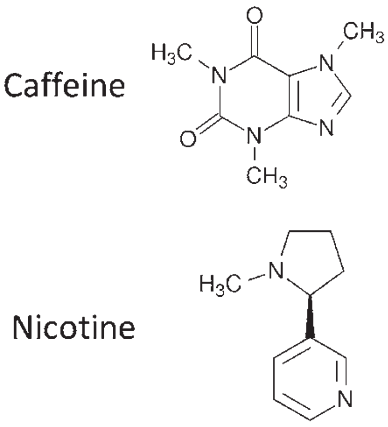


Fig. 2.18 Caffeine and nicotine are two examples of alkaloids

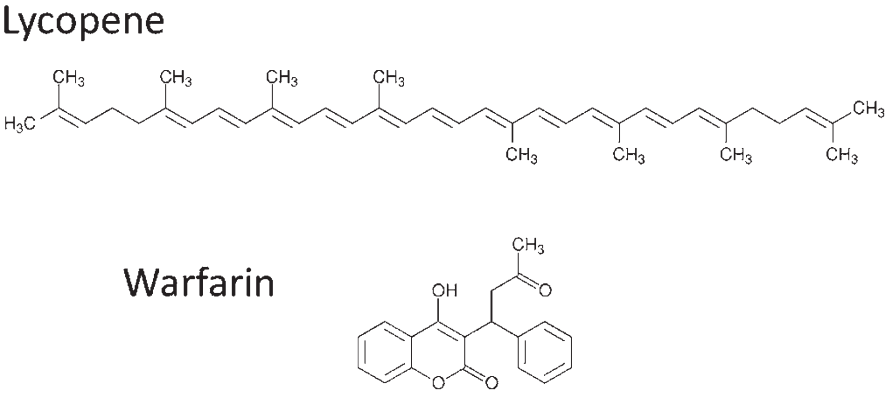


Fig. 2.19 Lycopene and warfarin are two examples of terpenoids

aromatic amino acids and have ring structures substituted with –OH groups. Resveratrol, an antioxidant in red wine, and vanillin, a flavor component of vanilla, are two examples of polyphenols (Fig. 2.20). Polyketides are produced from two- or three-carbon units in a process similar to fatty acid biosynthesis. Two examples include lovastatin, a cholesterol-lowering drug, and tetracycline, which is an antibiotic (Fig. 2.21).

2.8 Structural Diversity as a Major Advantage for Bioproducts

Petrochemicals are all based on a few simple hydrocarbon structures. The short-chain hydrocarbon, octane, is shown in Fig. 2.22. Petrochemicals can be straight chain, branched chain, cyclic, and aromatic with variations in chain length. In general,

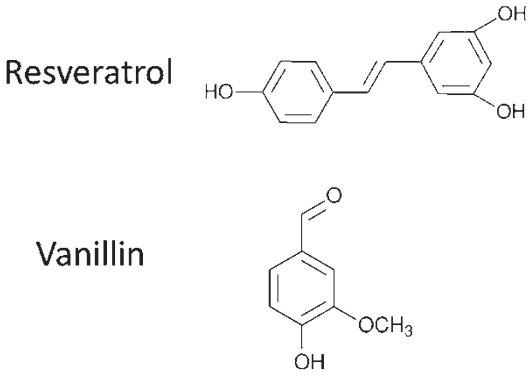
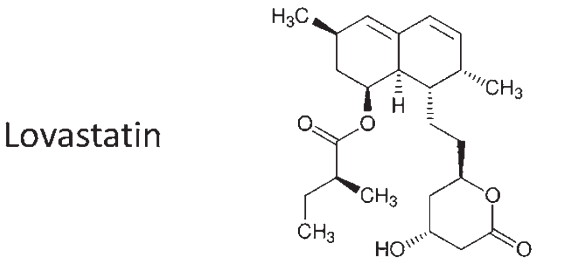


Fig. 2.20 Resveratrol and vanillin are two examples of polyphenols



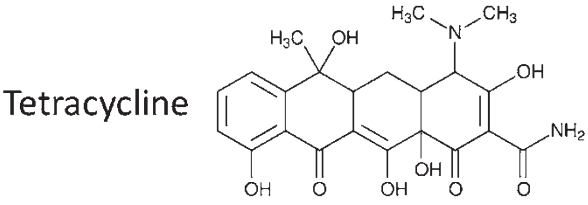


Fig. 2.21 Lovastatin and tetracycline are two examples of polyketides

Fig. 2.22 Octane, a straight chain petrochemical

there are a limited number of functional groups. As a result, petrochemicals require extensive processing, which necessitates expensive starting materials and catalysts. The synthesis of complex organic molecules from petrochemicals therefore tends to be time-consuming, expensive, energy intensive, low-yielding, non-stereoselective, and not environmentally friendly.

In contrast, there is an abundance of structural diversity in the plant kingdom. For example, there are over 100,000 different secondary metabolites representing 25 major structural groups. There is thus an enormous potential for bioproduct production, especially with regard to platform or building block biochemicals

(see Chap. 10). Proteins also have enormous structural diversity based on the fact that the 20 different amino acids can be linked in different sequences. With 20 amino acids to choose from, for a two amino acid combination, there are 20^2 or 400 possibilities for different amino acid sequences. With four and six amino acid combinations, the possibilities rise to 160,000 and 64 million, respectively. Thus, enormous diversity is possible with a moderately sized polypeptide consisting of 400 amino acid residues.

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Chapter 3
An Integrated Approach to Plant Bioproduct Production

Randall J. Weselake, Stacy D. Singer, and Guanqun Chen

Chapter Highlights

- The area of plant bioproducts comprises several interdependent and allied disciplines, including plant breeding, agronomy, biotechnology, biorefining, the social sciences, and legal expertise.
- Whereas plant breeding involves random genetic changes in many genes, genetic engineering specifically alters or introduces only a very small number.
- Plant breeding and biotechnology benefit from the widespread use of "omics" approaches, including genomics, transcriptomics, proteomics, and phenomics.
- Creative solutions need to be developed for the efficient utilization of biomass using a biorefinery approach, where waste is minimized.
- Social scientists can help determine where bottlenecks may lay in terms of public acceptance, the relevance of research to society, and possible ethical considerations.

3.1 Introduction

Bioproducts can be produced from feedstock derived from plant biomass in various ways. These processes can be chemical, biological, or combinations of chemical and biological processes. Chemists can use **green chemistry** to convert substances, such as plant triacylglycerol, into various polymers including nylon and foams. A classic example of a biological process is the use of yeast to convert glucose into ethanol.

R. J. Weselake (✉) · G. Chen
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
e-mail: randall.weselake@ales.ualberta.ca

S. D. Singer
Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

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Enzymes, which can be isolated from living systems, can also be used to catalyze bioconversions such as the breakdown of cellulose to provide glucose units for ethanol formation. The term **biocatalysis** refers to the use of enzymes or cells to speed up chemical reactions. In addition, enzymes, in their own right, often represent useful bioproducts. As we will see in later chapters, various bioproducts can be derived from plant lipids, carbohydrates, and proteins using chemical and biochemical processes. In addition, many small molecules from plants can serve as building blocks or platform biochemicals to produce a plethora of fine chemicals that are often generated from petrochemical sources.

The area of plant bioproducts would not be fully realized without the interdependence of several allied disciplines. Within the last several decades, **plant breeding** and **agronomy** have had substantial impacts on global crop production. More recently, plant **biotechnology** has shown promise in crop improvement as a complement to modern plant breeding and in the engineering of microorganisms involved in **bioconversion**s. In a **biorefinery**, processes are developed for maximum utilization of plant components. Finally, the social sciences and legal expertise are critical in dealing with issues such as consumer acceptance of bioproducts, intellectual property issues, freedom to operate, and dealing with trade barriers. This chapter provides insight into how bioproduct production can benefit from the integration of various disciplines.

3.2 Plant Breeding and Agronomy

In order to explain how plant breeding and agronomy have contributed to bioproducts, we will use corn (maize; *Zea mays*) as a notable example. Modern-day corn is very different from its wild ancestor (Fig. 3.1). It is estimated that the domestication of corn and selection for desirable traits began 6000–10,000 years ago (Gewin 2003). Today, corn is the most extensively grown field crop in the Americas. Corn is used as livestock feed, for human food (in raw or refined form), and as feedstock for producing various bioproducts. Corn can be separated into various fractions such as the endosperm, germ, and pericarp (the hull) (see Chap. 11 and Fig. 11.2 for details). The endosperm is enriched in starch and protein, whereas the embryo and pericarp are enriched in oil and **fiber**, respectively. These biochemical compounds have a variety of end uses which include the production of batteries, disposable diapers, and wallpaper (www.ontariocom.org/classroom/products.html).

Over time, the technique of crop improvement through plant breeding evolved into an elite science. Plant breeding involves the active selection of individuals with desirable traits. Plant breeding has been described in detail in textbooks such as Acquaah (2012). The discipline relies on an in-depth knowledge of genetics which is a branch of biology that deals with the heredity and variation of organisms (www.merriam-webster.com/dictionary/genetics). Methods used in plant breeding include basic selection for an individual trait, **hybridization**, **polyploidy**, and induced mutations. Plant breeding further involves controlled crosses between individuals

Fig. 3.1 Selection for kernels over time led to modern-day corn. (Source: Gewin 2003)

possessing desired traits. In cross-pollination, pollen from the anther of a flower from one plant is transferred to the stigma of a flower from another plant. In self-pollination, the pollen from the anther of a flower is transferred to the stigma of flowers on the same plant. Polyploid cells and organisms contain more than two paired sets of chromosomes (diploid) and often outperform their diploid relatives (Sattler et al. 2016). Hybridization involves the crossing of two highly inbred parental lines, each possessing one of the desired characteristics, to obtain progeny with both traits. Hybrids often exhibit increased hybrid vigor (often referred to as heterosis) which may include increased seed size, vigor, fertility, and overall productivity, involving gene-environment interactions. In contrast, the inbred parental lines used to produce the hybrids are often low-yielding. Natural mutations in the DNA of plants occur at low frequencies. Mutations, however, can be induced randomly through the use of chemical mutagens. Thus, plant breeders often use random chemical mutagenesis to develop sources of new traits.

Agronomy is defined as a branch of agriculture dealing with field crop production and soil management (https://www.merriam-webster.com/dictionary/agronomy). Breeding advances and various agronomic factors have contributed to a steady increase in corn yields over the past seven to eight decades. The application of commercial fertilizers to corn fields began in the mid-1940s with almost all of the

Table 3.1 Increases in USA corn yield since 1866

Year(s)	Approximate yield (bushels/acre)
1866–1940	24.3–28.9
1970	72.4
2017	176.6

Source: US Department of Agriculture (https://www.nass.usda.gov)

current acreage being fertilized. Herbicide application has also been critical for early season control of weeds so as to facilitate early planting of corn. Yield increases in USA corn since 1866 are depicted in Table 3.1. Today corn is grown in monoculture or alternated with soybeans (*Glycine max*).

3.3 Plant Biotechnology, Genomics, and Modern Plant Breeding

Biotechnology is the use of living organisms or biological processes for the purpose of developing useful agricultural, industrial, or medical products, especially through the use of methods such as genetic engineering (http://www.thefreedictionary.com/biotechnology). **Plant genetic engineering** involves modifying the DNA of a plant or introducing DNA from another source to generate a crop with a specific beneficial trait. The altered crops are referred to as being genetically engineered (GE) or genetically modified (GM). GE crop is a preferred term since plant breeding also involves genetic modification, especially chemical mutagenesis-assisted breeding where mutations are randomly introduced into the plant's DNA. Hildebrand (2008) provides a concise discussion of biotechnology and crop improvement. The reader is also encouraged to consult a special issue of the journal *Biocatalysis and Agricultural Biotechnology* which focuses on "trait introduction methods and innovation platforms in plant biotechnology" (Kovalchuk and Weselake 2014).

Genetic engineering is more precise than breeding with the generation of lines containing introduced traits occurring in a shorter time frame. Traditional breeding is a time-consuming process which requires generations of repeated and controlled selections and phenotyping. A **phenotype** is "the observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences" (http://www.thefreedictionary.com/Phenotyping). For example, a crop may have been genetically engineered with the intent of producing larger seeds, which is a phenotypic observation. On the other hand, **genotype** has to do with "the genetic makeup of the organism" (http://www.thefreedictionary.com/genotype).

In order to fully appreciate plant genetic engineering, it is worthwhile to have some insight into the flow of genetic information. A detailed discussion of the flow of genetic information can be found in various biochemistry and molecular biology textbooks (e.g., Buchanan et al. 2015; Moran et al. 2012). The nucleus of a plant cell contains

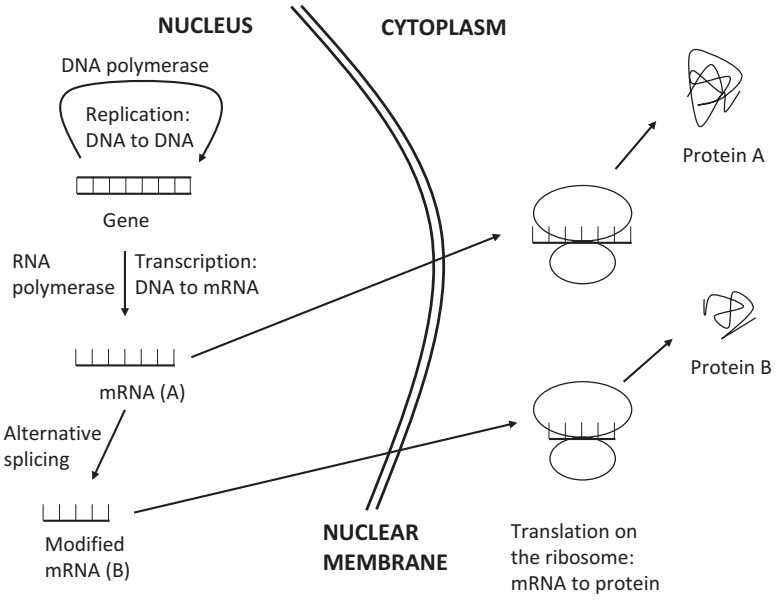

Fig. 3.2 The flow of genetic information in a eukaryotic cell and the involvement of alternative splicing. Replication, transcription, and alternative splicing occur in the nucleus, while translation (protein biosynthesis) occurs on the ribosomes in the cytoplasm. The image is based on information from Moran et al. (2012) and Syed et al. (2012)

chromosomes which in turn contain the "genetic blueprint" of the plant. This genetic blueprint contains genes which code for proteins and is reproduced during cellular replication. A **gene** can be defined as "the basic physical unit of heredity; it represents a linear sequence of nucleotides along a segment of DNA that provides coded instructions for the synthesis of mRNA, which, when translated into protein, leads to the expression of hereditary character" (http://www.dictionary.com/browse/gene).

More specifically, the DNA which makes up the genetic blueprint is replicated through the involvement of DNA polymerase, an enzyme driving the formation of a new double helix (Fig. 3.2). In turn, the conversion of DNA to mRNA is driven by RNA polymerase, which catalyzes the formation of a single strand of mRNA based on the information in the gene. In eukaryotic cells, mRNA, which also represents the information in the gene, moves outside of the nucleus to a protein formation factory where mRNA is converted to protein via a process known as translation or protein synthesis (Fig. 3.2). Individual genes are known to lead to the production of proteins and enzymes that can have biological functions in the plant. Initially, it was thought that one gene leads to the formation of one protein. We now know, however, that there are considerably more proteins than genes in a plant. **Alternative splicing** can lead to the formation of more than one protein based on the information from one gene (Fig. 3.2). In alternative splicing, "precursor mRNAs are spliced differentially to generate different mRNA isoforms" (Syed et al. 2012).

Unlike genetic engineering, in traditional plant breeding, thousands of genes are combined each time with the hope of changing traits. Often "backcrossing" is needed to compensate for unwanted side effects. In addition, plant breeding is only possible between closely related species. With genetic engineering, genes can be targeted and changed in specific ways. The process of producing mRNA from a gene can be enhanced and this is often referred to as overexpression. Through overexpression, the quantity of a specific enzyme in a plant cell can be increased. In contrast, through gene knockout, it is possible to eliminate the action of a specific enzyme in a plant cell. Genes can also be introduced from other sources through **heterologous expression** which involves the "expression of a gene or part of a gene in a host organism which does not naturally have this gene or gene fragment" (https://en.wikipedia.org/wiki/Heterologous_expression). The general methods involved in genetic engineering are often referred to as recombinant DNA technology since they involve the manipulation of DNA. Interestingly, many of the techniques used in modern plant breeding are based on molecular biology and recombinant DNA technology (Moose and Mumm 2008). It is useful to think of plant genetic engineering as being complementary to plant breeding.

Agrobacterium-mediated transformation and microprojectile bombardment are two commonly used approaches for introducing genes from other sources into crop plants (Hildebrand 2008; Weselake 2011). In the case of *Agrobacterium*-mediated transformation, the gene of interest is inserted into *Agrobacterium* along with an antibiotic resistance marker and then used to infect plant cells. In turn, antibiotic selection is used to identify plant cells that carry the gene of interest. In microprojectile bombardment, particles coated with the gene of interest are propelled into plant tissue followed by regeneration of the altered or "transgenic" plants. Both methods of transformation are dependent on techniques in plant tissue culture, which can also be thought of as part of plant biotechnology. Transformed plant cells are grown in culture under conditions to promote shoot and root development, and the plantlets are eventually transferred to soil. In plant biology research, initial transformations are often conducted with the model plant *Arabidopsis thaliana*, a member of the Brassicaceae family which has a relatively short propagation time and small genome, with extensive genetic and phenotypic resources available (Koornneef and Meinke 2010). A **genome** is an "organism's complete set of DNA, including all of its genes" (https://ghr.nlm.nih.gov/primer/hgp/genome).

The first major crop traits, introduced through genetic engineering, were insect resistance and resistance to broad-spectrum herbicides, such as Roundup™ (glyphosate) (Hildebrand 2008). Both of these traits have been of great benefit to producers. *Bacillus thuringiensis* (*Bt*) is a soil bacterium that occurs naturally and makes a protein that is toxic to certain types of insects. The bacterium has been used a biopesticide since the 1920s. The gene coding for the toxic protein from *Bt* was introduced into corn resulting in a transgenic crop with a reduced need for application of commercial pesticides, which also results in reduced greenhouse gas emissions due to fewer passes of pesticide-dispensing airplanes. The herbicide resistance trait allows producers to apply a broad-spectrum herbicide to a crop so as to give it a competitive advantage against weeds. Glyphosate-resistant soybean is grown

extensively in the USA (Hildebrand 2008), while glyphosate-resistant canola (*Brassica napus*) is grown extensively in Canada (Weselake 2011). Unintended benefits of herbicide-resistant canola have included diminished fuel costs for farmers and reduced soil erosion because of increased use of zero-tillage practices where dead plant material remains unplowed in the field (Weselake 2011).

More recently, genetic engineering has contributed to the development of crops producing nutritionally enhanced and value-added compounds for industrial applications. The latter is in line with the focus of this book; i.e., genetic engineering can be used to modify plant metabolism so as to produce an enriched source of feedstock for the production of bioproducts. The term **metabolic engineering** is often used to describe the redirection or modulation of carbon flow through a metabolic pathway (Stephanopoulos 2012; Venglat et al. 2014). It involves the adjustment and optimization of genetic and metabolic processes to produce a certain substance (https://en.wikipedia.org/wiki/Metabolic_engineering).

The development and commercialization of a GE crop is a very expensive undertaking which can only be effectively achieved by biotechnology companies with deep pockets. Extensive growth chamber, greenhouse, and confined field testing are conducted following the development of a GE line. GE corn, canola, soybean, and cotton (*Gossypium hirsutum*) account for 99% of the world's GE crops with the USA producing 40% of the global GE crop area (https://cban.ca/gmos/products/on-the-market/). Despite the resistance of the European Union (EU) to the adoption of GE crops, some insect-resistant GE corn is grown in Spain, Portugal, the Czech Republic, Slovakia, and Romania. GE crops grown in Canada include canola, corn, soybean, sugar beet (*Beta vulgaris*), and alfalfa (*Medicago sativa*; for animal feed), which accounts for about 6% of the global acreage of GE crops. In Canada, the Canadian Food Inspection Agency (CFIA) and Health Canada ensure that all GE products are safe for animals, people, and the environment. All **plants with novel traits** (PNTs) are regulated by the CFIA. A PNT is defined as "a plant that contains a trait which is both new to the Canadian environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health. These traits can be introduced using biotechnology, mutagenesis, or conventional breeding methods" (http://www.inspection.gc.ca/plants/plants-with-novel-traits/eng/1300137887237/1300137939635). The introduction of a PNT as a commercial crop involves several stages of testing and approval. Assessing the environmental biosafety of PNTs involves evaluation of potential to become a weed or to be invasive of natural habitats, for gene flow to wild relatives, and to become a plant pest. The potential impacts on nontarget species and biodiversity are also assessed. The seeds of GE crops are considered to be the intellectual property of the company who holds the patent on the novel trait. Producers must purchase seed under license with the company and are not allowed to save seed for planting in the next year.

Consumer acceptance of GE crops is a controversial topic with the EU being very resistant to the widespread introduction of these crops (Sprink et al. 2016). One area of intense discussion is whether or not food products obtained from GE crops should be labeled as such. Environmental activist groups have also been concerned with the effect of insect-resistant GE crops on nontarget insects such as the monarch butterfly

(*Danaus plexippus*) (Hildebrand 2008). Insect-resistant corn, however, causes no immediate harm to monarch butterflies under field crop conditions. The view of environmental groups is interesting given that these groups also argue against using chemical-based pesticides which can potentially contaminate the environment. There has also been concern whether the herbicide resistance of GE crops can be transferred to non-GE crops or weedy relatives of these crops growing in the vicinity of GE crops. Contamination of a regular crop by a GE version of the same crop can occur via pollen flow and/or seed movement (Jhala et al. 2009; Weselake 2011). Maintenance of both isolation distances and isolation in time can be used to reduce gene flow from engineered oilseed species. Control of volunteer transgenic plants appearing in subsequent years can also reduce undesirable gene flow.

The flax (*Linum usitatissimum*) cultivar Triffid represents an interesting case of a GE crop (https://cban.ca/gmos/products/not-on-the-market/flax/). Triffid is a herbicide-resistant GE flax that was developed at the Crop Development Centre of the University of Saskatchewan. The name Triffid was based on John Wyndham's 1951 horror novel entitled *The Day of the Triffids* (Wyndham 1970).

Although Triffid was approved for release in Canada in the late 1990s, it was never grown on a large scale and was voluntarily de-registered in 2001 because of pressure from flax producers who wanted to protect their European markets. In 2009, however, traces of Triffid were found in Canadian flax shipments to Europe. This resulted in severe consequences for the Canadian flax industry. At the time Triffid contamination was found, the European market accounted for about 70% of Canada's flax exports.

Although there has been great concern regarding the manipulation of a single gene, edible products from crop plants modified through chemical mutagenesis appear to fly under the radar. Unlike GE crops, in most countries, chemical mutagenesis-induced variation applied to breeding is not regulated. One notable example is the development of flax with seed oil enriched in linoleic acid (18:2$\Delta^{9cis,12cis}$) instead of α-linolenic acid (18:3$\Delta^{9cis,12cis,15cis}$) (Green 1986; Rowland et al. 1995). Flax enriched in linoleic acid is broadly known as Linola™ and as Solin in Canada (Hall et al. 2016). The decrease in unsaturation in high-linoleic acid flax oil resulted in oil more similar to corn oil and rendered it more suitable for cooking applications. Eventually, it was determined that this trait was the result of the inactivation of genes encoding fatty acid desaturase enzymes which govern the formation of α-linolenic acid from linoleic acid (Vrinten et al. 2005). Given that chemical mutagenesis was used to produce this type of flax, one would expect other mutations in the chemically treated genome. Random mutations introduced through chemical mutagenesis do not appear to be a concern with many of those who oppose the development of GE crops. Eventually, genetic engineering was used to further reduce unsaturation in high-linoleic flax so as to produce a GE line of flax with high oleic acid (18:1Δ^{9cis}) content in the seed oil (Chen et al. 2015). Further information on the use of genetic engineering to produce oleaginous crops with modified lipid biosynthesis is presented in Chaps. 4 and 5.

Plant breeding has also benefited from **genomics**, which is "the study of the way genes and genetic information are organized within the genome, the methods for collecting and analyzing this information, and how this organization determines

their biological functionality" (Campos-De Quiroz 2002). "Omics" research works toward generating a global picture of genome DNA sequence and mRNA sequences leading to a plethora of proteins (Weselake 2011; Venglat et al. 2014; Gupta et al. 2017). The global analysis of mRNA sequences is known as **transcriptomics**, whereas the analysis of proteins is called **proteomics**. Omics now also includes **metabolomics**, which attempts to take a snapshot of the metabolite status of cell or tissue type. Very large amounts of genomic, transcriptomic, metabolomic, and proteomic data are analyzed by bioinformaticians or computational biologists. The reader is encouraged to consult Venglat et al. (2014) for a discussion on the genomics of seed development. A detailed knowledge of crop genomes has led to genomics-assisted breeding (Varshney et al. 2005) and the identification of new gene targets for genetic engineering (Weselake 2011; Venglat et al. 2014).

Plant phenomics is a relatively new "omics," which involves the high-throughput analysis of the phenome (Groβkinsky et al. 2015; Tardieu et al. 2017). The crop phenome is represented by the structure and function of plants (i.e., all the phenotypic indicators). Gene variants and environmental changes can influence the plant phenome. Advances in plant phenomics are dependent on improvements in high-throughput noninvasive plant imaging and data analysis. In the future, investigations of plant phenomics could potentially lead to the generation of physiological predictors for complex traits, thereby linking genotype to phenotype for applications in plant breeding (Groβkinsky et al. 2015).

Genome editing represents a relatively new development in plant biotechnology and allows biotechnologists to create site-specific changes in the plant genome (Kathiria and Eudes 2014; Yin et al. 2017). In contrast, *Agrobacterium*-mediated transformation and microprojectile bombardment are based on the random integration of DNA into the plant genome. So far, most applications of genome editing have involved loss-of-function through gene inactivation. It has been suggested that genome editing may be more acceptable to government regulators and consumers (Kathiria and Eudes 2014). The potential acceptability of genome editing as a method for introducing new traits into crops, however, is under intense discussion in the EU (Sprink et al. 2016).

Another emerging area in plant biotechnology is research aimed at developing crops that reproduce through asexual reproduction or **apomixis** (Barcaccia and Albertini 2013; Gewin 2003; Lovell et al. 2013). Through apomixis, fertilization is avoided altogether through the production of seed without pollination. Although apomixis occurs naturally in a few hundred species of plants, this biotechnology still needs to be effectively applied in a crop. In essence, seeds of apomixis crops could become natural clones of the "mother" with hybrid quality maintained from year to year by the producer.

Biotechnology is a very powerful tool for developing crops with desirable traits. Science-based evaluations support the safety of GE crops, but negative consumer perceptions still need to be overcome. Future applications of biotechnology for bioproducts will need to balance good science with consumer acceptance, while navigating an increasingly complex intellectual property landscape. Since bioproducts, in their strictest sense, are used only for industrial applications, this may be less concerning for consumers than the acceptance of edible products from GE crops.

3.4 The Biorefinery

Heating crude oil combined with fractional distillation can lead to several products which are differentially volatile based on temperature (Fig. 3.3). Petroleum distillates are used for liquid transportation fuels, lubricants, heating oil, and raw materials for the chemical synthesis of polymers and solvents. The mindset and engineering practices used in petroleum distillation can be applied to the processing of biomass. In biorefining, biomass is the raw material which can be separated into lipid, carbohydrate, protein, and high-value building-block biochemicals. In terms of plant biomass, many different feedstocks are possible, including crops and crop wastes, wood, sawdust, grasses, and algae. Biorefining also involves the conversion of the separate components into high-value bioproducts.

The biorefinery can be defined as "the sustainable processing of biomass into a spectrum of marketable products and energy" (de Jong and Jungmeier 2015). Creative solutions need to be developed for efficient utilization of biomass where waste is minimized. As an example, biodiesel production (see Chap. 4) uses seed oil, but the meal, which contains fiber and protein, is left over. Biodiesel production on its own is not fully profitable unless value can also be derived from the meal. Neibergs et al. (2016) have suggested that canola and *Camelina sativa* meal can be substituted for well-established soybean to supplement low-protein forages in livestock rations.

The Canadian Triticale Biorefinery Initiative (CTBI) research network was an example of a Canadian-based multi-coinvestigator research project aimed at whole-plant utilization (Beres et al. 2013a, b; King 2014). The CTBI was co-led by

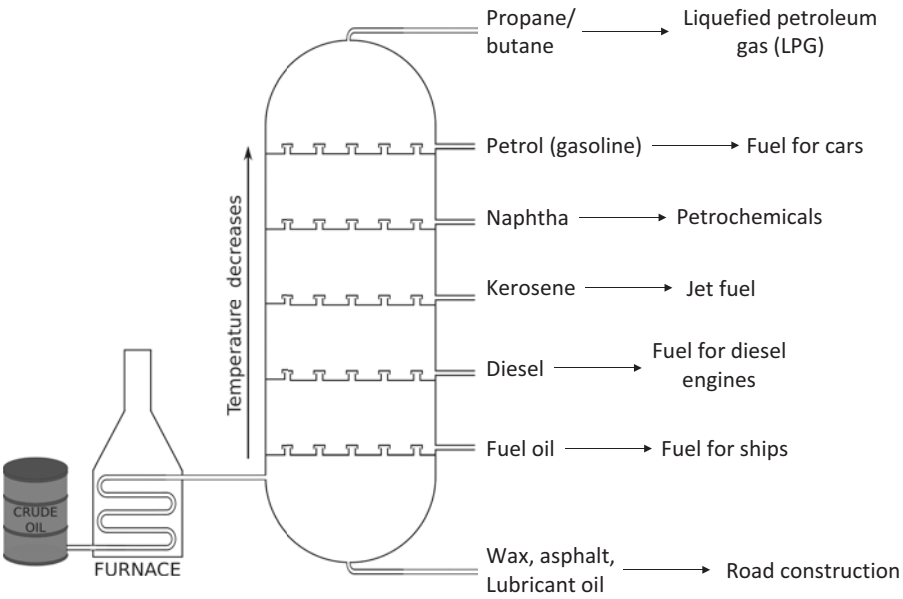

Fig. 3.3 Fractionation of crude oil (fossil fuel) by distillation to produce various products

Agriculture and Agri-Food Canada and Alberta Agriculture and Rural Development. Triticale (*Triticosecale* spp.) is a hybrid of wheat (*Triticum aestivum*) and rye (*Secale cereale*). The crop combines the high-yield potential of wheat with the disease and stress tolerance of rye. Lower input costs are required to produce triticale, and the crop does not naturally hybridize with other crops or wild species. Triticale has excellent potential for whole-plant utilization with applications in livestock feed, chemical production from green and mature biomass, bioethanol production from both the grain component and cellulosic material, polymer/fiber production, and pulp and paper production. Similar to corn (Fig. 11.2), the triticale grain has a relatively large starchy endosperm and small germ. Instead of a pericarp, the triticale grain has an outer bran layer. The grain contains about 65% starch, which can be used for bioethanol production, textile manufacturing, biodegradable packaging materials, and **adhesives**. Triticale straw, in turn, can be used for the production of cellulose, hemicellulose, and lignin. The cellulose can be used for bioethanol production, paper products, and cellulose-based composites. The hemicellulose and lignin fractions can be used to produce a range of specialty chemicals. Some of the genetic engineering objectives of the CTBI included modification of starch composition for specific applications, increasing seed oil content for livestock feed applications and reducing lignin content to increase the amount of cellulose harvested. The CTBI resulted in a valuable research platform which combined conventional and biotechnology-based breeding (King 2014). Chapter 11 of this book focuses on the biorefining of seeds of major crops to produce value-added substances.

Bioconversion (or biotransformation), which can be used in biorefining, is "the conversion of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes or agents, such as certain microorganisms" (https://en.wikipedia.org/wiki/Bioconversion). A notable example is the bioconversion of starch or cellulose into bioethanol (see Chap. 6). Bioconversion can involve chemical, enzymatic, and/or microbial-facilitated processes. Biotechnologists are also interested in modifying metabolism in microorganisms such as bacteria and yeast. Indeed, applications of genetic and metabolic engineering were applied to microorganisms before plants (Vitorino and Bessa 2017). The burgeoning field of **synthetic biology** overlaps with metabolic engineering in that both disciplines are concerned with the modification of biochemical pathways in cells. Synthetic biology, however, is focused more on the use of synthetic DNA and genetic circuits to produce value-added products (Stephanopoulos 2012). Most applications of synthetic biology have been in microbial systems.

3.5 Bioproduct Development and the Social Sciences

Agricultural biotechnology has also drawn upon the expertise of social scientists and legal experts. Indeed, large-scale agricultural genomics projects funded by Genome Canada routinely include a GE^{3}LS component where G = Genomics; E^3 = Ethical, Environmental, Economic; L = Legal; and S=Social Aspects (https://www.genomecanada.ca/en/programs/ge3ls-research). GE^{3}LS research occurs at the crossroads of genomics and society.

Brewin and Malla (2012) have examined the effects of biotechnology on the Canadian canola industry. The success of canola as a major Canadian crop is due largely to public research, but more recently the private sector has been heavily involved in further improving the crop. Only a few major companies appear to exert influence over research on canola and the development of new varieties. Although the introduction of biotechnology and modern plant breeding led to a large increase in private investment into canola research, basic research and development on canola has been affected by intellectual property rights and freedom to operate issues. For example, the need for gene trait cross-licensing agreements has led to economic barriers for commercialization of new varieties.

Grierson et al. (2011) have compiled "one hundred important questions facing plant science research," many of which are relevant to plant bioproduct production. Some of the relevant questions include:

- "When and how can we can we simultaneously deliver increased yields and reduce the environmental impact of agriculture?
- How do we ensure that sound science informs policy decisions?
- Can we improve algae to better capture CO_2 and produce higher yields of oil or hydrogen for fuel?
- How can we use plants as the chemical factories of the future?"

The above questions and the many other questions compiled by Grierson et al. (2011) can be useful exercises for senior undergraduate students in plant science. The final chapter of this book by Smyth and Lubieniechi (Chap. 12) addresses the food versus fuel debate. In essence, can we effectively produce biofuels without affecting the food supply for a growing global population?

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Chapter 4
Production of Biodiesel from Plant Oils

Stacy D. Singer and Randall J. Weselake

Chapter Highlights

- Biodiesel is composed of alkyl esters of vegetable oil or animal fat and is produced primarily through a process known as transesterification.
- The functional properties of biodiesel are determined by the composition and structure of the fatty acids it is derived from.
- Many strategies are currently being explored for the modification of seed oil content and composition for improved biodiesel supply and functionality.
- Several applications exist for the by-products of biodiesel production, making the large-scale production of biodiesel more economically feasible.

4.1 Introduction

Vegetable oils have been explored as a fuel source for automobiles since the advent of the earliest diesel engines. In a famous demonstration at the 1900 World Fair in Paris, a diesel engine ran on peanut oil, and the earliest known patent for palm oil biodiesel was issued in Belgium in 1937 (Knothe 2001, 2016). During World War II, several countries turned to plant oils as an alternative fuel source, including the Japanese, who reportedly used soybean oil as bunker fuel in their battleships (Knothe 2001). Despite this history, interest in biodiesel faded once petroleum became cheap and readily available.

S. D. Singer (✉)
Agriculture and Agri-Food Canada, Lethbridge, AB, Canada
e-mail: ssinger@ualberta.ca

R. J. Weselake
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

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While most of the global energy demand is currently met using petroleum from fossil reserves, escalating concerns with regard to the long-term sustainability of such a practice are driving research efforts to develop alternative fuel technology platforms based on renewable feedstocks. Today, biodiesel has resurfaced beside bioethanol (see Chap. 6) as an additional transportation fuel as petroleum stocks begin their decline. Between 2002 and 2006, biodiesel production increased 15-fold in the USA and nearly fivefold in the EU (Durrett et al. 2008), and several countries have set ambitious targets for continued growth. In this chapter, we examine the production and functional properties of biodiesel from plant and algal oils and explore strategies for improving both the quality and quantity of plant oils for biodiesel production.

4.2 What Is Biodiesel?

Today, biodiesel is strictly defined as "an alkyl ester of a vegetable oil or animal fat" (Knothe 2001). While there are many widely publicized accounts of vehicles running on neat vegetable oil or waste cooking oils, these are not considered "biodiesel" by definition and are not subject to the same quality standards. Intact vegetable oils are made up of triacylglycerols (TAGs; see Fig. 2.4) and are highly viscous compared to petroleum-based diesel, which, when used in unmodified engines, can result in incomplete combustion and carbon deposits that clog filters and reduce engine performance over time (Durrett et al. 2008).

As a result, biodiesel is now produced primarily through a process known as **transesterification** (Fig. 4.1), where plant oils are reacted with an alcohol in the presence of a strong alkali, such as potassium hydroxide, to produce alkyl esters with viscosity and ignition properties comparable to conventional diesel (Durrett et al. 2008). As methanol is the least expensive alcohol currently available, it is most commonly used for transesterification, resulting in the formation of fatty acid methyl esters; however, other alcohols such as ethanol, butanol, and isopropanol have been utilized (Knothe 2005; Wang et al. 2010) and confer different properties to the biodiesel.

4.3 Other Types of Fuel Produced from Plant Oils

Additionally, a very promising alternative lipid conversion technology for the production of renewable liquid hydrocarbons has recently been optimized for use on **bio-oils** and is termed pyrolysis, or thermal cracking (Maher et al. 2008; Asomaning et al. 2014). This method involves a high-temperature reaction in the absence of oxygen and converts free fatty acids (which can be obtained from crude TAG feedstocks through an initial hydrolysis step) to mainly alkanes and alkenes. Similar high-temperature-based approaches are used in the production of renewable jet fuel

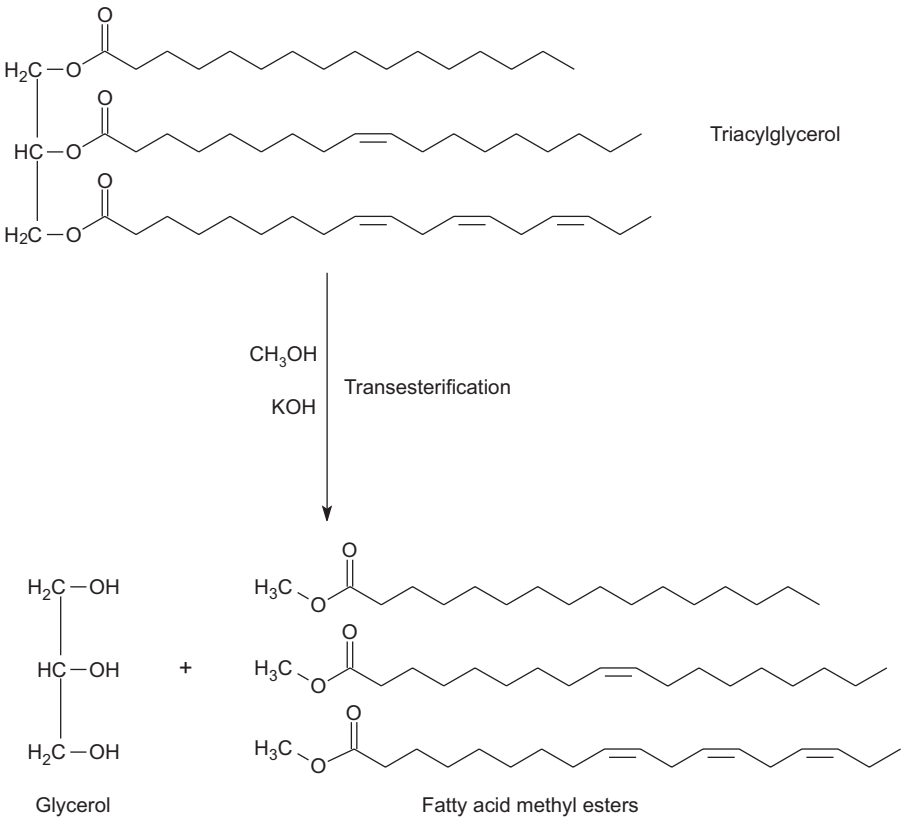

Fig. 4.1 Production of biodiesel from triacylglycerol. Triacylglycerol contains three acyl groups esterified to a glycerol backbone. In the production of biodiesel, the acyl groups are transesterified in the presence of a strong base (e.g., KOH) and an alcohol (e.g., methanol), yielding fatty acid methyl esters (biodiesel), and glycerol, a by-product with value-added potential

(Kallio et al. 2014; Zhao et al. 2015). Medium-chain fatty acids including caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), and myristic acid (14:0), along with long-chain palmitic acid, provide suitable feedstock for the hydrocarbon component of jet fuels, which consist of C8–C16 alkanes and aromatic hydrocarbons (Kallio et al. 2014; Kim et al. 2015). Plant biotechnologists are interested in metabolically engineering *Camelina sativa* to produce medium-chain fatty acids with the goal of making the oil an ideal feedstock for jet fuel production (Kim et al. 2015; Hu et al. 2017). *C. sativa* has minimal requirements for crop management, a short growing season, along with being drought- and frost-tolerant (Iskandarov et al. 2014; Murphy 2016). This plant species is a member of the Brassicaceae, producing seeds containing approximately 40% oil, and its meal has recently been approved by the US Food and Drug Administration for livestock feed, providing a market for products other than the oil.

4.4 Advantages and Challenges of Biodiesel Versus Petroleum-Derived Diesel

Besides being derived from a renewable resource, biodiesel has a number of advantages over petroleum diesel. Biodiesel contains no sulfur or aromatic compounds, and the presence of oxygenated functional groups tends to reduce emissions of carbon monoxide and particulate matter (McCormick et al. 2001; Hill et al. 2006). Blending of biodiesel with petroleum diesel at levels of 5–20% confers some of these advantages to the blend (Dincer 2008), while addition of smaller amounts (1–5%) of biodiesel has been shown to restore the lubricity of low-sulfur petroleum diesel (Geller and Goodrum 2004).

One of the major challenges with biodiesel, however, is its heterogeneous composition which reflects the diversity of fatty acids found in vegetable oils. Plant oils commonly contain five major fatty acids: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1Δ^{9cis}; hereafter 18:1), linoleic acid (18:2$\Delta^{9cis,12cis}$; hereafter 18:2), and α-linolenic acid (18:3$\Delta^{9cis,12cis,15cis}$; hereafter 18:3). These fatty acids occur in various proportions depending on the source (Table 4.1), along with many other fatty acids in minor quantities. As shown in Chap. 2, these fatty acids vary both in chain length and degree of unsaturation (see Figs. 2.2 and 2.3). Rapeseed (*Brassica napus* and *B. rapa*) is enriched in a very long-chain monounsaturated fatty acid known as erucic acid (22:1Δ^{13cis}; hereafter 22:1). Canola, which displays low erucic acid and glucosinolate content, with oil enriched in oleic acid, was developed from rapeseed in Canada (McVetty et al. 2016). In contrast, petroleum diesel contains primarily saturated hydrocarbon chains of varying chain lengths. The fatty acid composition is a critical determinant of the functional properties of biodiesel, such as its ignition quality, oxidative stability, and low-temperature performance (Knothe 2005, 2016).

Table 4.1 Fatty acid composition of some common biodiesel crops

Oil	Fatty acid composition (mol %)							Suitability for biodiesel
	16:0	18:0	18:1	18:2	18:3	C20–C24	Ref.	
Canola	4	2	56	26	10	2	Moon et al. (2001)	Preferred stock in Canada, excellent biodiesel performance
Rapeseed[a]	3	2	26	17	10	43	Taylor et al. (2009)	Preferred stock in Europe, excellent biodiesel performance
Soybean	9	4	24	54	8	0	Taylor et al. (2009)	Preferred stock in the USA; but high saturates (13%) reduce cold performance
Palm	48	4	36	10	0	0	Taylor et al. (2009)	Very high oil content but only suitable in tropical climates

[a]High erucic acid rapeseed

As indicated in Chap. 1, it is also important to take into consideration the nitrogen fertilizer requirements of a potential oil crop for biodiesel or other oil-derived fuel production. The manufacture of nitrogen fertilizer produces the GHG nitrous oxide, and there is an energetic cost to its production (Karmakar et al. 2010).

4.5 Structure/Function Relationships Influencing Biodiesel Performance

A number of functional characteristics influence the overall performance of a fuel, including ignition quality, heat of combustion, oxidative stability, lubricity, cold flow properties, and viscosity. In the case of biodiesel, these properties are a function of both the fatty acid composition and the type of ester linkage introduced during the transesterification process (Knothe 2016). In terms of fatty acid structure, the chief considerations are chain length, degree of unsaturation, and the presence of substituted functional groups. The influence of these structural characteristics on fuel performance is summarized in Table 4.2, and some of these are described in detail below.

Ignition quality in diesel fuels is usually described in terms of **cetane number (CN)**, which is a relative measure of the delay between fuel injection and ignition, which decreases as CN increases. Petroleum diesel typically has a CN in the range of 40–50, and biodiesel quality standards mandate a minimum CN of 47 in the USA (ASTM D6751) and 51 in Europe (EN 14214) (Knothe 2005). In general, CN increases with increasing chain length and decreases with increasing degree of unsaturation (McCormick et al. 2001). The length of the alkyl ester also influences CN, with increasing ester length generally resulting in increasing CN relative to the neat fatty acid, which further underscores the value of transesterification for enhancing biodiesel performance. Interestingly, although branched fatty acids tend to have a lower CN, branched esters of saturated fatty acids retain the high CN imparted by the saturated acyl group (Knothe 2005).

This observation may be particularly useful in improving the low-temperature performance of biodiesel, which is currently one of the major challenges to biodiesel use in temperate regions. The relatively long-chain length and the presence of long-chain saturated fatty acids in biodiesel produced in temperate regions, which are mostly derived from soybean (*Glycine max*) oil and canola/rapeseed oil (see Table 4.1), make it susceptible to crystallization and gelation at low temperatures. The temperatures at which these processes occur are termed the **cloud point** and **pour point**, respectively (Lang et al. 2001). Biodiesel quality standards (ASTM D6751 and EN 14214) do not specify a target cloud point or pour point, due to different geographical and seasonal demand (Knothe 2005), but in general, cold temperature performance of biodiesel is inferior to that of diesel fuel (Table 4.3). Cloud point increases with chain length and decreases with increasing number of double bonds or substitutions (Knothe 2005). Since double bonds tend to reduce the CN

Table 4.2 The effect of structural attributes on biodiesel performance parameters

Functional property		Structural attribute	Ref.
Cetane number	increases with	Chain length	Wang et al. (2010)
	decreases with	Unsaturation, branching	
Cloud point/pour point	increases with	Chain length	Wang et al. (2010)
	increases with	Saturation	Hill et al. (2006)
	decreases with	Unsaturation, branching	
Heat of combustion	increases with	Chain length	Wang et al. (2010), Hill et al. (2006)
Oxidative stability	decreases with	Unsaturation	Wang et al. (2010)
Lubricity	increases with	Hydroxy substitution, unsaturation	McCormick et al. (2001), Lang et al. (2001)

Table 4.3 Cold flow properties of several biodiesels

Oil	Ester	Cloud point (°C)	Pour point (°C)	Ref.
High oleic, low palmitic soy oil	Methyl	−5	−9	http://www.canolacouncil.org
	Ethyl	−7	−15	
	Isopropyl	−10	−18	
Soybean oil	Methyl	−2	−3	http://www.canolacouncil.org
	Ethyl	−2	−6	
	Isopropyl	−9	−12	
Canola oil	Methyl	1	−9	Hill et al. (2006)
	Ethyl	−1	−6	
	Isopropyl	7	−12	
Rapeseed oil	Methyl	0	−15	Hill et al. (2006)
	Ethyl	−2	−15	
Petroleum diesel	n/a	−5–−15	−15–−35	Dincer (2008), Geller and Goodrum (2004)

and the oxidative stability of the fuel, the use of branched esters is being explored as a means of decreasing the cloud point of biodiesels without sacrificing ignition quality, since branched esters (e.g., isopropyl esters) often have better cold flow properties than their equivalent methyl or ethyl esters (Table 4.3; Wang et al. 2010). In addition to optimizing the fatty acid composition and esterification for enhanced cold performance, these properties can be improved by blending with petroleum diesel (Joshi and Pegg 2007) or with the use of various fuel additives (Shrestha et al. 2008).

The **heat of combustion** of a fuel describes the energy density of the fuel (Lang et al. 2001) or the amount of energy released from the complete combustion of the fuel. Intuitively, heat of combustion increases with increasing chain length (Knothe 2005). **Lubricity** refers to the ability of a diesel fuel to lubricate engine surfaces. The lubricity of petroleum diesel has declined as a result of legislation to reduce the

sulfur content (Goodrum and Geller 2005), and additives must now be added to diesel fuel to reduce engine wear. Biodiesel has been successfully used for this purpose (Geller and Goodrum 2004). In terms of structure/function relationships, fatty acids containing hydroxyl groups increase the lubricity of biodiesel, even at very low proportions (<1%) (Goodrum and Geller 2005). While there is no consistent trend in terms of chain length, increasing unsaturation appears to enhance lubricity of non-hydroxylated fatty acids at proportions greater than one percent (Geller and Goodrum 2004). Hydroxy fatty acids naturally occur in a few oilseeds, such as castor bean (*Ricinus communis*) and *Lesquerella fendleri* (Moon et al. 2001), and efforts to produce hydroxy fatty acids in crop species are being aggressively pursued in the context of producing industrial oils (Smith et al. 2000; Burgal et al. 2008). These strategies are described in more detail in Chap. 5.

4.6 Sources of Plant-Derived Biodiesel

A relatively wide variety of plant-derived oils, such as rapeseed, canola, and soybean, can be utilized as feedstocks to produce biodiesel. Unfortunately, the current worldwide production of vegetable oil is nowhere near sufficient to completely replace petroleum-based fuels. In fact, if all oils from higher plants were used to make biodiesel, this would amount to about 4% of global petroleum consumption for fuel/energy purposes (Carlsson 2009). Although global plant oil production increased from 129 million metric tonnes in 2007 (Carlsson 2009) to 163 million metric tonnes in 2013 (Weselake et al. 2017), the situation is still similar. In addition, there are serious concerns regarding the conversion of arable land typically used for the production of food crops to biofuel crops (Table 4.4). In response to this apprehensiveness surrounding the use of food crop species for the production of biodiesel, several alternative strategies are currently being explored that would minimize this risk. The first is the use of vegetable oil waste (Asomaning et al. 2014),

Table 4.4 Comparison of several sources of biodiesel

Crop	Oil yield (L/ha)	Land area needed[a] (M ha)	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

Source: Chisti (2007)
[a]To meet 50% of all transport fuel needs of the USA
[b]30% oil (by wt) in biomass
[c]70% oil (by wt) in biomass

which is produced in abundance following frying and has the potential to serve as an environmentally friendly alternative feedstock for biodiesel production that would provide the added benefit of contributing to waste management. Unfortunately, difficulties in processing these recycled oils exist due to their variable quality, making their large-scale processing problematic. Several nonfood oilseed species with the ability to grow on marginal land that is not used for food crops are also currently being explored as candidates for the production of oil for use in the generation of biodiesel. In addition to an ability to grow on nonarable land, a nonfood biodiesel crop would ideally also possess a very high oil yield with a suitable fatty acid composition for biodiesel production along with favorable agronomic characteristics.

Pongamia pinnata, which is a leguminous oilseed-bearing tree, is one such species that yields very high levels of oil in its seeds; can grow on marginal, high-saline land; and requires very little fertilizer (Karmee and Chadha 2005). Similarly, several groups are also conducting research on *Jatropha curcas* as a biodiesel crop (Divakara et al. 2010), which is a perennial shrub grown mainly in Asia and Africa. As is the case for *P. pinnata*, this species can be grown in adverse conditions. There are challenges associated, however, with both species, including the toxicity and variable oil content of *J. curcas* and the fact that growth of these plants on marginal land tends to result in lower seed and/or oil yields (Yong et al. 2010), which could theoretically once again result in competition with food crops for better soil. Perhaps the forerunner in emerging alternative biodiesel crops though is *C. sativa* (Iskandarov et al. 2014; Murphy 2016).

Yet another potential alternative source of feedstock for biodiesel production is microalgae, which, like plants, are photosynthetic organisms. Certain species produce high quantities of oil, in some cases exceeding 70% of their weight (Spolaore et al. 2006), which means they have a much higher potential oil yield per unit land area than any oilseed crop (Table 4.4). The US Department of Energy has been researching algae as a source of fuel since the energy crisis of the 1970s, although their initial interest was in using algae to produce hydrogen. The high oil content of certain species, however, is now fueling research into its usefulness as a feedstock for the production of biodiesel.

There are several advantages of microalgae over terrestrial plants in terms of their applicability for use in the manufacture of biodiesel, including the potential reduction in land area required for their growth and their potentially superior oil yields. Algae can be cultivated in large ponds, which are not expensive to build but are prone to contamination, water loss, and low productivity (Chisti 2007). Interestingly, certain algae can even be grown using waste materials (such as in ponds at wastewater treatment plants), and it could therefore be possible to cultivate them without necessitating the use of fresh water (Menetrez 2012). Alternatively, photobioreactors can also be used as a platform to grow algae; they have high costs initially but yield high biomass productivity (Chisti 2007).

There are still several obstacles, however, with the use of algae for the production of biodiesel, and it has not yet been undertaken on a commercial scale. One of these challenges involves the fatty acid composition of their oil, which tends to be very high in polyunsaturated fatty acids (PUFAs) and is thus relatively unstable.

The hydrogenation of the oil can overcome this issue. The growth of these organisms also requires the input of relatively high levels of nutrient fertilizers, even in wastewater situations, and production costs are rather expensive compared to petrochemical-based biodiesel (Chisti 2013). Therefore, biodiesel derived from algal sources is not likely to be competitive with petroleum fuels in the short term; as a result, the present focus in this field is leaning more toward the production of high-value nutraceutical and pharmaceutical products rather than biodiesel (Guedes et al. 2011). As is the case for terrestrial plants, projects are ongoing to attempt to optimize algal production both through biotechnological approaches to, for example, increase oil content, modify fatty acid composition, and enhance growth characteristics, as well as classical engineering approaches in terms of enhanced oil extraction methods, biorefining strategies (see Chap. 11), and photobioreactor design.

4.7 Improving Biodiesel Through Modification of Plant Storage Lipid Biosynthesis

Efforts to improve biodiesel quality through crop biotechnology are typically focused on various strategies to alter the fatty acid composition of plant oils; however, rising demand for oils for both food and fuel is also driving research into increasing seed oil content. Prior to discussing metabolic engineering approaches to modify the fatty acid composition of seed oil or increase seed oil content, we provide some background information on the biochemistry of TAG formation, which will also be a useful background for the production of other bioproducts from plant oils discussed in Chap. 5.

4.7.1 Seed Oil Biosynthesis

The developing seeds of oleaginous plants are a major site for TAG accumulation. Oil biosynthesis can be broadly regarded as a two-stage process encompassing the reactions involved in fatty acid synthesis and those involved in glycerolipid assembly (reviewed by Singer et al. 2013; Chen et al. 2015). Oil biosynthesis can differ somewhat between different oil-forming plant species, and the process can be affected by environmental conditions (Singer et al. 2016). Fatty acid synthesis occurs in the plastids of the developing seed (Fig. 4.2). **Acetyl-CoA** is derived from sucrose, resulting from photosynthesis in leaves, which is transported to the developing seed. **Acetyl-CoA carboxylase (**ACCase) catalyzes the formation of malonyl-CoA from acetyl-CoA. The fatty acyl chain grows two carbons at a time while attached to the **fatty acid synthase (FAS) complex** via **acyl carrier protein** (ACP). In plants producing monounsaturated fatty acids or PUFAs, stearoyl (18:0)-ACP can be converted to oleoyl (18:1)-ACP by the catalytic action of a soluble **stearoyl-ACP**

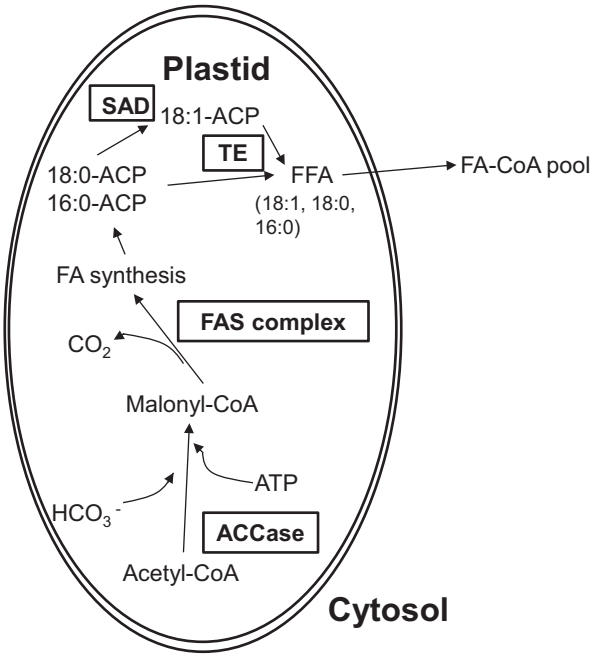

Fig. 4.2 Fatty acid biosynthesis in the plastid of an oil-forming developing seed. Acetyl-CoA is derived from sucrose, resulting from photosynthesis in leaves, which is transported to the developing seed. In an adenosine triphosphate (ATP)-dependent process, acetyl-CoA carboxylase (ACCase) catalyzes the formation of malonyl-CoA from acetyl-CoA. The FAS complex catalyzes the sequential addition of two carbon units from malonyl-CoA to the nascent fatty acid chain, until the chain is 16–18 carbons in length. In plants producing monounsaturated fatty acids or PUFAs, stearoyl (18:0)-ACP can be converted to oleoyl (18:1)-ACP by the catalytic action of a soluble stearoyl-ACP desaturase (SAD). The free fatty acids are then released from the FAS complex through thioesterase (TE) action. After moving across the plastidial envelope, the exported free fatty acids are reactivated to fatty acyl (FA)-CoAs on the outside of plastid. Other abbreviations: ACP acyl carrier protein, FFA free fatty acid, 16:0 palmitic acid. The image is based on information from Gurr et al. (2002)

desaturase (SAD). The fatty acids are then released from the FAS complex through the action of a **thioesterase** (TE). After moving across the plastidial envelope, the exported fatty acids are reactivated to **acyl-CoAs** on the outside of plastid.

The acyl-CoAs are now available as high-energy acyl donors to fuel membrane and triacylglycerol (TAG) biosynthesis in the endoplasmic reticulum (ER) (Fig. 4.3). In some cases, these acyl-CoAs can be further elongated (e.g., to 22:1-CoA) on the ER. In plants producing PUFA or other phosphatidylcholine (PC)-modified fatty acids (e.g., hydroxy fatty acids), TAG assembly involves a complex interplay between the Kennedy or *sn*-glycerol-3-phosphate (G3P) pathway and membrane metabolism. Most of these enzymes are membrane-bound with segments of the polypeptide traversing the lipid bilayer of the ER as opposed to being soluble cytosolic enzymes. The **Kennedy pathway** involves the sequential acylation of the

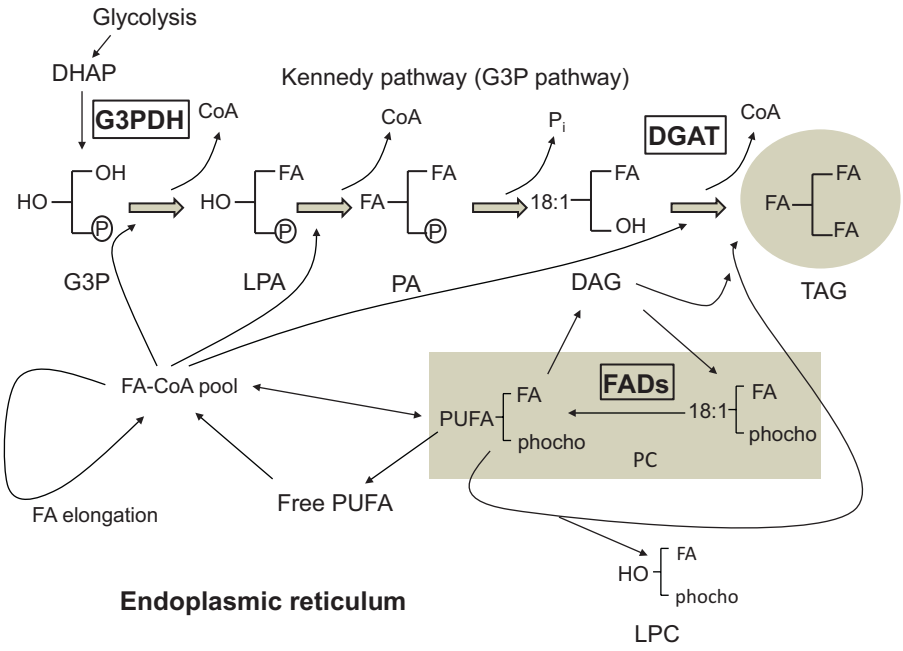

Fig. 4.3 Generalized scheme for triacylglycerol (TAG) assembly in the endoplasmic reticulum of oil-forming seeds which produce TAG containing polyunsaturated fatty acids (PUFAs). In the Kennedy or *sn*-glycerol-3-phosphate (G3P) pathway, G3P undergoes fatty acyl (FA)-coenzyme A (CoA)-dependent acylation. Inorganic phosphate (P_i) is released from the glycerol backbone prior to the addition of the final FA chain, which is catalyzed by diacylglycerol acyltransferase (DGAT). G3P for the Kennedy pathway is derived from glycolysis of glucose, which in turn is derived from sucrose imported into the seed. Diacylglycerol (DAG) formed via the Kennedy pathway can be converted to phosphatidylcholine (PC) where fatty acid desaturases (FADs) catalyze the formation of PUFAs starting with oleoyl (18:1)-PC. The fatty acyl chains of PUFA-enriched PC can be incorporated into TAG via various acyl-trafficking processes. Other abbreviations: G3PDH *sn*-glycerol-3-phosphate dehydrogenase, LPA lysophosphatidic acid, LPC lysophosphatidylcholine, P phosphate moiety; phocho, phosphocholine head group. The depicted scheme is based on information from Chen et al. (2015)

glycerol backbone, in the form of G3P (derived from glycolysis), to ultimately form TAG. **Diacylglycerol acyltransferase** (DGAT) catalyzes the acyl-CoA-dependent acylation of diacylglycerol (DAG) to produce TAG. DAG, however, represents the major branch point between the Kennedy pathway leading to TAG and membrane metabolism. In PUFA-enriched oilseeds, the DAG skeleton generated in the Kennedy pathway can be converted to PC where 18:1 chains undergo further desaturation to produce the PUFA, 18:2 and 18:3, in reactions catalyzed by fatty acid desaturase FAD2 and FAD3, respectively. Various acyl-trafficking processes are then involved in channeling PC-formed PUFA into TAG. The situation is becoming increasingly more complex with other processes being identified and with differences in acyl-trafficking between species (Chen et al. 2015; Fig. 4.3).

4.7.2 Strategies for Altering Plant Fatty Acid Composition for Improved Biodiesel Quality

As we have seen, the fatty acid composition of the feedstock has a tremendous impact on the performance characteristics of the biodiesel produced from it. Although no single feedstock is necessarily ideal for all biodiesel applications, reducing the amount of saturated fatty acids is a primary goal for improving the cold flow properties of biodiesel, while reducing PUFA content improves the oxidative stability and ignition quality. Thus, a feedstock containing high proportions of monounsaturated fatty acids such as palmitoleic acid (16:1Δ^{9cis}; hereafter 16:1) and oleic would likely perform favorably in biodiesel.

Saturated fatty acid content in plants is primarily determined during fatty acid synthesis in the plastid, through the joint action of the TE SAD (Fig. 4.2). Reduction of TE acting on 16:0-ACP using genetic engineering has been shown to prevent the early release of saturated acyl groups from the FAS complex, making them available for immediate desaturation, thus increasing the proportion of 18:1 while reducing the levels of 16:0 and 18:0 (Burh et al. 2002; Bonaventure et al. 2005). In an alternative approach, introduction of a soluble 16:0-ACP desaturase from a tropical forest vine into developing canola seed resulted in increased levels of 16:1 and its elongation products, but overall levels of saturated fatty acids did not decrease (Bondaruk et al. 2007).

Reduction of PUFAs has been accomplished through reduction of FAD2 action, preventing further desaturation of 18:1 (Buhr et al. 2002; Yang et al. 2006; Yin et al. 2007; Mietkiewska et al. 2008; Harwood et al. 2017). High 18:1 canola oil has primarily been marketed as high stability oil for food processing and frying applications (e.g., Nexera canola by Dow AgroSciences Inc.), but the same properties make it an excellent feedstock for biodiesel, especially since canola also has the lowest saturated fatty acid content of all commercial seed oils. Reduction of both 16:0-ACP TE and FAD2 action produced a similarly favorable fatty acid profile in transgenic soybean (Buhr et al. 2002).

It should be noted that alterations in fatty acid composition are often associated with developmental changes, probably as a consequence of changes in membrane lipid composition. Plants with increased saturated fatty acid content or reduced PUFA content tend to exhibit altered responses to low temperature, reduced growth rate, and compromised seed viability (Bonaventure et al. 2005; Yang et al. 2006). Future work in this area will undoubtedly involve developing strategies to mitigate these side effects to ensure that the agronomic characteristics of biodiesel crops are not adversely affected.

4.7.3 Strategies for Enhancing Seed Oil Content to Increase Supplies of Biodiesel Feedstocks

Increasing seed oil content in biodiesel crops has also become an important goal, as demand for biodiesel increases alongside increasing demand for edible oils. It has been estimated that converting the entire 2005 world supply of vegetable oil to

biodiesel would meet only about 80% of the annual demand for diesel in the USA (Durrett et al. 2008). While biodiesel clearly is not poised to become a complete replacement for diesel, this figure illustrates the supply challenges associated with the emerging biodiesel industry. One way of combating this is to choose higher oil feedstocks for the production of biodiesel. Canola, the preferred feedstock in Canada and Europe, has an oil content of around 42% (Durrett et al. 2008), while soybean, preferred in the USA, contains only about 20% oil (Lardizabal et al. 2008). The solution, however, goes beyond merely changing crops, since many other factors influence crop selection, including the value of by-products (e.g., protein, seed meal), crop subsidies, cost of inputs for production, and local geography and climate which may favor one crop over another. As a result, initiatives are under way to increase oil content in most of the major biodiesel feedstocks, including canola and soybean (Canola Council of Canada 2015).

There are several approaches to increasing seed oil content, which have been reviewed in detail (Weselake et al. 2009; Rahman et al. 2013; Singer et al. 2013; Woodfield et al. 2015). Most of these investigations, however, have been limited to growth chambers, greenhouses, and confined field trials, and a commercially available oil crop which has been metabolically engineered for increased seed oil content is still on the horizon. Several metabolic engineering strategies have focused on the activity of the acyltransferases involved in glycerolipid assembly, with DGAT being a primary target. The DGAT-catalyzed reaction shown in Fig. 4.2 has been implicated as a bottleneck in TAG biosynthesis, as a result of its low activity relative to other Kennedy pathway acyltransferases (Perry et al. 1999) and the observation that plants deficient in DGAT activity have severely reduced seed oil content (Katavic et al. 1995; Zou et al. 1999). As expected, overexpression of the gene encoding DGAT in transgenic plants resulted in elevated oil content (Jako et al. 2001; Lardizabal et al. 2008; Weselake et al. 2008; Taylor et al. 2009), but the increase in seed oil content is an order of magnitude lower than the observed increase in DGAT activity (Taylor et al. 2009), which suggests the bottleneck shifts elsewhere in the pathway. Larger increases in seed oil content were observed with overexpression of a gene encoding a yeast *sn*-glycerol-3-phosphate dehydrogenase (G3PDH), which catalyzes the formation of G3P from dihydroxyacetone phosphate produced during glycolysis, indicating that the availability of glycerol backbones may limit TAG accumulation (Vigeolas et al. 2007). Both of these observations are consistent with metabolic control studies demonstrating that in canola, glycerolipid assembly exerts more control over TAG accumulation than fatty acid synthesis (Ramli et al. 2002; Weselake et al. 2008). In addition, increasing the expression of genes encoding various **transcription factors** with roles in governing lipid biosynthesis (Liu et al. 2010), altering the partitioning of carbon from starch and protein to lipids (Meyer et al. 2012), and decreasing the breakdown of TAG (Van Erp et al. 2014) have all also been found to be useful approaches for increasing seed oil content. Transcription factors are proteins which interact with regulatory sequences in genes to influence the extent of gene expression.

Recently, **directed evolution** has been used to produce variants of type-1 DGAT exhibiting enhanced performance (Siloto et al. 2009; Roesler et al. 2016;

Chen et al. 2017). In directed evolution, enzyme variants are generated based on the introduction of random mutations into the DNA encoding the enzyme with the hope that some of the mutated genes result in the production of a more active enzyme when produced in a host such as yeast (Siloto et al. 2009). Many of the DGAT1 variants reported by Chen et al. (2017) only had single amino acid residue substitutions. The introduction of a soybean DGAT1 variant during seed development in soybean was shown to increase oil content in mature seed by 16% on a relative basis (Roesler et al. 2016). Recently, the activity of canola DGAT1 was shown to be regulated by its hydrophilic N-terminal region in response to specific metabolites (Caldo et al. 2017), thus opening the door to the development of additional strategies to improve the performance of this key enzyme. It is anticipated that performance-enhanced DGAT will become a part of the toolkit for boosting seed oil content in various oil crops.

Metabolic engineers have also developed strategies to increase the oil content of vegetative tissue (Chapman et al. 2013; Vanhercke et al. 2014; Weselake 2016). This is transformative technology in that it has the potential to substantially increase the supply of oil from higher plants. One can imagine the amount of extra plant oil that could become available if it was produced in plant species such as sugarcane and poplar!

4.8 Applications for By-Products of Biodiesel Production: Maximizing the Value of Biodiesel Feedstocks

Despite rising petroleum prices improving the cost-competitiveness of biofuel production in recent years, opportunities for marketing the by-products of biofuel production further improve the economics of their production (Hill et al. 2006). The use of oilseeds for both food and industrial purposes already yields high-value protein for use as animal feed. In the case of soybean, the oil has traditionally been considered the by-product of protein production, but this perception has changed in recent years as the value of most vegetable oils has doubled (Canola Council of Canada 2015).

The glycerol produced as a by-product of the transesterification of TAG for biodiesel production also has value as an industrial product, and new applications for glycerol are being explored as the supply now far exceeds demand for traditional applications (da Silva et al. 2009). Currently glycerol is used as a food additive and preservative and has a number of applications in the cosmetic and pharmaceutical industries (Dyer et al. 2008). Among the potential uses being explored for excess glycerol, many researchers are testing it as a carbon source for bacterial fermentation processes to produce a variety of other materials, such as polyhydroxyalkanoates (a bioplastic; see Chap. 5) (Bormann and Roth 1999), 1,3-butandiol, and citric acid (Papanikolaou et al. 2008). Polypropylene glycol may also be produced via thermochemical conversion of glycerol (Haveren et al. 2007).

In a curious turn of fortune, glycerol derived from biodiesel production is also now being used as a raw material for the synthesis of epichlorohydrin, which was, until recently, the primary feedstock in the commercial synthesis of glycerol (Haveren et al. 2007). The availability of glycerol from biodiesel production made this process uneconomical, and now it appears more commercially viable to produce epichlorohydrin from glycerol than it was to produce glycerol from epichlorohydrin.

4.9 Closing Comments

In this chapter, we have examined the production of biodiesel from plant oils, its advantages over petroleum diesel, the structural characteristics required to obtain a high-performance biodiesel, and strategies to exploit plant lipid biosynthesis for enhancing biodiesel quality and quantity. As biodiesel production and consumption increase, additional applications for the by-products of biodiesel production must be developed, which will likely result in more economically competitive bio-based industrial feedstocks derived from glycerol. Even so, it seems likely that biodiesel will not completely replace petroleum diesel as a transportation fuel; rather, it is more feasible that we will see expanded use of biodiesel-petroleum diesel blends, reducing our dependence on petroleum diesel while more advanced fuel cell technologies mature.

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Chapter 5
Production of Other Bioproducts from Plant Oils

Stacy D. Singer and Randall J. Weselake

Chapter Highlights

- Plant oils have many additional applications other than biofuel.
- Potential industrial applications of plant lipids include the production of lubricants, solvents, surfactants, bioplastics, and rubber.
- There has also been a surge of interest in the utilization of plants as "biofactories" for the production of bioactive oils for human nutrition.
- There are several types of plant-derived lipids used for such purposes, including fatty acids within triacylglycerol, wax esters, and lipid-based polymers.
- Attempts to engineer plants that synthesize high levels of useful lipids are ongoing; however, this approach has proven challenging.

5.1 Introduction

Although the production of plant-derived biodiesel has seemingly been at the forefront of nontraditional uses of oilseed crops, there are several additional applications for plant lipids that have been gaining momentum in recent years. As is the case for fuel, our society relies heavily on petroleum as a feedstock for numerous industrial products, such as lubricants, solvents, agricultural chemicals, surfactants, polymers, and food processing compounds (Fig. 5.1). As petrochemical stocks dissipate and concern for the environment grows, however, the manufacture of industrial petrochemicals is sure to be hard hit. The fact that many of these

S. D. Singer (✉)
Agriculture and Agri-Food Canada, Lethbridge, AB, Canada
e-mail: ssinger@ualberta.ca

R. J. Weselake
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

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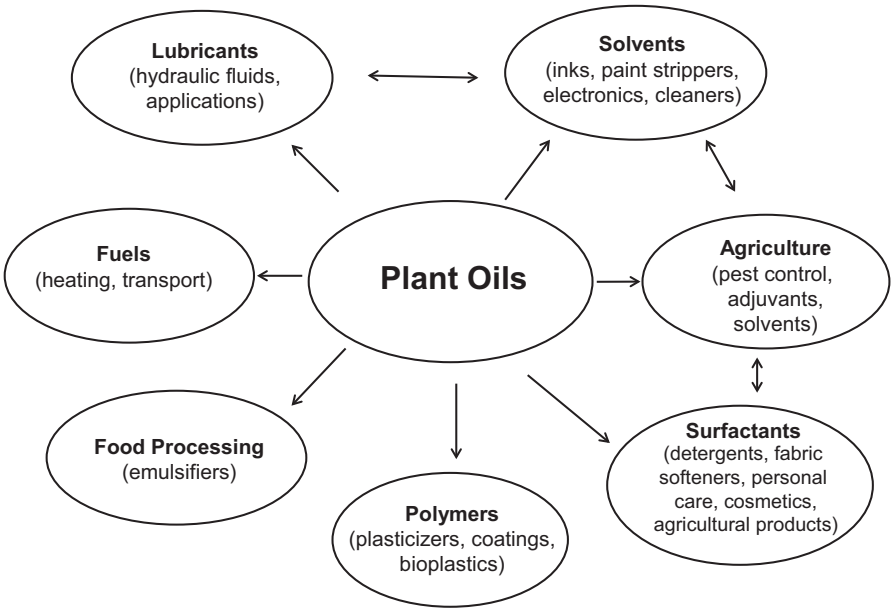

Fig. 5.1 Existing and emerging industrial applications for plant oils

industrial products have an even greater value per unit than hydrocarbon fuels means that the development of sustainable bio-based raw materials for industrial use is becoming increasingly important (Vanhercke et al. 2013). Only about 10% of the global petrochemical feedstock is used for production of these value-added industrial products (Carlsson 2009). Therefore, unlike the development of biofuel for plant oils, the use of plant oils for production of value-added materials will have less of an effect on the global supply of edible oils.

While the majority of plant lipids currently valued for these purposes are derived from storage triacylglycerol (TAG), they are by no means the only lipids of importance that can be obtained from plant sources. In this chapter we will be discussing various types of industrially important lipid-related molecules, including fatty acids, wax esters, and lipid-based polymers. Although not bioproducts in the strictest sense, we will also consider the production of **bioactive oils** in plants for human and animal nutrition. Finally we will touch upon the challenges that have been encountered in attempts to use biotechnology as a means to harness the lipid biosynthetic pathways of plants for the production of molecules of interest.

5.2 Industrial Feedstocks

While there are a wide range of bio-based molecules that could be used for industrial purposes, plant lipids are particularly compatible for replacing petrochemical-based feedstocks due to their similar linear carbon chain structures. In fact, many plant-derived

industrial feedstocks offer a variety of advantages over fossil fuel-based molecules. For example, while petroleum must be broken down and rebuilt into specialty chemicals, plants begin the process from simple precursors and build the complex molecules themselves, which has the potential to be very beneficial in terms of time, energy, and processing. Furthermore, a number of plant-based chemicals are actually known to perform better than their synthetic counterparts, as is the case for natural rubber.

Unfortunately, there are presently also several drawbacks to the use of plant oils for industrial purposes. The first of these is price – plant oils are currently more expensive than petroleum; however, they are becoming more competitive as petroleum prices rise. The second is availability – the majority of plant oils of industrial interest are often not present in domesticated crops and therefore tend to have limited distribution and variable supply.

5.2.1 Fatty Acids

Currently, the major food oil crops dominate in terms of agricultural production and include canola-type *Brassica napus*, oil palm (mainly *Elaeis guineensis*), soybean (*Glycine max*), cotton (*Gossypium* spp.) seed, peanut (*Arachis hypogea*), corn (*Zea mays*), sunflower (*Helianthus annuus*), and olive (*Olea europaea*), along with several less significant crops such as coconut (*Cocos nucifera*), flax (*Linum usitatissimum*) seed, sesame (*Sesamum indicum*), and safflower (*Carthamus tinctorius*) (Weselake et al. 2017). Industrial oils for various applications make use of the natural diversity of fatty acid structures found within plant storage TAG (Fig. 5.2); however, the majority of the food-use oils derived from the crops mentioned above consist of only five main fatty acids, including palmitic, stearic, oleic, linoleic, and α-linolenic acids (Fig. 4.2). Indeed, most fatty acids of interest for industrial use

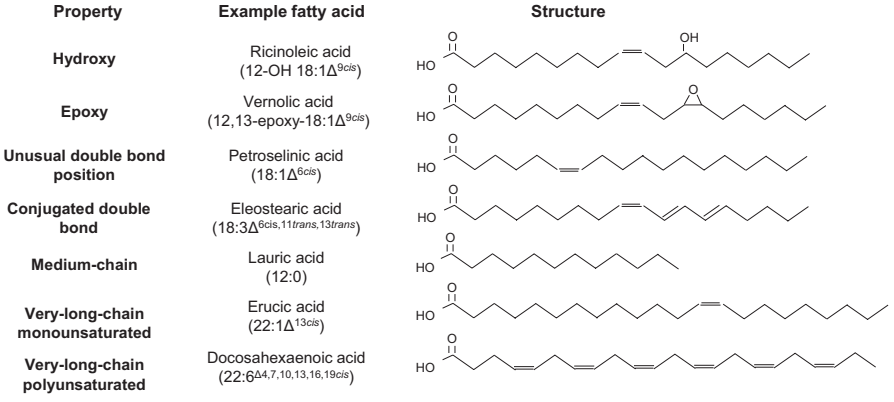

Fig. 5.2 Examples of uncommon fatty acids with commercial importance. A large number of industrially important fatty acids are produced naturally; however, they are generally only found in a limited range of species

Table 5.1 Examples of oilseed crops grown for industrial applications

Common name	Major cultivated species	Class of FA	Key FA	Major use
Castor bean	*Ricinus communis*	Hydroxy FA	Ricinoleic acid	Chemical feedstock
Linseed	*Linum usitatissimum*	PUFA	Linolenic acid	Drying oil
HEAR cultivars	*Brassica napus*	VLC-FA	Erucic acid	Chemical feedstock
Tung	*Aleurites fordii syn. Vernicia fordii*	Conjugated FA	α-Eleostearic acid	Drying oil
Potential new crops now under development				
Crambe	*Crambe abyssinica*	VLC-FA	Erucic acid	Chemical feedstock
Rain daisy	*Dimorphotheca pluvialis*	Hydroxy FA	Dimorphecolic acid	Chemical feedstock
Cuphea	*Cuphea* spp.	Medium-chain FAs	C8:0 to C14:0	Chemical feedstock
Lesquerella	*Lesquerella fendleri*	Hydroxy FA	Lesquerolic acid	Chemical feedstock
Coriander	*Coriandrum sativum*	Monounsaturated FA	Petroselinic acid	Chemical feedstock
Calendula	*Calendula officinalis*	Conjugated FA	Calendic acid	Drying oil
Euphorbia	*Euphorbia lagascae*	Epoxy FA	Vernolic acid*	Chemical feedstock

PUFA polyunsaturated fatty acid, VLC-FA very long-chain fatty acid, FA fatty acid
Adapted from Taylor et al. (2011)

occur in a limited range of species (Table 5.1) or only in very minor quantities in domesticated crop species. As such, only a small number of fatty acids are actually used for industrial purposes at present compared with the large number produced in nature (over 300 types) (Badami and Patil 1980).

5.2.1.1 Medium-Chain Saturated Fatty Acids

One major use of plant oils for industrial purposes relies on the presence of a high proportion of saturated fatty acids of medium chain length, which are used for the production of surfactants such as detergents, soaps, and other personal care products. As mentioned in the previous chapter, the synthesis of fatty acids in plants normally occurs through the sequential addition of 2 carbon units at a time and terminates when the chain reaches 16 or 18 carbons. This occurs through the action of an acyl-acyl carrier protein (ACP) thioesterase (TE), which catalyzes the release of the new fatty acid chain for subsequent export from the plastid to the cytosol and incorporation into TAG (see Fig. 4.2). In certain tropical plants, specialized chain-length-specific TEs catalyze the release of fatty acids at an earlier stage in their synthesis, when the chain is 10, 12, or 14 carbons in length (Voelker et al. 1997).

The main fatty acid of this type currently in use industrially is lauric acid (12:0), which is found naturally in plants such as oil palm and coconut (Fig. 5.2, Table 5.1). Caprylic (8:0) and capric (10:0) acids are also useful in a range of industrial applications. Unfortunately, there are currently few commercially viable plants that are rich in any of these fatty acids, and they are therefore either fractionated out as very minor components of palm kernel or coconut oils or produced from petroleum. To make matters worse, the expanding production of oil palm is causing a considerable amount of habitat destruction in tropical regions, which makes finding an alternative source of these fatty acids of the utmost importance. Interestingly, members of the genus *Cuphea*, which produce extremely high levels of these three fatty acids (Badami and Patil 1980; Kim et al. 2015), may offer a potential source in temperate regions. Initial attempts to genetically engineer oilseed crops to produce these valuable fatty acids have also met with some success in certain cases, which bodes well for their future production in agronomically amenable plant species.

The production of medium-chain saturated fatty acids in transgenic plants represents an early success story. Lauric acid is typically found in tropical oils such as coconut and palm kernel oil and is widely used in food, pharmaceutical, and cosmetic industries. One of the earliest examples of metabolically engineering plants for oil quality was achieved through the expression of a 12:0-ACP-specific TE from the seeds of California bay laurel (*Umbellularia californica*) in canola, whereby they achieved up to 60% lauric acid in the second generation. However, the vast majority of this fatty acid was only present in the *sn*-1 and *sn*-3 positions (the two outer positions on the glycerol backbone) of TAG, suggesting that these plants were unable to incorporate 12:0 at the middle *sn*-2 position, which limited further increases in yield (Voelker et al. 1996; Wiberg et al. 2000).

In nature, only select plants are capable of introducing saturated fatty acids into the *sn*-2 position of TAG, which greatly influences the levels at which the plant can accumulate this type of fatty acid. Members of Lauraceae, Myristicaceae, and Lythraceae, as well as coconut, are known to produce seed TAG with a predominance of medium-chain saturated acyl groups at all three *sn* positions and are thus able to amass up to 90% of these fatty acids. This is very likely a direct result of their unusual lysophosphatidic acid acyltransferase (LPAAT) enzymes, which show a selective preference for this type of acyl substrate. LPAAT catalyzes the acyl-CoA-dependent acylation of lysophosphatidic acid (LPA) to produce phosphatidic acid (PA) in the Kennedy pathway (Fig. 5.3). While enzyme **specificity** refers to the ability of an enzyme to use a particular substrate when presented in isolation from others, and is thus not all that reflective of what occurs in a living plant cell, enzyme **selectivity** refers to the preference of an enzyme to use a particular substrate when presented with a mix of possible substrates. As one may expect, when transgenic plants were generated that produced both the specialized California bay laurel TE and an LPAAT from coconut, lauric acid was also observed at the *sn*-2 position of the glycerol backbone of TAG, and total levels of this fatty acid were increased (Knutzon et al. 1999; Wiberg et al. 2000). This approach involving the introduction of an LPAAT with a preference for particular fatty acids may also prove useful for overcoming low yields when

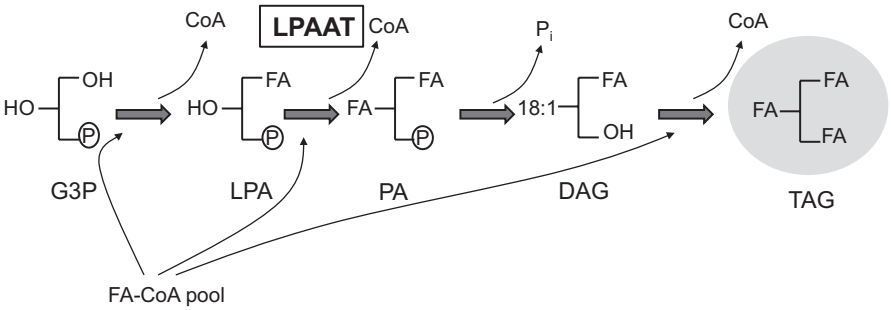

Fig. 5.3 The role of lysophosphatidic acyltransferase (LPAAT) in the Kennedy pathway leading to triacylglycerol (TAG). Other abbreviations: CoA coenzyme A, DAG diacylglycerol, FA-CoA fatty acyl-coenzyme A, G3P *sn*-glycerol-3-phosphate, LPA lysophosphatidic acid. Information on this pathway can be found in numerous reviews on plant lipid biosynthesis (e.g., Chen et al. 2015)

metabolically engineering plants to produce other fatty acids that are naturally excluded from the *sn*-2 position, such as erucic acid (Lassner et al. 1995).

5.2.1.2 Monounsaturated Fatty Acids

Monounsaturated fatty acids are also incredibly useful for industrial purposes (Harwood et al. 2017). This is due, at least in part, to the fact that when compared to oils rich in polyunsaturated fatty acids (PUFAs), those containing high levels of monounsaturated fatty acids are far more resistant to oxidation. This makes for more stable oil and allows its direct use in such industrial products as biolubricants. In addition, monounsaturated fatty acids can be easily cleaved at their double-bond sites through chemical processing to yield many highly desirable feedstocks for industrial use, such as monomers that can be used to produce various types of nylon (polyamides).

Oleic acid ($18{:}1^{\Delta 9cis}$; hereafter referred to as 18:1) is probably the most common of the monounsaturated fatty acids and typically makes up approximately 25–55% of the oil from major oilseed crops (Table 4.2). It is generated via the action of a stearoyl-ACP desaturase (SAD) enzyme, which catalyzes the addition of a double bond at a specific position within a fatty acid chain (see Fig. 4.2). In this case, the enzyme is termed as Δ9 SAD, which catalyzes the insertion of a double bond at the Δ9 position of the stearoyl moiety (18:0) of stearoyl-ACP. Although high oleic acid cultivars (75–85%) of several oilseed crops have been bred for food use (Takagi and Rahman 1996; Schierholt et al. 2000), the significant residual levels of PUFAs that remain in these oils cause issues for industrial applications.

There are also a number of naturally occurring plant oils that possess monounsaturated fatty acids with double bonds at alternative positions. For example, seeds from Umbelliferae species, such as *Daucus carota* (carrot) and *Coriandrum sativum*, produce seed oil with the major constituent being petroselinic acid ($18{:}1^{\Delta 6cis}$). This fatty acid has the potential to be very significant for industrial use as it can be

split using ozonolysis to yield lauric acid and adipic acid (6:0), which is a building block of 6,6 nylon, a product that is currently generated from petroleum and has a very large annual production. The synthesis of this unusual monounsaturated fatty acid results, at least in part, from the activity of a plastidial Δ4 16:0-ACP desaturase that catalyzes the addition of a double bond to palmitoyl-ACP, with the resulting molecule then being elongated to petroselinic acid (Cahoon and Ohlrogge 1994). This desaturase has distinct substrate specificity in terms of acyl chain length compared to other acyl-ACP desaturases.

Additional examples of acyl-ACP desaturases with unique substrate specificities also exist, including the Δ9 18:0-ACP desaturase from *Thunbergia alata* (Cahoon et al. 1994) and Δ9 16:0-ACP desaturases from *Doxantha* spp. and *Asclepia syriaca* (Cahoon et al. 1997a, 1998), which in the latter case allows the production of palmitoleic acid ($16{:}1\Delta^{9cis}$). This fatty acid and its elongation product *cis*-vaccenic acid ($18{:}1\Delta^{11cis}$) are two additional monounsaturated fatty acids that have garnered industrial interest due to the recent development of olefin metathesis. This technology allows the production of 1-octene from either of these fatty acids, which is a very valuable industrial feedstock with particular use in the generation of polyethylene and plasticizers. Both palmitoleic acid and *cis*-vaccenic acid are normally present in only very small amounts (<2%) in the majority of plant oils, with the exception of such species as *Hippophae rhamnoides* (sea buckthorn) and *Doxantha unguis-cati* (cat's claw), which can accumulate up to 80% of these two fatty acids in its seed oil (Chisholm and Hopkins 1965). As has been the case for the aforementioned monounsaturated fatty acids, the major determinant of whether these fatty acids are present at high levels in a particular species appears to be the presence of a specific acyl-ACP desaturase. Interestingly, very few amino acid differences need to exist in these various desaturase enzymes for distinct changes in substrate specificities to occur; in fact, it has been found that the alteration of as few as five amino acid residues can result in the conversion of a Δ6 16:0-ACP desaturase into a Δ9 18:0-ACP desaturase (Cahoon et al. 1997b).

Another relatively uncommon monounsaturated fatty acid is the very long-chain erucic acid ($22{:}1^{\Delta 13cis}$), which is used to produce erucamide (a slipping agent used in the production of polyethylene and propylene films) (McVetty et al. 2016). This fatty acid is generated through the sequential elongation of oleoyl-CoA by an extraplastidial **elongase system** (see Fig. 4.3). Modern-day canola, in which erucic acid is essentially absent, is the result of a nonfunctional fatty acid elongase 1 (Katavic et al. 2002). Erucic acid is produced by such plants as *Limnanthes douglasii* and members of the Brassicaceae. Currently, it is derived mainly from the oil of high-erucic rapeseed (HEAR), which contains 45–55% of this fatty acid. Unfortunately, the continued production of HEAR is set to become increasingly difficult due to the ever-expanding growth of food-use canola (low-erucic acid rapeseed), as the two crops are cross-fertile. This means that HEAR must be grown in complete isolation from canola crops to preclude contamination with erucic acid, which is thought to be detrimental to our health when ingested (Zhang et al. 1991), a feat that will be highly challenging. As a result of this, biotechnological approaches to produce high levels of erucic acid in alternative oilseed crops are underway (Zhu et al. 2016).

Indeed, the production of up to almost 80% erucic acid in transgenic *Crambe abyssinica* was achieved using a gene stacking strategy involving multiple expression cassettes (Li et al. 2012).

5.2.1.3 Unusual Fatty Acids

In addition to the fatty acids described above, there are several specialty industrial oils that are derived from fatty acids found only in very specific plants that contain unusual functional groups, such as hydroxyl groups or epoxy bridges, unusual double-bond structures, or cyclic structures (Fig. 5.2). Many of these fatty acids result from the activity of divergent members of the fatty acid desaturase (FAD) 2 family, which is typically an endoplasmic reticulum (ER)-bound enzyme that catalyzes the addition of the second double bond at the Δ12 position of oleic acid to produce linoleic acid ($18{:}2^{\Delta 9cis,12cis}$; hereafter referred to as 18:2) (Okuley et al. 1994). As indicated previously, FAD2 and FAD3 catalyze PUFA formation at the level of phosphatidylcholine (PC) (see Fig. 4.3). While functional variants of this enzyme are rare in nature, there are several plant species that accumulate storage TAG with extremely elevated amounts of these unusual fatty acids.

One fairly well-known example is castor oil, which is obtained from the castor bean plant (*Ricinus communis*) and contains very high levels (90%) of the hydroxy fatty acid ricinoleic acid (12-hydroxy-$18{:}1\Delta^{9cis}$) (Fig. 5.2, Table 5.1) (Badami and Patil 1980), which is synthesized from oleic acid through the action of a **FAD2-related hydroxylase** (Broun and Somerville 1997). This rare fatty acid is becoming an increasingly important industrial feedstock, as it has the ability to undergo pyrolytic cleavage at the Δ12 position, resulting in the production of undecylenic acid ($11{:}1\Delta^{10cis}$), which is used in the production of various cosmetics, pharmaceuticals, and nylon (McKeon 2016). Castor oil can also be used directly in several applications, including the production of polyurethane and high-performance lubricants (McKeon 2016; Vanhercke et al. 2013). Unfortunately, the potential for castor as a future industrial oil crop is very limited due to the presence of a highly toxic protein (ricin) in its seeds, along with many allergens; as a result, attempts to produce alternative sources of this fatty acid through genetic engineering are currently under way.

When the castor fatty acid hydroxylase, which catalyzes the hydroxylation of oleic acid to ricinoleic acid, was introduced during seed development in the model plant *Arabidopsis thaliana*, only 18% ricinoleic acid was produced (Broun and Somerville 1997). While this result was promising conceptually, much higher levels are required for the plant to be useful in an industrial sense. Interestingly, both castor diacylglycerol acyltransferase (DGAT) 2 and phospholipid:diacylglycerol acyltransferase (PDAT) were subsequently shown to prefer substrates containing ricinoleic acid and have thus been implicated in the accumulation of high amounts of this fatty acid in this species. The DGAT-catalyzed reaction was discussed in Chapter 4 and is shown again in Fig. 5.4 in the context of a plant cell that has been engineered to produce TAG containing ricinoleic acid. In contrast, PDAT catalyzes

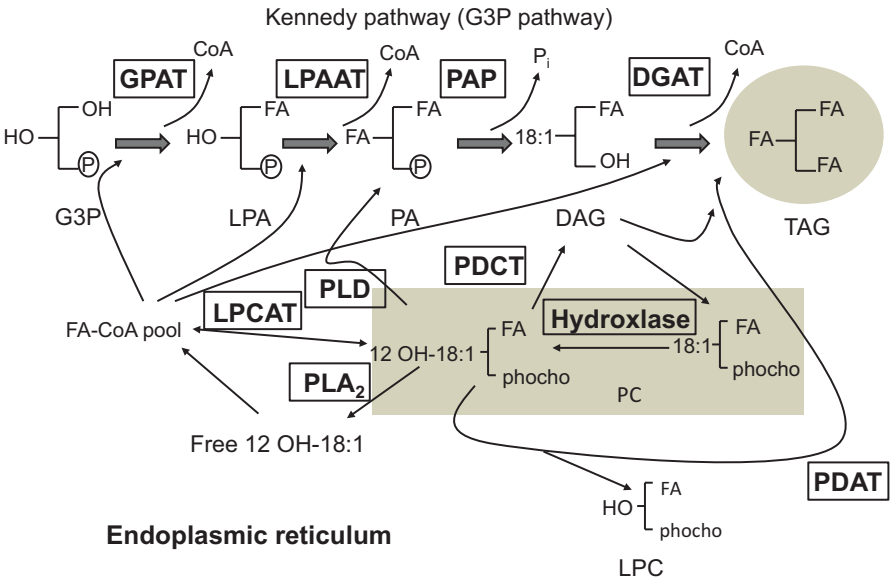

Fig. 5.4 The interplay of triacylglycerol (TAG) biosynthesis with membrane metabolism in a hypothetical plant system producing TAG enriched in hydroxy fatty acids. These pathways may also apply to other fatty acids produced on phosphatidylcholine (PC) such as α-linolenic acid. Diacylglycerol (DAG) produced in the Kennedy pathway can be incorporated into PC where hydroxylation of oleic acid (18:1) to form ricinoleic acid (12-OH 18:1Δ^{9cis}) occurs at the middle position of the glycerol backbone. Various acyl-trafficking processes can lead to the formation of TAG enriched in ricinoleic acid. Other abbreviations: CoA coenzyme A, DAG diacylglycerol, DGAT diacylglycerol acyltransferase, FA-CoA fatty acyl-coenzyme A, G3P *sn*-glycerol-3-phosphate, LPA lysophosphatidic acid, LPAAT lysophosphatidic acid acyltransferase, LPC lysophosphatidylcholine, PA phosphatidic acid, P_i inorganic phosphate, PAP phosphatidic acid phosphatase, PDAT phospholipid:diacylglycerol acyltransferase, PDCT phosphatidylcholine:diacylglycerol cholinephosphotransferase, phocho phosphocholine head group, PLA_2 phospholipase A_2, PLD phospholipase D. This figure is based on information from the following sources: Vanhercke et al. (2013), Bayon et al. (2015), Chen et al. (2015), Yang et al. (2017)

the acyl-CoA-independent formation of TAG using PC as a fatty acyl donor (Fig. 5.4) (Ståhl et al. 2004). Co-introduction of castor DGAT2 and PDAT, along with the castor fatty acid hydroxylase, resulted in a significant boost in ricinoleic acid levels in transgenic *A. thaliana* seeds (Burgal et al. 2008; Kim et al. 2011; Van Erp et al. 2011). Similarly, when castor phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) was co-introduced along with the castor fatty acid hydroxylase in *A. thaliana*, ricinoleic acid levels in the seed oil were also increased compared to when the hydroxylase was introduced by itself (Hu et al. 2012). PDCT catalyzes the transfer of the phosphocholine head group of PC-modified fatty acids, such as ricinoleic acid, to diacylglycerol (DAG) produced in the Kennedy pathway (Fig. 5.4) (Lu et al. 2009). As a result, hydroxy fatty acid-enriched DAG is now available for the Kennedy pathway leading to TAG, and DAG enriched in oleic acid originally produced in the Kennedy pathway is now available for further

hydroxylation at the level of PC. Even with these further modifications, the ricinoleic acid content still only reached about 27%, indicating that additional engineering work was required to achieve higher levels. In this regard, additional enzyme-catalyzed reactions are being targeted for modification. For example, phospholipase A_2 (PLA_2) catalyzes the release of fatty acyl chains from the middle position (*sn*-2) of PC. Interestingly, a low molecular mass PLA_2 from castor has been shown to exhibit preference for hydroxy fatty acyl chains (Bayon et al. 2015). Introduction of this castor PLA_2 into transgenic *A. thaliana* expressing a gene encoding castor oleic acid hydroxylase resulted in a significant decrease in the ricinoleic acid content of PC.

The long-chain monounsaturated epoxy fatty acid vernolic acid (12,13-epoxy-18:1Δ^{9cis}) is produced in abundance in the seed oil of *Asteraceae*, *Vernonia*, and *Euphorbia* spp. (Fig. 5.2, Table 5.1). This unusual fatty acid, which contains an epoxy group, can be used as a feedstock for the production of adhesives, varnishes, paints, and industrial coatings. The epoxy group of vernolic acid is added through the catalytic action of either a **FAD2-related epoxygenase** or a **cytochrome P450**, depending on the species (Cahoon et al. 2002). Yet another example of FAD2-like enzymes with a divergent function are the **FAD2-related conjugases** commonly found in *Momordica charantia*, *Impatiens balsamina*, and *Vernicia fordii* (tung tree) (Dyer et al. 2002). These enzymes catalyze the formation of conjugated fatty acids, which contain non-methylene-interrupted double bonds within their structure, from either linoleic acid or α-linolenic acid (18:3$\Delta^{9cis,12cis,15cis}$; hereafter referred to as 18:3) acid. *M. charantia* and tung tree seed in particular contain very high levels of the conjugated α-eleostearic acid (18:3$\Delta^{9cis,11trans,13trans}$), which accumulates to 60% and 82% of their total seed oil, respectively. Tung tree oil possesses very unique drying properties and is highly valued for the protection of furniture. As is the case for castor, the potential for the future expansion of the production of the majority of plants that synthesize these unusual fatty acids is very limited due to their unsuitability as commercial crops. Unfortunately, transgenic approaches in which these genes have been introduced into non-native plant species have shown only minimal success as of yet. For example, introduction of a *Vernonia galamensis* Δ12-epoxygenase, along with a DGAT2 from the same species, in transgenic plants has resulted in the production of seeds containing up to only 26% vernolic acid (Li et al. 2010).

5.2.2 Plant-Derived Wax Esters

TAGs and their associated fatty acids certainly dominate in terms of the plant lipids most commonly used for industrial applications at present. However, **wax esters**, which are made up of a long-chain fatty acid esterified to a long-chain fatty alcohol, are another class of plant lipid that is used in the production of specialized industrial products. Plant waxes are present at high levels in their cell walls, where they function in light absorption and reflection, and also provide protection from desiccation and resistance to pathogens (Kolattukudy 1970). These functional properties are a

result of their solid state, high hydrophobicity, and excellent resistance to hydrolytic degradation, all of which are also incredibly useful for potential industrial applications, especially in the form of lubricants (Heilmann et al. 2012).

The plant wax that is most commonly used for industrial purposes at present is obtained from the leaves of the carnauba palm tree (*Copernicia prunifera*), and is highly valued as a surface polish and protectant, with many additional industrial uses (Vanhercke et al. 2013). Wax esters derived from the seeds of the desert shrub jojoba (*Simmondsia chinensis*), however, are also gaining interest. The seed oil produced by this species is unique, as straight-chain wax esters that are in liquid form at room temperature serve as the predominant storage lipid in their seeds in place of TAG, accumulating to levels of up to 50% of their weight (Ohlrogge et al. 1978). Currently, they are used as ingredients in cosmetics and personal care products such as moisturizers, shampoos, and conditioners, as they are quite similar to human sebum. They are also used as an alternative of sperm whale oil, which is likewise made up mainly of wax esters and was widely used in high-pressure and high-temperature lubricants prior to the ban on importing whale oil to the USA in 1971. Unfortunately, since jojoba wax esters are made up mainly of relatively short monounsaturated C20 and C22 fatty acids and fatty alcohols, they have too high of a melting point (approximately 9 °C) for their widespread application as lubricants, particularly in colder climates. While this plant is grown commercially for its seed wax in certain parts of the world, its agricultural expansion is limited by its low yield and requirement for a warm climate. In addition, harvest of the beans is typically carried out by hand as maturation occurs sporadically, which makes it a very labor-intensive crop. Therefore, its wax esters are prohibitively expensive and are not able to compete with cheaper petroleum-based lubricants (Vanhercke et al. 2013).

Similarly to TAGs, the specific properties of wax esters result from their carbon chain length and in some cases from the occurrence of functional groups such as methyl branches or diesters (Biester et al. 2012). However, it appears that wax ester synthesis may be a more straightforward process than TAG biosynthesis, and in principle only three additional enzymes would be required for their production in seeds: a fatty acid elongase to yield high levels of fatty acids with a chain of at least 20 carbons in length, a **fatty acid reductase** (FAR) to convert fatty acids to fatty alcohols, and a **wax synthase** (WS) to esterify a fatty alcohol to a fatty acid. This final step in the biosynthesis of wax esters is somewhat analogous to the final DGAT-catalyzed acylation of DAG to produce TAG in the Kennedy pathway, and in some cases, DGAT enzymes possess dual WS/DGAT activity (Kalscheuer and Stienbüchel 2003; Li et al. 2008). As expected, the transgenic production of wax esters in plant seeds has proven to be very promising.

In theory, the transgenic production of wax esters would require the activity of only a fatty acid elongase to generate high levels of fatty acids with chain lengths of C20 or longer, as well as those of a FAR and WS. This was indeed confirmed in transgenic *A. thaliana* through the introduction of a fatty acid elongase, along with a jojoba FAR and WS (Lardizabal et al. 2000), whereby a major proportion of seed TAG was replaced by wax esters (up to 70% of the oil by weight). Since the

introduced wax ester biosynthesis pathway was running alongside the naturally occurring (endogenous) TAG biosynthetic pathway in these plants, it is reasonable to assume that suppression of TAG assembly could result in even better results.

In addition to their use as biolubricants, wax esters can also be hydrolyzed to yield fatty acids and fatty alcohols. Indeed, wax ester biosynthesis may provide a more amenable system for engineering the accumulation of useful fatty acids. Therefore, other fatty acid-modifying enzymes, such as hydroxylases or TEs, could be incorporated into the mix to provide substrates for the synthesis of various wax esters. By combining various permutations of genes encoding these enzymes, a very large number of wax esters with different compositions and functionalities could thus be achieved. In line with this, the worldwide EU-FP7 ICON (Industrial Oil crops producing added value Oils for Novel chemicals) project was established in an attempt to further research in this area (http://icon.slu.se).

5.2.3 Lipid-Derived Polymers

Polymers are large molecules that are made up of a number of repeated monomer subunits covalently linked through the process of polymerization. Examples of lipid-derived polymers include many plastics, Styrofoam, and rubber. The properties of a particular polymer are influenced by several factors including structure and architecture, as well as chain length.

5.2.3.1 Bioplastics

In the USA alone, two million plastic bottles are used every 5 min, and 60,000 plastic bags are used every 5 s (Rauber 2011). The majority of this plastic is petroleum-based and is not recycled nor is it biodegradable. Therefore, the bulk of it ends up either in landfills or the ocean. Plastics are composed of high molecular weight polymers of simple monomers, such as ethylene and styrene. While most are hydrocarbon-based with N, O, S, or halogen (chlorine or fluorine) substitutions, a minority are based on silicone. There are many different types of plastics, including polyethylene, polypropylene, polyvinylchloride, polytetrafluoroethylene, nylon, and polystyrene, which are classified according to their composition or functional properties.

One class of plastic that is generated in nature are the **polyhydroxyalkanoates** (PHAs), which, unlike starch-based plastics such as **polylactic acid**, are biodegradable and UV-stable. PHAs are linear polymers of 3-hydroxy fatty acids with various side chains that give them different properties. They are a storage compound produced in response to nutrient deficiency or excess carbon supply by certain soil bacteria through the fermentation of either lipids or sugars. The particular polymer generated depends upon both the bacterial species and growth conditions, with the resulting molecules accumulating as intracellular granules (Fig. 5.5) (Suriyamongkoi et al. 2007).

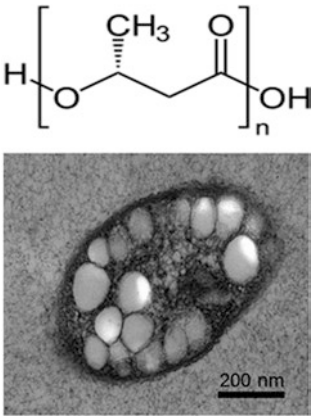

Fig. 5.5 Polyhydroxyalkanoates (top), which accumulate as granules within the bacteria that produce them (bottom) (Image source: AOCS Lipid Library http://lipidlibrary.aocs.org/Biochemistry/content.cfm?ItemNumber=41298)

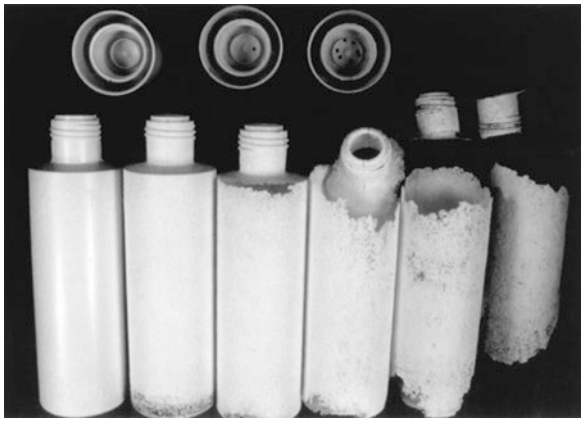

Fig. 5.6 Degradation of polyhydroxyalkanoate containers in aerobic sewage sludge after treatment for 0, 2, 4, 6, 8, and 10 weeks (left to right) (Reproduced from Madison and Huisman (1999) with permission from the American Society for Microbiology)

There are two main classes of PHAs, which are based upon the chain length of their hydroxy fatty acids. Short-chain PHAs comprise hydroxy fatty acids with three to five carbon atoms and have properties that are similar to polypropylene. Medium-chain PHAs comprise hydroxy fatty acids with 6–14 carbon atoms and resemble elastomers and rubbers. The simplest and most commonly occurring form of PHA is poly-β-hydroxybutyrate (PHB), which is made up of 1000–3000 short-chain hydroxy fatty acid monomers. While PHB on its own is rather brittle and stiff, copolymerization with other fatty acids, such as β-hydroxyvalerate, results in increased flexibility (Suriyamongkoi et al. 2007). PHA containers undergoing aerobic biodegradation (Madison and Huisman 1999) are shown in Fig. 5.6.

Although plants are not natural producers of PHAs, oilseed crops have been targeted as potential "factories" for this polymer as the products of fatty acid breakdown can provide abundant substrate for its synthesis. In bacteria, there are three

Table 5.2 Summary of PHA production in transgenic plants

Plant.	Tissue	PHA produced	PHA yield (% dcw)
Arabidopsis	Shoot	PHB	14–40
	Shoot	PHB-co-HV	1.6
	Whole plant	PHA_{MCL}	0.6
Alfalfa	Shoot	PHB	0.2
Corn	Shoot	PHB	6
Cotton	Fiber	PHB	0.3
	Fiber	PHB	0.05
Potato	Shoot	PHB	0.02
Rapeseed	Shoot	PHB	0.1
	Seed	PHB	8
	Seed	PHB-co-HV	2.3
Tobacco	Shoot	PHB	0.01
	Shoot	PHB	0.04

PHA polyhydroxyalkanoate, PHB poly-β-hydroxybutyrate, HV β-hydroxyvalerate, MCL medium-chain length, dcw dry cell weight. Adapted from Philip et al. (2007)

enzymes required for the conversion of propionyl-CoA and/or acetyl-CoA to PHA: **β-ketothiolase**, **acetoacetyl-CoA reductase**, and **PHA synthase**. Plants already possess their own ketothiolase, which is involved in isoprenoid metabolism, and would therefore theoretically only require the introduction of bacterial acetoacetyl-CoA reductase and PHA synthase. To date, several approaches have been used to successfully produce PHAs in plants at moderate levels (Table 5.2) (Bohmert-Tatarev et al. 2011); however, further research will likely be necessary to increase the yield of this product in order for these plants to be of industrial use.

5.2.3.2 Natural Rubber

Natural rubber consists of a high molecular weight polymer of isoprene (most often *cis*-1,4-polyisoprene), with other ill-defined minor components that contribute to its functionality, and is used extensively in many products with a global consumption of about 11 million metric tons per year. Natural rubber is synthesized within microscopic particles produced in the cytosol of certain plants and fungi, although the precise genetic mechanism of its biosynthesis has yet to be fully elucidated. What is known is that it comprises a rather complicated side branch of the isoprenoid pathway involving a **rubber transferase** (or rubber transferase complex) that is embedded within a membrane surrounding the rubber particle core (Cornish and Xie 2012). To confuse matters further, it appears that different species tend to exhibit variations in their exact mode of rubber biosynthesis, accumulation, and compartmentalization.

Currently, the main commercial source of natural rubber is the latex of the rubber tree (*Hevea brasiliensis*). Unfortunately, rubber tree plantations are very labor-intensive due to the fact that the latex cannot be harvested mechanically, and they are limited to warm, humid, and sunny climates such as Thailand, Indonesia, and

Malaysia, which together account for the majority of its production. While *H. brasiliensis* is native to South America, it is not cultivated widely there due to the presence of South American leaf blight. While this fungal pathogen appears to remain limited to South America at this time, the fact that the genetic base of the various plantations worldwide is extremely narrow makes the commercial production of *H. brasiliensis* incredibly susceptible to widespread failure if/when this disease were to spread (Onokpise and Louime 2012). To make matters worse, rubber tree plantations are quickly being replaced with palm oil plantations to meet biofuel demands and increase profit to the farmers, and allergies to *H. brasiliensis* latex are very common and on the rise (Bousquet et al. 2006). As a result of all these factors, the bulk of rubber produced today is generated synthetically from petroleum. Natural rubber, however, is known to have superior performance to synthetic rubber in some applications, such as aircraft tires and medical devices (Imle 1978), making synthetic rubber not only an unsustainable and environmentally unfriendly source of this product but also an inadequate one.

Rubber-containing latex is produced in varying amounts by approximately 2500 plant species and is generally exuded following injury where it mainly plays a role in disease resistance, tolerance to environmental stress, and wound healing. However, in the vast majority of these species, the plant is difficult to cultivate and/or tap, or the rubber is not suited for industrial purposes (Belcher et al. 2004). Unfortunately, due to the complexity of the rubber biosynthetic pathway, little progress has been made as of yet in the metabolic engineering of plants capable of producing this product.

There are two major candidate species that are currently being studied for their potential as alternative sources of natural rubber: guayule (*Parthenium argentatum*) and Russian dandelion (*Taraxacum kok-saghyz*) (Table 5.3) (Van Beilen and Poirier 2007). Guayule is a flowering shrub in the Asteraceae family that is native to the southwestern USA and northern Mexico. It has been explored as an alternative source of rubber several times throughout history, but following World War II, its expense compared to *H. brasiliensis*-derived rubber was considered to be prohibitive. Despite its cost, as well as issues with its ability to only produce rubber seasonally and technical challenges with extraction and processing, it has seen a growing resurgence in interest due to the fact that unlike rubber extracted from *H. brasiliensis*, it is hypoallergenic, which makes it an ideal candidate for various medical applications (Van Beilen and Poirier 2007). Another attraction of this species is its potential as a biofuel crop, as it is a nonfood species and can be grown in arid regions where food crops would fail, thus limiting the food versus fuel conundrum.

Russian dandelion was grown on a rather large scale in the Soviet Union in the 1930s and 1940s, as well as in other parts of the world during World War II when supplies of *H. brasiliensis*-derived rubber were threatened. During this time, it was found that tires produced using rubber derived from Russian dandelion were as resilient as those from *H. brasiliensis* rubber and better than those generated from guayule. As is the case for guayule-derived rubber, its expense compared to that of *H. brasiliensis* has prohibited its further development, although there has been a resurgence of interest in this species. While it is potentially even more allergenic

Table 5.3 Potential plant sources of natural rubber

Rubber source	Content (%)	Rubber mw (kD)[a]	Production T y^{-1} (year)	Yield (kg $ha^{-1}y^{-1}$)
Rubber tree	30–50 in latex			
H. Brasiliensis	2% of tree dw	1310	9,000,000 (2005)	500–3000
Guayule				
P. argentatum gray	3–12	1280	10,000 (1910)	300–2000
Russian dandelion				
T. Kok-saghyz	Trace-30	2180	3000 (1943)	150–500
Rubber rabbitbrush				
Chrysothamnus nauseosus	<7	585	–	–
Goldenrod				
Solidago virgaurea minuta	5–12 of root dw	160–240	–	110–155
Sunflower				
Helianthus sp.	0.1–1	69–279	–	–
Fig				
Ficus carica	4 in latex	190	–	–
F. Benghalensis	17 in latex	1500		
F. Elastica	18 in latex	1–10		
Lettuce				
Lactuca serriola	1.6–2.2 in latex	1380	–	–

[a]Average molecular weight
dw dry weight, mw molecular weight, kD kilodalton, T tonne, Y year, kg kilogram, ha hectare. Adapted from Van Beilen and Poirier (2007)

than rubber obtained from *H. brasiliensis* and would require domestication, its short life span and amenability to tissue culture and transformation would facilitate agronomic improvement (Van Beilen and Poirier 2007).

5.3 Production of Bioactive Oils in Plants

Over the past few decades, a great number of studies have shown a link between the consumption of certain lipids, such as fat-soluble vitamins, phytosterols, carotenoids, and particular polyunsaturated fatty acids, and improved human health. Indeed, many of these compounds have been associated with the prevention, delay, or treatment of such serious diseases as cancer, osteoporosis, cardiovascular disease, and a number of immune disorders, to name a few (Chen et al. 2013). These oils occur naturally in certain foods and are termed bioactive, which refers to their potential ability to promote human health. Bioactive oils are often categorized as **nutraceuticals** since they have the potential to offer health benefits beyond normal

nutrition. While milk products, fish, and particular vegetables and seeds can often be a rich source of these healthy molecules, it has been widely reported that the general public is in fact not consuming enough of many of them. Therefore, it is now becoming the norm within the food industry to fortify foods, either for human consumption or animal feed, with bioactive lipids. In an attempt to produce some of these compounds at higher levels in a more sustainable manner, metabolic engineering of plants is being explored (Weselake et al. 2017). Although bioactive oils are not bioproducts in the strictest sense, it is useful to discuss the molecular strategies leading to their production in transgenic plants.

5.3.1 Very Long-Chain Polyunsaturated Fatty Acids

One of the most beneficial types of bioactive lipids are believed to be the **omega-3 very long-chain polyunsaturated fatty acids (VLC-PUFAs)**, which are 20 carbons or more in length with at least three methylene-interrupted double bonds in the *cis*-position, the first of which is located three carbons from the methyl end of the chain (Venegas-Calerón et al. 2010). In particular, eicosapentaenoic acid (EPA; $20{:}5\Delta^{8cis,11cis,14cis,17cis}$) and docosahexaenoic acid (DHA; $22{:}6\Delta^{4cis,7cis,10cis,13cis,16cis,19cis}$) (Fig. 5.2) have been shown to have a myriad of health benefits. For example, clinical trials have demonstrated protective roles for these fatty acids in the prevention of cardiovascular disease, and they are also linked to the prevention of obesity and type 2 diabetes, as well as having a role in neonatal development (Horrocks and Yeo 1999; Innis 2000; Das 2002). Furthermore, they are currently being studied for a vast number of additional health-promoting properties, such as their ability to protect against certain types of cancer (Rose and Connolly 1999), attention-deficit disorder (Richardson and Puri 2002), and dementia (Morris et al. 2003). Interestingly, stearidonic acid ($18{:}4\Delta^{6cis,9cis,12cis,15cis}$), which is an intermediate in the pathway leading to EPA and DHA, and flax oil containing this PUFA have also been shown to have anticancer properties (Subedi et al. 2015; Yu et al. 2015). While EPA and DHA are vital components of human metabolism, most animals (including humans) have a very limited ability to produce them from their α-linolenic acid (18:3) precursor, and therefore, their intake via food is a necessity (Riediger et al. 2009). The principal source of EPA and DHA for human nutrition is cold-water marine fish such as salmon, mackerel, and tuna, which are used for both direct consumption and the isolation of nutritional additives. Like the majority of other animals, these fish do not synthesize these fatty acids themselves but accumulate them from their dietary intake of marine microbes such as algae, which are able to generate VLC-PUFAs de novo (Williams and Burdge 2006).

Unfortunately, due to decades of overfishing (Pauly et al. 2005), as well as concerns relating to marine pollution and the resulting accumulation of dioxins, heavy metals, and polychlorinated biphenyls in fish (Yokoo et al. 2003), there is a dire need to develop alternative and sustainable sources of these VLC-PUFAs. Indeed, even the use of farmed fish for this purpose has proven problematic in terms of

sustainability, since farmed fish require greater input amounts of EPA- and DHA-containing feed than what can be harvested from the finished product. Several strategies are currently under study in an attempt to overcome these challenges. For example, approaches in which algal sources themselves are utilized as a production platform for the synthesis of these fatty acids are currently used for certain high-value applications such as infant formula. Such cultures, however, are expensive to maintain, necessitate specialized facilities, have a significant environmental footprint, and are limited in terms of scalability (Lee 2001). In contrast, the metabolic engineering of plants to produce relatively high levels of EPA and/or DHA is proving to be achievable, which holds out promise for a sustainable means of generating these bioactive fatty acids.

While certain lower plants (such as mosses) have the capacity to synthesize significant amounts of VLC-PUFAs, these fatty acids are virtually absent from higher plants, although they can provide a rich source of their precursor fatty acid, α-linolenic acid (Venegas-Calerón et al. 2010). In theory, the conversion of native plant fatty acids to VLC-PUFAs requires a minimum of three additional enzymes, including two ER-bound FADs and an elongase. To date, genes encoding these enzymes have been successfully isolated from a range of VLC-PUFA-synthesizing organisms, and several have been expressed in oilseed crops, providing proof of concept that the VLC-PUFA pathway could operate in a higher plant system (Abbadi et al. 2004; Qi et al. 2004); however, only very low levels of EPA and in some cases DHA were obtained. Additionally, the majority of these transgenic plants also contained high levels of undesirable omega-6 metabolic intermediates, which are completely lacking in marine oils. Very recently, a new study has been published in which researchers successfully generated transgenic *Camelina sativa* that produced levels of both EPA and DHA that were equivalent to those seen in fish oils through the introduction of multigene constructs expressing five to seven cassettes encoding various desaturases and elongases (Ruiz-Lopez et al. 2014). This study provides promise that generating a sustainable, terrestrial, source of EPA and DHA will indeed be feasible in the future.

5.3.2 Conjugated Linolenic Acids

Although PUFAs typically possess double bonds that are separated by at least one methylene group, the seeds of certain plant species produce oils that are rich in PUFAs bearing conjugated double bonds. Conjugated linolenic acids (CLNAs) bear three conjugated double bonds and can be found in the seed oils of plants from several families, including Rosaceae, Punicaceae, Chrysobalanaceae, Lythraceae, Cucurbitaceae, and Euphorbiaceae (Badami and Patil 1980). Many of these oils are currently under intense study for their nutraceutic applications, especially due to growing evidence of their cytotoxic and antiproliferative effects on tumor cells (Igarashi and Miyazawa 2000; Tsuzuki et al. 2004; Shinohara et al. 2012), as well as their alteration of lipid metabolism in animals (Koba et al. 2002).

Punicic acid (18:3$\Delta^{9cis,11trans,13cis}$) is one such fatty acid and is found predominantly in pomegranate (*Punica granatum*) seeds where it constitutes approximately 65% of the oil. Pomegranate oil/punicic acid has demonstrated anticancer activity against prostrate (Gasmi and Sanderson 2010) and breast cancer cells (Sturgeon and Ronnenberg 2010) and has also been linked to the prevention of obesity (Arao et al. 2004), type 2 diabetes (Vroegrijk et al. 2011), osteoporosis (Spilmont et al. 2013), and inflammatory disease (Bassaganya-Riera et al. 2011). Furthermore, it has also been established in human clinical trials that the consumption of pomegranate seed oil has a positive effect on cardiovascular health (Mirmiran et al. 2010). Tropical and subtropical pomegranate crops are very limited in their agronomic range, require warm climates, exhibit susceptibility to various insect pests and fungal pathogens, and currently require hand-harvesting. Therefore, the transfer of genes responsible for the biosynthesis of punicic acid into other crop plants would be advantageous.

Like other conjugated fatty acids, punicic acid is synthesized by a divergent FAD2 enzyme termed a conjugase (FADX). This enzyme acts to convert the Δ12 double bond of linoleic acid (18:2) into two conjugated double bonds (Δ11*trans* and Δ13*cis*). As has been the situation in many other attempts to produce non-native fatty acids in transgenic plants, only very low levels of punicic acid were obtained in initial studies whereby a *FADX* gene was expressed alone in transgenic plants (Koba et al. 2007), which is almost surely the result of a requirement for a network of additional enzymes exhibiting a preference for this fatty acid. Indeed, the production of up to 21% punicic acid in the seed oil of *A. thaliana* was demonstrated when the *P. granatum FADX* gene was expressed along with *P. granatum FAD2* in a background where the elongation of C18 fatty acids was suppressed (Mietkiewska et al. 2014). Once again, these results suggest that even higher amounts of punicic acid could be generated in desired crop species through the incorporation of further genes within the network.

5.4 Challenges Associated with the Metabolic Engineering of Lipid Composition in Plants

The specialty lipids described above each yield tremendous potential for an expansion of use as industries as a whole make a shift toward the production of value-added compounds. Unfortunately, in most cases the species that synthesize these molecules tend to suffer from various limitations in terms of further agronomic expansion, including a requirement for narrow growth conditions, labor-intensiveness, and lack of domestication. Alternatively, as is the case for PHAs and VLC-PUFAs, the native producer is not a land plant species. As a result, a substantial amount of research is now being conducted in an attempt to engineer the production of various molecules with commercial applications in plant species with superior agronomic traits. Several target oilseed species have been recognized for this

purpose, such as *C. sativa*, *C. abyssinica*, and *B. carinata*, which tend to have many of the same attributes as alternative biodiesel crops (Zhu et al. 2016). Ideally, these target species are nonfood crops that would not outcross with any food crop, are amenable to genetic transformation and tissue culture, and would allow large-scale production and incorporation into existing agricultural practices. Furthermore, an ability to grow on marginal land that is not suitable for other agricultural purposes would be an incredible advantage, as it would provide the added benefit of reducing competition with current food crops.

In the case of industrial oils, the hope is that plant oils, waxes, and lipid-derived polymers could 1 day be produced on a scale that would significantly reduce the use of petrochemicals for these purposes. As discussed above, many genes involved in the biosynthesis of industrially useful fatty acids and lipids have been cloned, and in certain instances (e.g., the production of unusual fatty acids and wax esters) their synthesis is under relatively simple genetic control. Therefore, it would seem that expressing these genes in transgenic plants would be fairly straightforward, and in certain cases, as discussed, results have been promising. However, as a whole, the results of these experiments have been fairly unpredictable, and the amounts of value-added product generated have virtually always fallen short of levels present in the species from which the transgenes were derived (Dyer et al. 2008). There are likely many reasons for these disappointing results but most almost certainly stem from the complexity of lipid biosynthetic pathways. Since it is necessary to have a relatively high content of the desired lipid molecule within the plant in order to maximize yield for most applications, the development of these transgenic plants will not only involve achieving the synthesis of the target molecule but will also necessitate genetic modification to enable very high levels of accumulation (see Sect. 4.7).

5.4.1 Preference of Kennedy Pathway Acyltransferases for Particular Substrates

In many cases where metabolic engineering of TAG composition has not yielded impressive gains in the fatty acid of choice, it has been suggested that the transgenic seeds lacked the enzymes required to efficiently acylate the novel fatty acids onto the glycerol backbone of TAG, resulting in an accumulation of these fatty acids in the acyl-CoA pool and their subsequent degradation via **beta-oxidation**. Conversely, in the native plant species that naturally produce these fatty acids, their Kennedy pathway acyltransferases are often specialized to preferentially utilize these fatty acids as substrates (Davies et al. 1995; Kroon et al. 2006; Yu et al. 2006). In particular, LPAAT and DGAT2 enzymes, which catalyze the acylation of the *sn*-2 and *sn*-3 positions of TAG (see Figs. 5.3 and 5.4), respectively, from species that produce unusual fatty acids, often appear to exhibit clear substrate preferences. As such, the co-introduction of these enzymes along with the target catalytic enzyme will be of the utmost importance in the production of unusual fatty acids at high levels (Burgal et al. 2008; Nath et al. 2009; Li et al. 2010; Kim et al. 2011; Van Erp et al. 2011).

5.4.2 *The Importance of Acyl Transfer from Phosphatidylcholine to Triacylglycerol in Fatty Acid Composition*

Until fairly recently, it was believed that the biosynthesis of fatty acids and their incorporation into TAG was an essentially linear process that included the generation of saturated or monounsaturated acyl chains of various lengths in the plastid via the fatty acid synthesis pathway, followed by their export to the cytosol, modification of the acyl groups (mainly while bound to phosphatidylcholine [PC]), return of acyl groups to the acyl-CoA pool, and subsequent assembly onto the *sn*-1, *sn*-2, and *sn*-3 positions of a glycerol backbone through the activity of ER-bound acyl-CoA-dependent Kennedy pathway enzymes to form TAG (Vanhercke et al. 2013). This process is now understood to be far more complex than originally thought, with complicated networks mediating the movement of acyl groups between pools of acyl-PC, acyl-CoA, and TAG precursors. The pathways outlined in Fig. 5.4 give us a glimpse of this complexity. Indeed, several enzymes have been identified that are involved in acyl trafficking between PC, which is the site of many fatty acid modification reactions, and DAG, TAG, or the acyl-CoA pool (Chen et al. 2015).

The role of PDAT in channelling hydroxy fatty acids into TAG, along with PDCT, was discussed earlier in this chapter (Fig. 5.4). Yet another acyl-CoA-independent route for DAG synthesis involves the enzyme phospholipase D (PLD) which catalyzes the removal of the choline group from PC to form PA (Yang et al. 2017) (Fig. 5.4). PA is a substrate for the Kennedy pathway enzyme phosphatidic acid phosphatase (PAP), which catalyzes the removal of phosphate (P_i) from PA to generate DAG.

In terms of acyl movement between PC and acyl-CoA, lysophosphatidylcholine acyltransferase (LPCAT) appears to catalyze acyl exchange between the *sn*-2 position of PC and the acyl-CoA pool (Yurchenko et al. 2009). There is also evidence that the DGAT-catalyzed reaction may encourage the removal of modified fatty acids from the *sn*-2 position of PC through **biochemical coupling** of the reverse reaction catalyzed by LPCAT (i.e., formation of acyl-CoA and lysophosphatidylcholine [LPC]) and the forward reaction catalyzed by DGAT (i.e., formation of TAG and CoA) (Pan et al. 2015).

The extent of acyl movement through PC probably differs markedly between plant species and could play a key role in the ability of a plant to incorporate unusual fatty acids into TAG. Perhaps even more importantly, particular routes through this complicated network have likely become specialized for unusual acyl chains (such as hydroxyl fatty acyl chains) in different plants, possibly in an effort to channel potentially damaging acyl groups away from membranes and into storage lipids. While the precise mechanisms of these adaptations remain to be fully elucidated, they almost certainly involve the concerted action and specialization of enzymes involved in PC editing and PC to DAG conversion (Napier and Graham 2010).

The lack of efficient removal of unusual fatty acids from the site of their synthesis on PC has also been identified as a bottleneck in many transgenic plants

(Bates and Browse 2011). As a result, it is not at all surprising that transgenic oil species that do not normally synthesize these unusual fatty acids are often suboptimal in attempts at their biosynthesis. As a result, it is becoming clear that in order to produce these target fatty acids at useful levels in transgenic plants, several enzymes in addition to the Kennedy pathway acyltransferases will require modification, including PDAT, PDCT, PLA_2, PLD, and LPCAT (Van Erp et al. 2011; Hu et al. 2012; Bayon et al. 2015; Chen et al. 2015; Yang et al. 2017).

5.5 Closing Comments

Plant oils have the potential to be used for a large number of industrial and nutraceutic applications and could provide a sustainable replacement for current sources of these molecules. However, it will be necessary to produce these lipids on a much larger scale than they are presently in their native species, and metabolic engineering will likely play a strong role in this expansion. The engineering of oils and waxes, as well as other plant lipids, is currently set to flourish due to a number of major advances in this field. These advances have taken into account the complex nature of lipid biosynthesis in plants and have resulted in the combination of various engineering strategies to enable maximization of both the efficiency and specificity of the introduced pathway. Indeed, the generation of plants producing the VLC-PUFAs EPA and DHA (Ruiz-Lopez et al. 2014) may be the most complex genetic engineering goal achieved to date in plants.

Further advances in this field are also likely on the horizon, as additional genes necessary for the high-level production of these compounds are uncovered and put to use. Furthermore, the majority of transgenic approaches utilized to date have been carried out through the addition of a pathway, whereby it must compete with existing pathways in the target plant. This means that the introduced pathways are not likely operating at their full potential; as such, inactivating the function of naturally occurring networks and including novel pathways as substitutions rather than additions could provide an enhancement in the accumulation of useful lipids (Chapman and Ohlrogge 2012). Future studies will almost certainly take this into consideration, and it is anticipated that 1 day soon, the engineering of plants to produce non-native lipids at levels that are equivalent to or better than those present in their native host species will be a reality.

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Chapter 6
Plant Carbohydrates and Production of Renewable Biofuel from Starch, Sugar, and Cellulose

Lucas J. Falarz, Michael K. Deyholos, and Guanqun Chen

Chapter Highlights

- Carbohydrates are a group of molecules with various structures that are composed of carbon, oxygen, and hydrogen.
- Sugars and polysaccharides (e.g., starch, cellulose, hemicellulose, and pectin) are major plant carbohydrates that are important feedstocks for bioproducts.
- Sugarcane and corn are the most widely cultivated crops and are important sources of sugar and starch, respectively, whereas fiber-rich switchgrass is an emerging energy crop.
- Corn starch and sugarcane sucrose are the most important feedstocks for ethanol production.
- Lignocellulosic biomass has a high potential to be used as feedstock for ethanol production, but technologies need further development for commercial-scale production.

L. J. Falarz
Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

M. K. Deyholos
Department of Biology, University of British Columbia, Kelowna, BC, Canada

G. Chen (✉)
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
e-mail: guanqun.chen@ualberta.ca

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6.1 Introduction

Biologists define carbohydrates as molecules with three or more carbon atoms that are composed of only carbon (C), oxygen (O), and hydrogen (H), typically with a general formula of $(CH_2O)_n$. Carbohydrates are abundant in most plant organs and tissues since they are the primary products of photosynthesis and form polymers that are important in carbon and energy storage, as well as in mechanical support. The starch that accumulates in potato tubers, for example, is used as an energy supply in the initial development of the next generation of potato plants (Turesson 2014). Carbohydrates also have other roles in plants, for example, making up components of nucleic acids and glycoproteins (Nelson and Cox 2017).

Due to their properties, carbohydrates are also important feedstocks for the production of food, food additives, pharmaceuticals, biofuels, paper, construction materials, textiles, and various chemicals. Plant-derived carbohydrates have been gaining attention in recent years as a source of industrial products due, at least in part, to the depletion of fossil fuel-based resources and threat of global warming. Plant carbohydrates have the potential to provide renewable substitutes for fossil-based raw materials, and as an added bonus, their biosynthesis consumes large amounts of carbon dioxide. This means that they form part of a closed carbon cycle, whereby carbon dioxide released by burning the fuels can be removed from the atmosphere through the regrowth of the plant source of feedstock. While carbohydrates (usually in the form of wood) have been burned as fuel for tens of thousands of years and combustion remains a simple method to extract energy from carbohydrates, recent surges in fuel and energy costs, as well as environmental concerns, have spurred interest in the conversion of carbohydrate feedstocks to liquid fuels (especially ethanol). Indeed, recent legislation (e.g., Brazil) requires the addition of fuel ethanol to gasoline, which highlights the importance that carbohydrates will have in the near future supply of fuels and in the reduction of greenhouse gas (GHG) emissions (Table 6.5). This chapter builds upon the information on carbohydrates presented in Chap. 2 and discusses the relevance of carbohydrates to bioproducts and production of fuel ethanol. The discussion on carbohydrates that follows is based on information from Nelson and Cox (2017), Voet et al. (2016), and Heldt and Piechulla (2010).

6.2 Structure and Classification of Carbohydrates

Carbohydrates are classified according to their chemical structures (Table 6.1). Many carbohydrates are polymers, or polysaccharides, made up of monomers called monosaccharides (Nelson and Cox 2017). A disaccharide is a dimer of two monosaccharides, and an **oligosaccharide** is a polymer of several (e.g., roughly 3–20) monosaccharides. Monosaccharides and disaccharides are also commonly known as sugars. With reference to the general formula $(CH_2O)_n$, a monosaccharide may be classified based on the number of carbons it contains; **pentoses** ($n = 5$) and **hexoses** ($n = 6$) are most relevant to our discussion.

Table 6.1 Classification of carbohydrates according to their number of monomers, carbons, and identity of functional groups

According to number of monomers		
Classification	*Number of monomers*	*Example*
Monosaccharide	1	Glucose, fructose
Disaccharide	2	Maltose, lactose
Oligosaccharide	3–20	Raffinose, fructo-oligosaccharides (FOS)
Polysaccharides	>20	Starch, cellulose
According to functional group		
Classification	*Functional group*	*Example*
Aldose	Aldehyde	Glucose, mannose
Ketose	Ketone	Fructose, ribulose
According to number of carbon		
Classification	*Number of carbons*	*Example*
Pentose	5	Arabinose, xylose
Hexose	6	Glucose, fructose

6.2.1 Monosaccharides

All monosaccharides contain a chain of C atoms, to which hydroxyl (-OH) groups, or simply H or O atoms, are attached. The C chain can take either a linear or cyclic form. In the linear form, one of the C atoms is attached by a double bond to an O atom, forming a carbonyl group (C=O). If the carbonyl is at the end of the carbon chain, the monosaccharide is considered an **aldose**; otherwise it is a **ketose** (Fig. 6.1; Nelson and Cox 2017). In both aldoses and ketoses, the carbonyl group and a hydroxyl group may form an intramolecular covalent bond, which changes at least part of the open chain into a ring. It is the cyclic forms of monosaccharides that polymerize to form di-, oligo-, and polysaccharides. If the ring contains five members (4 C, 1 O), it is called a **furanose**. If the ring contains six members (5 C, 1 O), it is called a **pyranose** (Fig. 6.1). In both furanoses and pyranoses, the term anomeric carbon is used to refer to the C atom that formed the carbonyl group when the molecule was in the linear form. The C atoms of a cyclic molecule are often numbered for easy reference, and the anomeric carbon is always designated as C1 (see Fig. 2.7 in Chap. 2). The anomeric carbon is also central to defining α- and β-forms of sugars as described below.

Glucose (Glc, $C_6H_{12}O_6$) is an example of a hexose and is itself also an aldose. Glc is produced during photosynthesis by the reduction and fixation of CO_2 (Nelson and Cox 2017). Glc, however, is not the only hexose present in plants. For example, galactose, mannose, and fructose also each have the formula $C_6H_{12}O_6$ but differ from each other structurally. Like Glc, galactose and mannose are both aldoses, whereas fructose is a ketose. Pentoses that are most relevant to bioproducts are arabinose, xylose, and apiose, which are all aldoses.

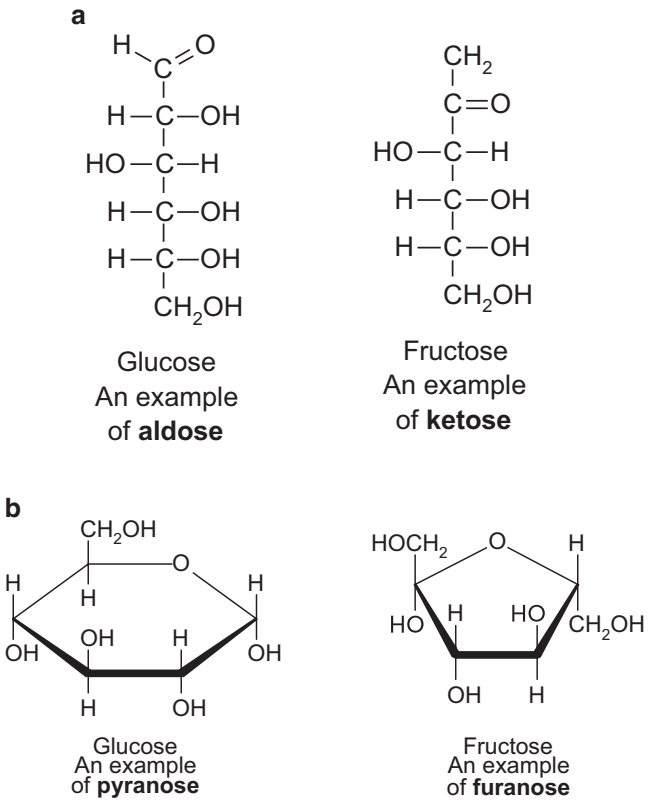


Fig. 6.1 Classification of monosaccharides. (**a**) Example of an aldose and a ketose. (**b**) Example of a pyranose and a furanose

Deoxysugars and acidic sugars are two derivatives of monosaccharides (Nelson and Cox 2017; Voet et al. 2016). The major **deoxysugars** relevant to bioproducts are rhamnose and fucose, which are both deoxyhexoses with the formula $C_6H_{12}O_5$, and have an H atom in place of the terminal hydroxyl group found in other hexoses. The major **acidic sugars** are galacturonic acid and glucuronic acid, which are also both derived from hexoses and have a carboxyl (-COOH) group in place of the $-CH_2OH$ in the sixth carbon position. The Haworth projection of the monosaccharides is shown in Fig. 6.2.

Although the common monosaccharides are all very similar in their elemental compositions, differences in their structures have profound effects on their biological functions. For example, Glc, galactose, and mannose are stereoisomers of each other, because they differ only in the arrangement of some hydroxyl groups relative to other hydroxyl groups in the same molecule (Nelson and Cox 2017). It is also important to note that for each monosaccharide, there are two possible configurations that are mirror images of each other (enantiomers), known as the **D- and L-forms** (Fig. 6.3). Usually, only one of the enantiomers is found within

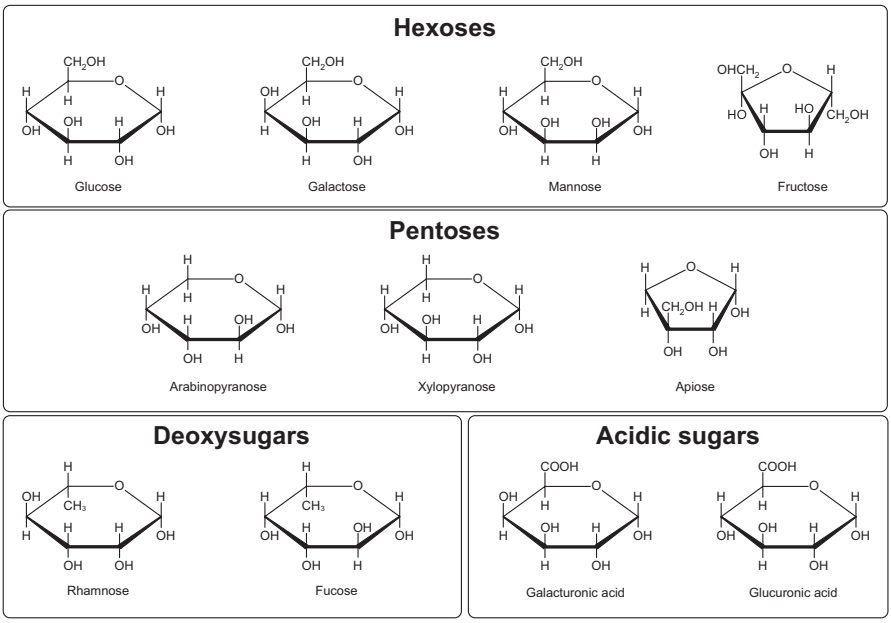
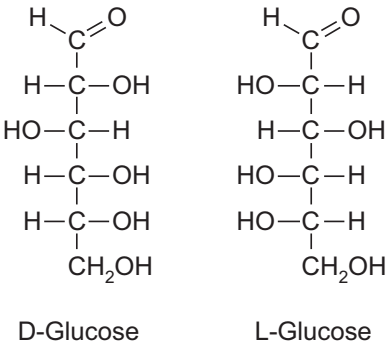


Fig. 6.2 Haworth projection of relevant monosaccharides

Fig. 6.3 Example of linear D- and L-glucose



a cell. For the main hexoses (Glc, galactose, mannose), pentoses (xylose, apiose), and sugar acids (galacturonic acid, glucuronic acid), it is the D-*form* that is biologically relevant. For arabinose, rhamnose, and fucose, it is the L-*form* that is most relevant. Finally, in cyclic molecules, the anomeric carbon is the C atom that was part of the carbonyl group when the molecule was in the linear form. The anomeric carbon is always bound to a hydroxyl group, and the position of the hydroxyl relative to other groups on the ring defines a cyclic monosaccharide as being in either α- or β-anomeric configuration. Most monosaccharides convert freely between the open-chain, α- and β-configurations when in solution, although one of these forms may be more favored than another.

6.2.2 *Oligo- and Polysaccharides*

Monosaccharides can form covalent bonds with other molecules (including other monosaccharides). The most important type of bond is a **glycosidic bond** or glycosidic linkage, which is formed between the anomeric carbon of a monosaccharide and the oxygen of a hydroxyl group of another molecule (Fig. 6.4; Nelson and Cox 2017). When a glycosidic bond forms between two monosaccharides, a disaccharide is produced (see Fig. 2.8 in Chap. 2), and additional glycosidic linkages produce an oligosaccharide and ultimately a polysaccharide. Any glycosidic bond between sugars can be described by identifying the carbons that are linked and by the relative orientation of the monosaccharides. If the monomers are in the α-form, the linkage is classified as α. If the monomers are in the β-form, the linkage is β. For example, starch and cellulose are both polymers of Glc but differ in their linkages. Starch is a mixture of **amylose** and **amylopectin**. Amylose are chains of glucosyl moieties linked α-1,4, whereas amylopectin contains both α-1,4 glycosidic bonds and α-1,6 glycosidic bonds (see Fig. 2.9 in Chap. 2). In contrast, cellulose contains only β-1,4 glycosidic bonds (see Fig. 2.10 in Chap. 2). Starch is readily digestible

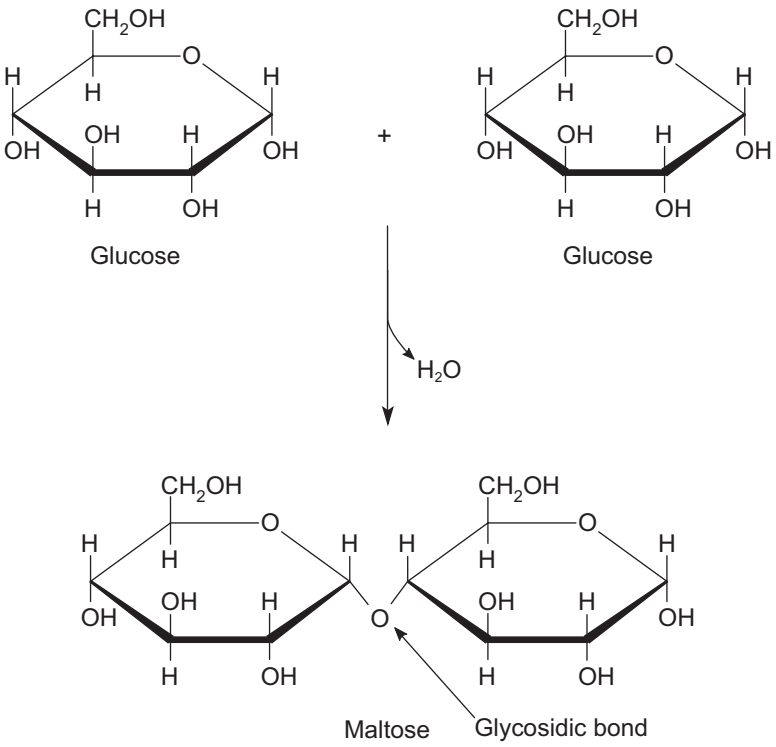


Fig. 6.4 Condensation of two molecules of glucose to form a maltose. This process releases a molecule of water

by most animals, whereas cellulose is insoluble and indigestible (Voet et al. 2016). Thus, the types of glycosidic bonds present in a polysaccharide are of great significance to its function. As shown in amylopectin, a single monosaccharide molecule can form glycosidic bonds with more than one other molecule, which gives polysaccharides the potential for forming highly branched structures.

Many polysaccharides are named based on their constituent sugars, using the suffix -an. For example, mannan is a polymer of mannose, and xylogalacturonan is a polymer of xylose and galacturonic acid. However, to fully describe a polysaccharide, it is necessary to know the identity of all of the constituent monosaccharides; their individual configurations (D- or L-, α- or β-, furanose or pyranose); their sequence in the polymer, including any branches; and the configuration (α- or β-) of the glycosidic bonds. Fortunately, many of the major plant polysaccharides are polymers of fairly simple repeating units, so their primary structures can be inferred without describing every one of their constituents.

6.3 Plant Origins of Polysaccharides

Plant polysaccharides can be categorized as structural or nonstructural polymers. These categories are not mutually exclusive, as some polysaccharides (such as those found in the cell walls of some legumes) provide both energy reserves and mechanical support.

The major structural polysaccharides are located in the cell wall and in secretions such as the mucilage released by the seed coat. All plant cells are surrounded by a primary cell wall, and some cells undergo additional thickening to produce a secondary cell wall. Secondary walls are found most prominently in xylem, where strength is required to support the plant body and to resist the tension created during water transport (transpiration; Plomion et al. 2001). Wood is made of xylem. In softwoods (i.e., conifers), xylem consists mostly of cells called tracheids, whereas hardwood (i.e., woody angiosperms) xylem is composed primarily of cells called vessel elements and non-transporting cells called fibers. Thick-walled cells in other parts of the plant are also commonly called fibers, including the phloem (bast) fibers of crops such as flax and hemp [*Cannabis sativa*] and the hairs (trichomes) on the surface of cotton seeds.

6.3.1 Cell Walls

Cell walls are made mostly of carbohydrates and their derivatives, plus small amounts of other molecules, especially proteins (Heldt and Piechulla 2010). Secondary walls are often also abundant in lignin. The carbohydrate component of the primary cell wall consists of cellulose embedded in a matrix of other polysaccharides, which are classified as either hemicelluloses or pectins (Fig. 6.5).

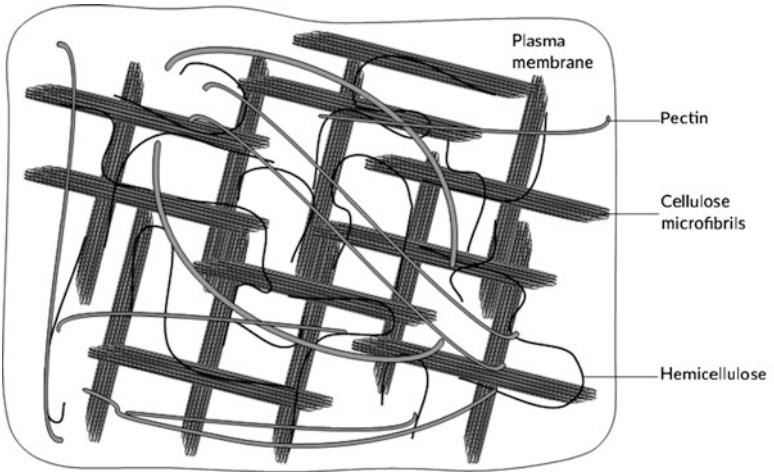


Fig. 6.5 Structure of a plant cell wall

As previously shown, cellulose is an unbranched β-1,4 glucan (see Fig. 2.10). It is the most abundant terrestrial organic polysaccharide (Wüstenberg 2015). Within the cell wall, cellulose is found in microfibrils, which are bundles of approximately 36 cellulose molecules (Voet et al. 2016; Rayon et al. 2014). The parallel alignment of the cellulose chains allows for the formation of many intra- and intermolecular hydrogen bonds within the microfibril. Regions of the microfibrils with extensive hydrogen bonding are considered to be crystalline and are particularly strong and resistant to chemical or enzymatic degradation. Microfibrils may also contain amorphous regions in which the cellulose molecules are not crystalized. The degree of crystallinity, therefore, affects the use of cellulose in bioenergy and bioproduct applications. Microfibrils are themselves bundled together to make macrofibrils, which form a network throughout the cell wall.

Hemicelluloses usually make up less than half of the dry mass of cell walls. Unlike cellulose, they do not form microfibrils or extensive crystalline regions. In the primary walls of cereals and other grasses, the major hemicellulose is arabinoxylan, which comprises a β-1,4 xylan backbone to which side chains consisting mostly of arabinose are attached (Grandis et al. 2014). In most other seed plants (i.e., eudicots, gymnosperms), the major hemicellulose is xyloglucan. Xyloglucan has a β-1,4 glucan backbone, to which are attached short side chains that begin with xylose and may additionally contain arabinose or terminal galactose or fucose. Other hemicelluloses include mannans, glucomannans, and galactoglucomannans.

Pectins are defined by a polymer backbone that is rich in galacturonic acid (Heldt and Piechulla 2010). Pectins comprise part of the cell wall and are also abundant in the middle lamella, which connects adjacent plant cells. Under specific circumstances, carboxyl residues of galacturonic acid form intramolecular ionic linkages, mediated by Ca^{2+}. Within the plant, these linkages stiffen cell walls. Methylation of the carboxyl group prevents the formation of ionic linkages, and therefore the degree

of methylation of a pectin molecule greatly affects its function. The simplest pectin is homogalacturonan, which is a α-1,4 polymer of galacturonic acid. Pectins of the rhamnogalacturonan II group have a backbone similar to homogalacturonan, to which are attached complex side chains consisting of combinations of up to ten sugars. Rhamnogalacturonan I is also a type of pectin, but its backbone is made of repeats of a rhamnose, galacturonic acid disaccharide. Many different polysaccharide chains may be attached to the rhamnogalacturonan backbone.

6.3.2 Starches

Starches are the most important nonstructural polysaccharides and are a major source of stored carbon and energy. Starch is found in many cell types throughout the plant but is most abundant in vegetative storage tissues (e.g., tubers of potatoes) and in the seeds of some species (e.g., endosperm of cereals; Heldt and Piechulla 2010). Starch is synthesized mainly within the chloroplast, or in specialized organelles called **amyloplasts**, although some synthesis may also occur in the cytosol. Starch is deposited in granules, the diameter of which affects their digestibility and physical properties.

As indicated previously, starch is made up of two types of polymers (see Fig. 2.9 in Chap. 2), namely, amylose (α-1,4 glucan) and amylopectin (α-1,4 glucan with α-1,6 branch points; Heldt and Piechulla 2010). Despite its name, amylopectin is chemically unrelated to pectin. The highly branched structure of amylopectin gives it very different physical properties than amylose. The linear nature of amylose allows for adjacent molecules to tightly associate and form hydrogen bonds; thus amylose tends to have higher crystallinity and lower solubility and digestibility than amylopectin. Natural starches contain more amylopectin than amylose, and some crop varieties have been selected for specific starch characteristics. For example, starch in sticky rice (also called glutinous rice) is made almost entirely of amylopectin and contains no amylose, whereas short grain rice is approximately 85% amylopectin/15% amylose and long grain rice is 75% amylopectin/25% amylose (Lian et al. 2014; Cameron and Wang 2005; Ayres et al. 1997). Waxy-type starches (~ 100% amylopectin) are produced by varieties of several species and are preferred in some bioproduct applications.

6.4 Plant Carbohydrates as Industrial Feedstocks

Any component of the plant that is rich in polysaccharides is a potential industrial feedstock. Many factors affect the utility of a feedstock (US Department of Energy 2012), including **uniformity** (feedstocks that do not vary in their composition are preferred), **availability** (ideally a reliable supply should be available year-round), **proximity** (distance between the harvest location and the end user), and

extractability (the ability to separate desirable and undesirable components of the feedstock). Effort may also be expended for **densification** (i.e., compaction) of the feedstock prior to transportation. The price of a feedstock therefore depends not only on the cost of growing and harvesting the crop but also any necessary densification and transportation and many other market factors. Not all feedstocks are suitable for all applications, and most established industries are reluctant to adopt new feedstocks (e.g., a different crop or plant component) for existing processes.

Tuber or seed-derived starch is usually used as a food source because starch is easily digested by humans and animals. Although starch and cellulose are both polymers of Glc, cellulose is not digestible by animals (except ruminants and others with specialized microbial symbionts). Cellulose and other cell wall polysaccharides are abundant in wood and straw and other crop or forest residues. These are normally less expensive than starch and are also commonly referred to as biomass.

Some major crops are cultivated to provide components that are rich in carbohydrates. Sugarcane [*Saccharum* spp.] is rich in sucrose, corn accumulates starch, and switchgrass [*Panicum virgatum*] is a source of cellulose. Corn and sugarcane are the most widely cultivated crops, while switchgrass is being developed as a potential energy crop. Due to their relevance as sources of carbohydrates, these plants will be discussed as representatives in the sections below. Although all three are agronomically important crops, both sugarcane and switchgrass in particular are very much limited by the fact that their growth is restricted to warm climates.

6.4.1 Corn/Maize

Corn or maize [*Zea mays*] is a crop of great relevance because it is an important source of carbohydrates in the western diet and it serves a feedstock for many industrial applications. Corn was the second most widely cultivated crop in 2014, and its production was estimated to reach more than 1 billion tonnes (FAO 2017). The production of corn is highly concentrated in a few countries, such as the United States (USA) and China, which make up approximately 55% of total production. Indeed, ten countries (Table 6.2) make up more than 80% of the global production of this crop. Corn produced in the United States is very important because it can be used as a feedstock in the production of biofuels and animal feed. In the 2015/2016 production cycle, 38% of the corn harvested in the United States was consumed in the production of fuel ethanol, and 37% was used in livestock feed (USDA 2017). Exports (14%), food, and other industrial uses (10%) also represent important markets for the corn produced in the United States (Table 6.3).

Corn kernels contain around 70% of their weight (on a dry weight basis) as starch, which is composed mainly of amylopectin (73%; Corn Refiners Association 2006). The composition of this starch can be altered using breeding and genetic techniques, allowing the amount of amylose to be reduced or increased according to the application. The remainder of the corn kernel is composed of fibers, proteins, and oils, which also have commercial value.

Table 6.2 Main producers of corn in the world – 2014 (FAO 2017)

Country	Corn production (million tonnes)
United States of America	361.1
China	215.8
Brazil	79.9
Argentina	33.1
Ukraine	28.5
India	23.7
Mexico	23.3
Indonesia	19.0
France	18.3
South Africa	14.3

Table 6.3 Consumption of corn in the United States by segment in the 2015/2106 production cycle (September to August; USDA 2017)

Segment	Consumption (%)
Fuel ethanol	38.2
Feed and residual use	37.4
Exports	13.9
High-fructose corn syrup (HFCS)	3.5
Glucose and dextrose	2.5
Starch	1.8
Cereals and other products	1.5
Alcohol for beverages and manufacturing	1.0
Seed use	0.2

Technological advances over the last century, such as the adoption of fertilizers and herbicides, mechanization, and the use of hybrids, have resulted in increased production yield per area and reduced cultivation costs (Crow 1998; Pruitt 2016). Genetically engineered plants have also contributed to increased yields and profit more recently. For example, varieties that are simultaneously resistant to herbicides and insects (via the introduction of Bt protein, which is toxic to insects but not humans) are currently on the market, which has diminished the use of insecticides and has improved management practices (Vercesi et al. 2006; Pruitt 2016).

Despite these advances, there are certain areas that could still use improvement, such as resistance to drought, which often affects production and therefore impacts corn prices and the cost of associated bioproducts (Adonizio et al. 2012). In addition, although high levels of starch are stored in kernels, for certain industrial processes, the polysaccharide must first be broken down into its monomers, which means additional steps and costs. The enzymatic hydrolysis of starch, for example, releases monomers of Glc that can be used as a substrate for microbial fermentation (Lin and Tanaka 2006; Vohra et al. 2014). However, genetics and breeding techniques for corn plants are well-established, and their application may provide new varieties that overcome this limitation in the future.

6.4.2 *Sugarcane*

Sugarcane is mainly cultivated for the production of sucrose, which is an important feedstock in the food and manufacturing sectors. The production of sugarcane in 2014 was 1.9 billion tonnes, making it the most abundantly produced crop in the world (FAO 2017). Similar to corn, most of its production is concentrated in a handful of countries such as Brazil and India, which make up almost 60% of its production, with 83% of its global production occurring in only ten countries (Table 6.4).

Ethanol and sugar are the most relevant products of sugarcane, with 1 hectare of sugarcane yielding 140 kg of sugar (Vohra et al. 2014). The dry matter within the sugarcane juice, which is the syrup extracted from pressed sugarcane, comprises 77–90% of total sugars (Carneiro et al. 2015). **Bagasse** is the biomass residue, which is usually burned to generate power in processing plants. Bagasse, however, contains cellulosic fibers (8–14%) that also have the potential to be used in the production of cellulosic ethanol (Bizzo et al. 2014).

The advantages of using sugarcane as a feedstock for industrial processes are its high content of sucrose, the high yields of the crop, and low processing costs (Vohra et al. 2014). Sugarcane, however, rapidly deteriorates after harvest. Consequently, it must be processed no longer than 72 h postharvest to prevent a loss of sugars in the stalks (Solomon 2009; Vohra et al. 2014). Thus, the proximity of sugarcane fields to processing plants is an important consideration.

While traditional breeding methods and the use of genetic markers have allowed the development of diverse varieties of sugarcane (Jackson 2005; Mohan 2016), the complexity of its genome in terms of the number of repeats, polyploidy, and degree of heterozygosity has limited the use of advanced molecular techniques (Mohan 2016).

Table 6.4 Main producers of sugarcane in the world – 2014 (FAO 2017)

Country	Sugarcane production (million tonnes)
Brazil	736.1
India	352.1
China	126.2
Thailand	103.7
Pakistan	62.8
Mexico	56.7
Colombia	36.5
Australia	30.5
Indonesia	28.6
United States of America	27.6

6.4.3 Switchgrass

Switchgrass is a perennial plant that is currently used in the production of forage hay or the control of erosion (Parrish et al. 2012). In addition, there is a growing interest in the development of switchgrass for the production of bioenergy because the cultivation of switchgrass has particular advantages compared to other crops. For example, certain upland ecotypes are adapted to drought conditions and grow well in marginal areas (Perlack et al. 2011; Parrish et al. 2012; Sands et al. 2017), which means that competition with other crops that are used for food production can be avoided. Furthermore, its production requires low inputs (such as fertilizers or pesticides) and does not require specialized machinery (Parrish et al. 2012).

Unlike corn and sugarcane, switchgrass is composed mainly of fibers (77–84%) and does not accumulate sugars or starch (Brown et al. 2016). Instead, cellulose and hemicellulose composed mainly of Glc and xylose are the main carbohydrates in this plant, representing an average 35% and 29% of the dry weight, respectively (David and Ragauskas 2010). Due to this property, switchgrass is mainly cultivated for the production of biomass, and most interest revolves around its potential to be developed as a bioenergy crop. Indeed, switchgrass has the potential to either be burned to generate electricity or used in the production of cellulosic ethanol. These technologies, however, are not viable on a commercial scale as of yet, and further research will be necessary to render production economically viable. In addition to its potential use for bioenergy production, the use of switchgrass as a raw material for pulp and the manufacturing of other bioproducts has also been proposed (Parrish et al. 2012).

6.5 Plant Carbohydrates as Feedstocks for Ethanol Production

6.5.1 Applications of Ethanol

One of the oldest uses of ethanol is in the production of fermented alcoholic beverages such as wine and beer. It is also used in many industrial sectors, such as the food and beverage industries, whereby it is used in the extraction of flavors and aromas that will be used in food products (ePURE 2017a). In the pharmaceutical industry, ethanol is used in cosmetics and medicines, while in the chemical industry, it is used as a solvent in the production of different chemicals and paints. Ethanol is also an important intermediate in the synthesis of valuable chemicals, such as ethylene, which is the precursor of a myriad of compounds and can be obtained from the dehydration of ethanol (Harmsen et al. 2014). Despite having many applications, the majority of ethanol produced is used as a fuel. While many vehicles use a blend of gasoline and ethanol, there are also those that are able to run using only ethanol, while others accept ethanol and gasoline in any proportions (flex-fuel vehicles), which are especially popular in Brazil.

Table 6.5 Blend of gasoline and ethanol in selected regions

Region	Amount of ethanol in gasoline (% v/v)
United States of America[a]	10
Canada[b]	5
European Union[c]	5
Brazil – regular gasoline[d]	27
Brazil – fuel ethanol only[e]	100[f]

[a]US Department of Energy (2017b), [b]ECCC (2017), [c]ePURE (2017b), [d]Petrobras (2017), [e]ANP (2015)
[f]This fuel is not pure ethanol, but a mixture with up to 7% of water (ANP 2015)

The recent increase in the production of ethanol is in part due to regulations to diminish pollution and emissions of GHGs. For example, methyl tertiary butyl ether has been used in gasoline as an antiknocking agent (to avoid engine knocking), octane enhancer (to substitute lead), and to oxygenate the fuel to improve its combustion (Keller et al. 2000; Shapouri and Salassi 2006). However, due to groundwater contamination, it has been banned in the United States. When added to gasoline, ethanol provides the same functions as methyl tertiary butyl ether and is even considered superior due to the fact that it contains higher amounts of oxygen, which may lead to more complete combustion (Bothast and Schlicher 2005).

Fuel ethanol is typically sold as a mixture with gasoline. These blends are termed E5, E10, E15, and E85, with the number indicating the percentage of ethanol in the mix (US Department of Energy 2017a). Some countries, such as Brazil, have regulations concerning the amount of ethanol in gasoline blends (Table 6.5), where all gasoline sold must contain a certain percentage of ethanol (Petrobras 2017). Brazil also provides hydrated ethanol (a mixture of 95% ethanol and 5% water) as a fuel (ANP 2016); since most cars in Brazil are flex-fuel vehicles and ethanol prices are competitive with that of gasoline, there is a high demand for ethanol-based fuels.

6.5.2 Industrial Production of Ethanol

Ethanol (or alcohol) is a flammable liquid that has the chemical formula C_2H_5OH. It is naturally produced by some yeasts under anaerobic conditions, a process that has been exploited by humans since ancient times for the production of beer and wine. Currently, ethanol has many uses and is produced on a very large scale, with global production reaching approximately 100 million m^3 in 2016 and 85% of this production (Fig. 6.6) being contributed by the United States (57%) and Brazil (28%; RFA 2017).

Currently, most ethanol is produced via the **fermentation** of agricultural feedstocks, followed by distillation. A smaller fraction, however, is synthetically produced from petroleum derivatives and is used mainly for industrial purposes (IEA – Industrial Ethanol Association 2007; Schobert 2013). This synthetic route

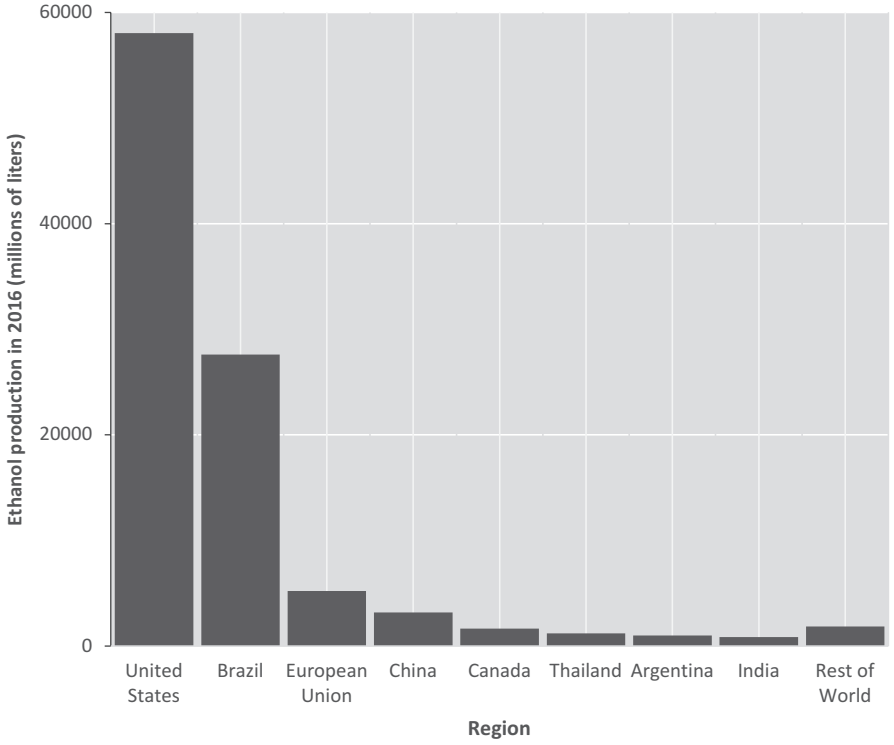


Fig. 6.6 Production of ethanol by country in 2016. Based on RFA (2017)

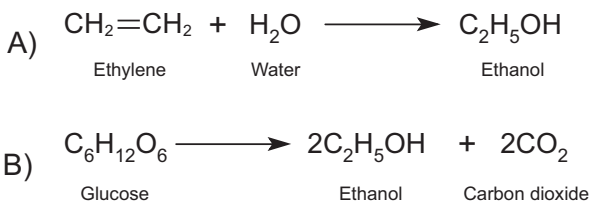


Fig. 6.7 Ethanol production via chemical synthesis (**a**) and biological fermentation (**b**)

involves the hydration of ethylene in the presence of an acid catalyst (Fig. 6.7a) at high temperatures. This process produces acetaldehyde as a by-product, but the process is not economically viable, and petroleum-derived ethanol is not considered a renewable fuel (Schobert 2013; Mohsenzadeh et al. 2017). When produced from agricultural sources, on the other hand, the resulting ethanol is considered a renewable fuel (also known as bioethanol). In this case, the fermentation is performed by certain microorganisms under far milder conditions.

Fermentation is an intracellular process that occurs in the absence of oxygen, whereby cells use organic compounds (e.g., pyruvic acid and Glc or other carbohydrates) for the production of ethanol or lactic acid (depending on the microorganism or cell type) and carbon dioxide (Fig. 6.7b). It guarantees electron flow in the cell

and the consequent production of energy (in the form of ATP) under anaerobic conditions. Humans have long been exploiting microorganisms that are capable of fermentation for the manufacture of a myriad of products, such as alcoholic beverages, yogurt, bread, antibiotics, and fuels.

Saccharomyces cerevisiae is an example of a fast-growing yeast that produces ethanol during fermentation and is widely used in industrial fermentation. Mankind learned how to exploit microorganisms in the manufacturing of a myriad of products, including alcoholic beverages, yogurt, bread, antibiotics, and fuels.

Feedstocks for first-generation bioethanol production are sucrose (mostly from sugarcane, but also from sugar beet [*Beta vulgaris*] and sorghum [*Sorghum bicolor*]) or starch (mostly from maize, but also from barley [*Hordeum vulgare*], wheat [*Triticum aestivum*], and other cereals). Starch that is released from milled grains must first be saccharified to yield Glc, and the resulting Glc or sucrose is fed to yeast growing in large liquid cultures (refer to Chap. 11 for the biorefinery process). The ethanol produced through alcoholic fermentation is then purified through distillation. By-products of this process include bagasse (leftover biomass) or distillers dried grains (the remnants of cereals from which the starch has been removed). An important economic consideration to the use of bioethanol is going to be our ability to find a market for these by-products in order to prevent their accumulation and yield maximal value from the crops. Currently, distillers dried grains have some value as animal feed, while bagasse can be used in energy production, in place of low-quality wood fiber in manufacturing, or in second-generation ethanol production as described below.

6.5.3 Industrial Production of Ethanol from Plant Carbohydrates

Ethanol derived from sugarcane and corn grains may not be able to meet our fuel demand, and there are also ethical concerns regarding the use of food crops for the production of biofuels. Therefore, the use of agricultural residues and other sources of cellulose is a promising alternative in the production of renewable fuels. Agricultural residues such as sugarcane bagasse or **corn stover** are by-products in the processing of these crops that usually have few applications (Tuck et al. 2012). Corn stover refers to the cobs, leaves, and stalks that remain in the field after harvest. Indeed, the production of these by-products often exceeds their demand, which makes these feedstocks very abundant and cheap. Other cellulosic sources, such as fast-growing trees (e.g., poplar [*Populus* spp.]) and switchgrass, also have potential to be used in the production of biofuels. As is the case with agricultural residues, they would not compete with food crops because they could be grown in marginal areas.

Despite these advantages, technology for the production of lignocellulosic-derived ethanol is not currently developed enough to allow commercial-scale production. Saccharification of cellulose is the main challenge, since biomass fibers are difficult to break. In addition, enzymes that catalyze the hydrolysis of cellulose and

hemicellulose are very expensive; lowering this cost will be essential for large-scale production (Kim and Kim 2014). Fermentation using lignocellulosic-derived sugars also has some limitations, since *S. cerevisiae*, which is commonly used for ethanol production, is not able to use pentoses as substrate (Gupta and Verma 2015). While other microorganisms do have this ability, their conversion times are slower than *S. cerevisiae.* Therefore, metabolic engineering has the potential to yield an ideal microorganism that can ferment both the hexoses and pentoses with a high productivity.

A major challenge when dealing with lignocellulosic materials is the variation in composition and structure of biomass, since different sources of biomass have distinct proportions of cellulose, hemicellulose, and lignin. **Softwoods** (e.g., spruce [*Picea* spp.], pine [*Pinus* spp.]), and **hardwoods** (e.g., poplar) are made up of woody biomass that is usually denser than agricultural residues, which is better for transportation (McKendry 2002; Zhu and Pan 2010). They also produce a lower content of pentoses after hydrolysis, which may contribute to fermentation efficiency. The higher content of lignin, however, increases the cost of pretreatment because it increases the energy requirements of these processes (Zhu and Pan 2010). Agricultural residues, such as corn stover, sugarcane bagasse, and rice hulls, have a lower lignin content and are therefore easier to break down compared to woody materials. Agricultural residues, however, have to undergo a compaction process prior to transportation, which can increase the price of the final product (McKendry 2002). **Energy crops** (or biomass crops), such as switchgrass and miscanthus [*Miscanthus sinensis*], have only biomass as their final product. They exhibit high yields, low lignin content, and fast growth. **Industrial waste**, especially from cellulose-processing plants, and **municipal solid waste**, such as paperboard products and woody materials, can also be used as feedstock for the production of bioethanol.

6.6 Pyrolysis of Lignocellulosic Biomass

Thermochemical and biochemical conversions of biomass are the two main routes for the transformation of this feedstock into fuels and other valuable compounds. While biochemical conversion uses enzymes and microorganisms to break down biomass (e.g., fermentation), thermochemical conversion is able to directly generate energy or other compounds from feedstock using high pressure, temperature, and/or chemicals (Basu 2013). Combustion, gasification, torrefaction, and pyrolysis are all used in the thermochemical conversion of biomass. Burning lignocellulosic biomass in the presence of oxygen releases heat in an exothermic oxidation reduction called combustion. Gasification of biomass produces gases (H_2, CO, CH_4) that can be utilized for the production of energy or for the production of liquid fuels. Torrefaction reduces the oxygen content of biomass and produces solid fuels that have a higher energy density (Basu 2013).

Pyrolysis is similar to combustion; however, biomass conversion occurs in the absence of oxygen and under lower temperatures (400–600 °C; Mettler et al. 2012; Basu 2013). The type of biomass, as well as temperature and length of reaction

(residence time), affects the final composition of the products. Conventional pyrolysis usually yields gaseous, liquid, and solid products, but their proportions can be adjusted by altering parameters of the process (Basu 2013; Collard and Blin 2014; Stefanidis et al. 2014). The most common solid phase product is biochar, which is mainly composed of carbon and has a higher energy density than biomass. It is the main product of slow pyrolysis and can be used as a form of carbon sequestering (Basu 2013). The gas phase is composed of noncondensable gases such as carbon dioxide, carbon monoxide, hydrogen, and hydrocarbons (Aho et al. 2013; Basu 2013). The liquid phase is made up of a complex mixture that contains water and a high content of oxygen known as bio-oil. Along with water, which can make up to one quarter of its mass, acids, ketones, phenols, aldehydes, ethers, carboxylic anhydrides, furans, and saccharides are among the compounds typically found in this mixture (Alvarez et al. 2014). While phenolic compounds are derived from lignin degradation, the degradation of cellulose and hemicellulose yields ketones. Acids, on the other hand, are derived from the decomposition of all the three polymers. A long residence time at high temperatures favors the production of gases, while lower temperatures favor the production of biochar (Bridgwater 2010). Fast pyrolysis, on the other hand, has a short residence time at intermediate temperatures and maximizes the production of bio-oil (Bridgwater 2010; Basu 2013).

Bio-oil is not suitable for direct use as a fuel in most engines (there are some furnaces and diesel engines that can use bio-oil) because it has a low pH and high moisture and oxygen contents (Bridgwater 2010). However, it can be upgraded to conventional fuels such as diesel or gasoline by converting oxygen to water or carbon dioxide, which are removed in downstream steps (Bridgwater 2010; Si et al. 2017). Gasification to synthesis gas, hydrodeoxygenation, and catalytic cracking are some processes that can convert bio-oil into fuels. Bio-oil can also be used for the production of chemicals for use in many different applications (Bridgwater 2010), although separation processes still need to be developed to effectively and efficiently separate out the most valuable compounds.

Co-pyrolysis is a technology that allows biomass to be processed with fossil fuel polymers to increase the final quality of bio-oil by reducing water and oxygen contents and increasing yield (Abnisa et al. 2017). In this case, tires and plastics are added to biomass, which increases both the energy and oil content of the bio-oil. The use of fossil fuel-derived materials in this process could also reduce the amount waste in landfills, making co-pyrolysis an attractive potential alternative in the production of biofuels.

6.7 Closing Comments

Carbohydrates are a very important output of many cultivated crops. The abundance of resulting feedstocks and the variety of chemical structures are very promising in terms of the potential use of plant-derived carbohydrates for many industrial applications. Among them, the production of biofuel demands enormous amounts of carbohydrates. Although corn starch and sugarcane sucrose are widely used for the

production of fuel ethanol, the use of lignocellulosic biomass as an alternative source is gaining attention. This is happening especially because of ethical implications concerning the production of fuels from potential food sources. Unfortunately, the production of ethanol from lignocellulosic materials is not as economically viable as ethanol production from corn or sugarcane, and therefore, virtually every step in the process currently requires improvement to reduce costs and increase yields. The use of plant carbohydrates as feedstocks for the production of bioproducts other than biofuel will be discussed in the next chapter.

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Chapter 7
Materials and Related Bioproducts from Plant Carbohydrates

Lucas J. Falarz, Michael K. Deyholos, and Guanqun Chen

Chapter Highlights

- Carbohydrate applications vary from papermaking to the synthesis of fine chemicals.
- Plant fibres are used as structural materials in textiles, construction materials and papermaking.
- Bioplastics such as polylactic acid and thermoplastic starch can be produced from carbohydrates.
- The interaction of polysaccharides with water form viscous mixtures that are used in food products or as adhesives.
- Some unique plant carbohydrates, such as the small-granule starches from cow cockle seeds, have special applications.

7.1 Introduction

Carbohydrates have been consumed in human nutrition or used as structural materials since ancient times. They also have many industrial applications in modern society due to properties derived from their diversity in terms of structure and composition

L. J. Falarz
Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

M. K. Deyholos
Department of Biology, University of British Columbia, Kelowna, BC, Canada

G. Chen (✉)
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
e-mail: guanqun.chen@ualberta.ca

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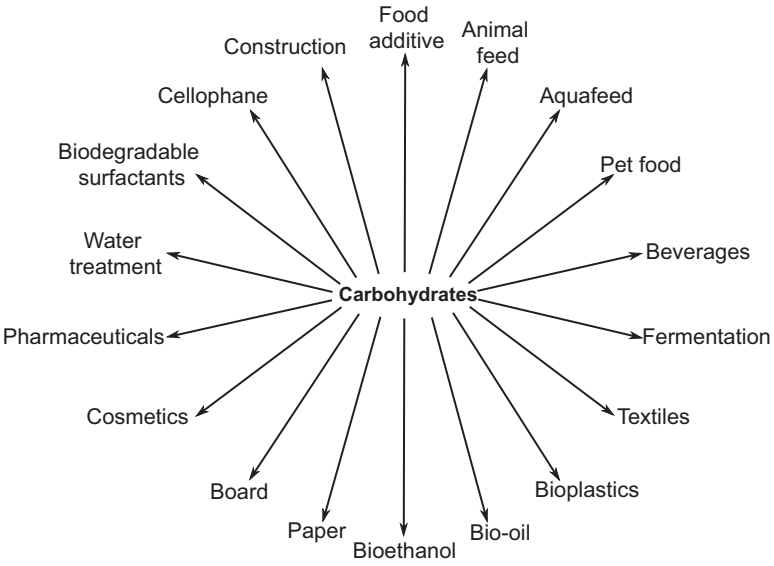

Fig. 7.1 Industrial applications of carbohydrates. (Adapted from Pal et al. (2005), Agrosynergie (2010), Wüstenberg (2015), Starch Europe (2017))

(Fig. 7.1) and can provide resistance, stiffness, thickness and sweetness. Plant carbohydrates can be also used as precursors for the production of chemicals and drugs. An advantage of their use as raw materials for industry is their abundance in nature; they can be easily obtained from wood, cellulose, fibres and crops.

With methods generally similar to those used to produce bioethanol, but with different microorganisms and catalysts, high-value chemicals can also be produced from sugar, starch and lignocellulosic biomass. These products include platform chemicals, which are simple molecules (such as lactic acid, isoprene or 1,3-propanediol) used as starting materials in the manufacture of other chemicals and products. Methods are currently under development to produce polyhydroxyalkanoates (PHAs) and other bioplastics from carbohydrate-rich feedstocks. Carbohydrates are also important in papermaking, textile production, livestock feeding and the synthesis of chemicals and health-related products. The use of plant carbohydrates in the manufacturing of some of these bioproducts will be discussed in detail in the following sections.

7.2 Structural Material

Plant fibres of various types have historically been a source of structural material. For example, the xylem fibres of wood are harvested for lumber and papermaking, with its functional qualities depending on its density and strength, lignin

Table 7.1 Methods of plant fibre treatment

Treatment method	Description
Mercerization	An alkaline solution is used to increase cellulose exposure, decrease surface roughness and decrease fibre diameter
Acetylation	Hydroxyl groups of the fibre are esterified with acetic anhydride to increase hydrophobicity
Benzoylation	Benzoyl groups are added to the hydroxyl groups of the fibre to increase hydrophobicity and improve adhesion properties
Graft copolymerization	Various molecules can be covalently bonded to the fibres to improve their surface properties
Silane treatment	Silane acts as a coupling agent to link fibres with the matrix. It provides a better adhesion to the composite and reduces hydrophilicity
Fungi	Some fungi degrade lignin, which reduces hydrophobicity and increase surface roughness
Bacterial cellulose	Some bacteria deposit nanofibres of cellulose onto the surface of plant fibres when grown in their presence. It modifies surface properties and may improve adhesion
Enzymes	Enzymes can catalyse specific modifications (e.g. laccase can modify lignin content) under milder conditions
Plasma	Plasma is a mixture containing free radicals and ions. When applied to the fibre, it can change the surface structure and properties such as hydrophobicity and adhesion

Based on Bhattacharya and Misra (2004), Li et al. (2007), Kabir et al. (2012), Kalia et al. (2013)

composition, degree of crystallinity of cellulose and microfibrillar angle (i.e. the orientation of cellulose fibres relative to the long axis of the cell). Conversely, the seed coat fibres of cotton (*Gossypium* spp.), and phloem (bast) fibres of crops including flax (*Linum usitatissimum*), hemp (*Cannabis portal*) and jute (*Corchorus* spp.), are used to make textiles and cordage. In addition, within the past few decades, methods have been developed for producing novel, advanced materials from fibre feedstocks. For example, there is interest in replacing the glass fibres of fibreglass with bast fibres. Compared to glass, natural fibres have the potential advantages of being renewable, biodegradable, less abrasive (and therefore less hazardous to personnel), and having a higher strength-to-weight ratio. However, the hydrophilic nature of the natural fibres (which limits their ability to bind to most resins) and the lack of uniformity and certainty of supply have thus far prevented their widespread use in composite materials. In order to overcome these limitations, natural fibres can be subjected to chemical or biological treatments (Table 7.1; Kalia et al. 2013), such as a plasma treatment of cellulose fibres, which renders the fibre narrower in diameter, thus increasing the surface area and hydrophobicity. In addition, changes in some functional groups are also apparent following treatment, which also impacts the properties of the fibres (Kolářová et al. 2013).

Cellulose nanocrystals (CNC; also referred to as nanocellulose or nanocrystalline cellulose) are another promising bioproduct obtained from plant carbohydrate feedstocks. As the name implies, CNCs are nanoscale (~5 nm wide, ~1000 nm long), needle-like particles of crystalline cellulose generated by mechanically and/or

chemically treating wood pulp or other natural fibres (especially cotton, which is very rich in cellulose; George and Sabapathi 2015). Essentially, this process extracts the strongest parts of the cell wall (crystalline cellulose) and discards the weaker, amorphous components. Accordingly, CNCs have tensile strength and stiffness that may exceed steel and Kevlar, respectively. One application of CNCs is therefore in the reinforcement of composite materials and paper products. The dimensions and surface characteristics of CNCs give them other interesting properties, including the ability to form gels, foams and liquid crystals. Because CNCs are indigestible, they have potential application as noncaloric thickeners. CNCs can also be formed into thin films with distinct optical and permeability properties. Many other applications of CNCs and chemically modified CNCs are still being developed.

7.3 Bioplastics

In 2015, the world produced 322 million tonnes of plastics (PlasticsEurope 2016). Considering that plastics are derived from petroleum, it is necessary to find new feedstocks for their production. In addition, oil-derived plastics are not biodegradable and cause significant environmental problems, which emphasizes the importance of developing future plastics that are environmentally friendly, which means that they have to be biodegradable and their raw materials have to be sustainable and renewable (Abolibda 2015). Thermoplastic starch (TPS) and polylactic acid (PLA) are some examples of carbohydrate-derived bioplastics that have those characteristics.

When mixed with water and plasticizers (such as glycerol) and exposed to heat, starch can be molded or otherwise formed into a variety of products. While TPS is not as strong or water resistant as plastics that are derived from petroleum, it offers advantages in terms of being a renewable and potentially biodegradable alternative to conventional plastics. In order to improve the properties of TPS, different plasticizers and coatings could be utilized, such as the addition of bacterial cellulose nanofibres (Fabra et al. 2016), which reduced water vapour and oxygen permeability. Indeed, the higher the amount of cellulose nanofibres, the lower the oxygen permeability, which is an important property for materials used in food packaging because the presence of oxygen reduces the shelf life of food products. Currently, commercial products made from TPS include disposable cutlery and various types of containers and packaging. In order to improve the TPS properties, different plasticizers and coatings are used. For example, bacterial cellulose nanofibres were added to TPS derived from corn to test the improvement in the barrier properties (Fabra et al. 2016). The properties of this composite showed a reduction in water vapour and oxygen permeability. Indeed, the higher the amount of cellulose nanofibres, the lower the oxygen permeability. Low gas permeability is an important property for materials used in food packaging because the presence of oxygen reduces the shelf life of food products.

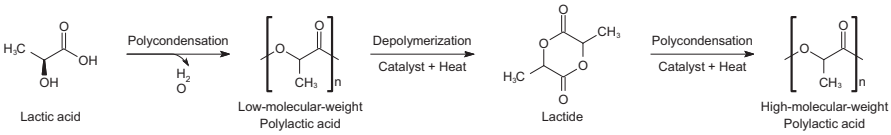

Fig. 7.2 Chemical synthesis of polylactic acid. (Adapted from Jamshidian et al. (2010))

PLA is a polymer of lactic acid that is used in the production of biodegradable plastics. The emissions of greenhouse gases and the consumption of fossil fuel-derived energy are lower in the production of PLA in comparison to the production of petroleum-based plastics (NatureWorks LLC 2017a). The lactic acid used in PLA production is obtained from the bacterial fermentation of sugars and starches, since many bacteria are efficient in the conversion of monosaccharides into lactic acid. The condensation of this compound then forms low-molecular-weight PLA as longer polymers cannot be efficiently obtained in this process (Jamshidian et al. 2010). The production of high-molecular-weight PLA requires the use of an intermediate (lactide) that permits a higher control in the size of chains. Lactide rings are obtained from the thermal depolymerization of PLA oligomers, and the lactide is then polymerized in the presence of catalysts and under high temperature, which produces high-molecular-weight PLA (Fig. 7.2).

PLAs exhibit some similar properties to fossil fuel-derived plastics and thus can be used in many applications, including the production of fresh food packaging, disposable cups and cutlery, construction materials and medical applications (Jamshidian et al. 2010; NatureWorks LLC 2017b). Disposed PLA is sent to composting facilities, where it can be biodegraded; however, the conditions for its degradation are very specific, requiring warm temperatures (~60 °C) and the presence of microorganisms. Under these conditions, PLA is decomposed in 45–60 days (Tokiwa and Calabia 2006). As such, PLA materials may not degrade well in landfills, since there is less water and lower temperatures, which does not allow for the hydrolysis of the polymer (Karamanlioglu et al. 2017). In addition, there are concerns that PLA discarded in landfills could release methane, which is a greenhouse gas (Washam 2010).

7.4 Gums and Functionally Related Products

Gums, pectins and other functionally related bioproducts share an ability to organize water in mixtures, which is useful in thickening, gelling, stiffening, emulsifying and stabilizing agents (Izydorczyk et al. 2005). This function of carbohydrates involves the formation of hydrocolloids, either as sols (polysaccharides dispersed in a liquid) or gels (liquids dispersed in a semisolid network of polysaccharides). Polysaccharides are well-suited to these applications because they are long hydrophilic polymers rich in polar (e.g. hydroxyl) or charged (e.g. carboxyl) groups, and

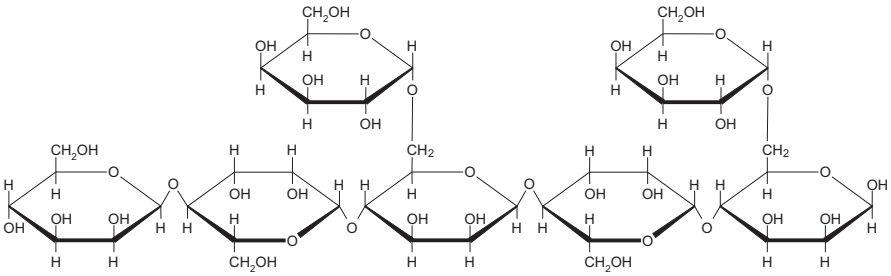

Fig. 7.3 Partial structure of galactomannans. (Adapted from Kramer (1988))

therefore each polysaccharide molecule is able to interact with many water molecules, and with other polysaccharide molecules, either directly or through cross-linkages (e.g. Ca^{2+}; BeMiller 2008). The structural diversity of polysaccharides allows a range of functionalities to be obtained depending on pH, ionic strength, temperature, polymer concentration and the composition of the mixture.

Galactomannans are an important class of gums and contain β-1,4 mannan polymers to which galactose (Gal) groups are attached through α-1,6 galactose linkages (Fig. 7.3; BeMiller 2008). The average ratio of Gal to mannose (Man) in the polymer varies between species, which affects the properties of the polymer. For example, locust bean gum has a Gal:Man ratio of approximately 1:4, while guar gum has a ratio of approximately 1:2. In general, the Gal side groups prevent hydrogen bond formation between the Man constituents of the galactomannan backbone, and as such, guar gum is more soluble than locust bean gum because it contains more Gal. Both locust bean and guar gums are obtained from seeds and are used to increase the viscosity of food products, paints and coatings and petroleum drilling fluids. In addition, guar gum is utilized in hydraulic fracturing (fracking) to increase the viscosity of the fluid that is injected at high pressure into a well (Barati and Liang 2014). This helps to supply sufficient pressure to fracture the rock and more importantly maintains particles (proppants) in suspension in the fluid until they can become lodged in the fractures and maintain them in an open state when the pressure is removed.

Cellulose itself does not possess the properties of a gum because most of the hydroxyl groups are entrapped in its crystalline structure, which limits water access. Therefore, chemical modifications are necessary to increase its solubility. Carboxymethyl cellulose (CMC) is an example of a cellulose-derived gum (Fig. 7.4), which is prepared through a reaction of cellulose in alkaline solution followed by a reaction with chloroacetic acid (BeMiller 2008). CMC has many applications in the food and pharmaceutical industries, functioning to stabilize, thicken, gel, suspend and bind (Wüstenberg 2015). In toothpaste, for example, CMC is used as thickener, binder and suspending aid, while in pharmaceuticals, it can be used as a tablet coating, viscosity controlling agent in creams and ointments and to stabilize suspensions in antacids (Dow 2014).

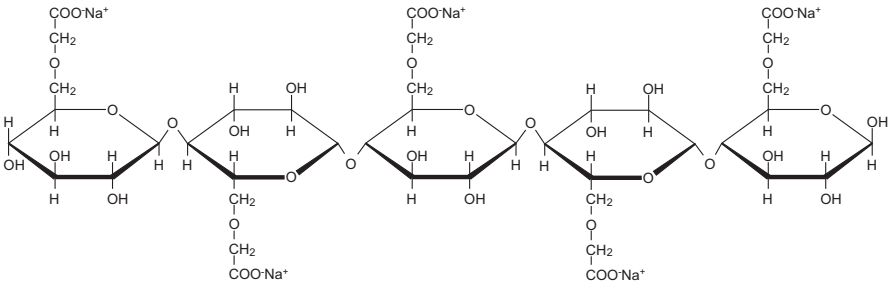

Fig. 7.4 Partial structure of carboxymethyl cellulose. (Adapted from Wüstenberg (2015))

There are other plant polysaccharides that can be used to prepare gums. Pectins, for example, do not have the necessary molecular weight to allow its use as a thickener. They are then mainly used as stabilizer and gelling agents in the food industry. Arabinoxylans are hemicelluloses present in cereal flours that have good water-binding properties and are used mainly in bakery applications due to their effect on dough rheology and the texture of the final product (Izydorczyk et al. 2005; BeMiller 2008). Cereal brans contain β-glucans, which are polysaccharides that contain β-1,3 linkages and can form gums (e.g. oat gum). β-glucans have positive health effects such as the reduction of serum cholesterol, but they negatively impact the manufacturing of some products such as beer, as they increase the viscosity of the mixture (BeMiller 2008). Gums can also be found in plant exudates, such as that from *Acacia* spp., which contains gum arabic, a gum that is widely used in the food industry due to its low-viscosity and stabilizing properties (Izydorczyk et al. 2005).

7.5 Adhesives

Adhesives are compounds that have the ability to bind materials and are used in many products such as paper packaging, vehicles, construction, electronics and textiles. The most popular commercial adhesives are currently derived from petrochemicals, such as urea-formaldehyde and phenol-formaldehyde (Imam et al. 2013); however, cellulose and starch can also be used as adhesives following certain modifications. Starches (especially amylose) are not immediately soluble in water because their polysaccharides are aggregated in closed granules that require chemical and physical treatments to improve their solubility through gelatinization. Commonly, such treatments include subjecting the starch mixture to elevated temperatures, which breaks up the granules, or the use of alkaline solutions, which causes the granules to swell. Unfortunately, the utilization of these techniques often causes the starch solution to undergo a retrogradation process, whereby the viscosity of the adhesive becomes unstable (Imam et al. 2013). In order to avoid this, the addition of additives or further chemical modifications is necessary. Following gelatinization, an acid is added to neutralize the mixture and borax is added to improve

adhesive properties (ASI 2005; Imam et al. 2013). A polymer with a higher molecular weight is thus formed, improving its adhesive properties and allowing its application in the production of paper packages and boxes.

Conversely, acid or enzymatic hydrolysis of starch releases oligomers of glucose called **dextrins**, which re-polymerize to form highly branched polymers that can also be used as an industrial adhesive with very good properties. In comparison to starch adhesives, dextrin adhesives display some superior properties, including reduced drying time (which helps avoid mold) due to the reduced amount of water required to prepare the adhesive. Dextrin adhesives can also be applied hot or cold, which facilitates handling (ASI 2005; Imam et al. 2013).

Additives can also be added to starch or dextrin formulations in order to improve their adhesive properties. For example, polyvinyl acetate is an adhesive widely used in wood and porous materials that when mixed with CNCs exhibits higher shear strengths even at elevated temperatures (Chaabouni and Boufi 2017).

7.6 Papermaking

The papermaking and pulp industries use carbohydrates for a variety of applications. Polysaccharides, for example, can replace mineral-based fillers, which are denser and more difficult to recycle. Indeed, the recycling and waste treatment of papers with carbohydrate-based additives are much easier than those containing mineral-based components (that generate inorganic materials in the sludge) since the additives are biodegradable. In addition, petrochemical-derived pigments are not suitable for papermaking since they decompose under heat (Shen et al. 2011). Paper may also contain a high content of starch, which is used to increase strength (since it will be a component of the paper web), to bind coatings or as filler to improve certain paper properties such as printability, wettability, whiteness and writability (Shen et al. 2011; ADM 2017). For these purposes, starch must first be modified through reaction with epichlorohydrin, acetic anhydride or acetic monoglyceride, which improves the light-scattering properties of the starch and creates an insoluble polymer that is also stable at elevated temperatures (Shen et al. 2011).

The use of CNC in papermaking is also gaining attention in recent years because of the properties it provides. When added to the pulp, for example, CNCs increase the strength of the paper and do not damage the structure of the fibres during the processing. They also improve the barrier properties of paper since they reduce porosity, which lessens permeability to air, water and oil, depending on the treatment (Boufi et al. 2016). The use of hemicelluloses in papermaking has been investigated, with some findings showing improvements in mechanical properties. For example, xyloglucans from the seeds of jatoba (*Hymenaea courbarils*), copaiba (*Copaifera langsdorffii*), nasturtium (*Tropaeolum majus*) and tamarind (*Tamarindus indica*) and galactomannans from the seeds of *Dimorphandra* spp. yield improvements in paper strength and porosity when added to the pulp (Lima et al. 2003).

7.7 Biomedical Materials Produced from Plant Carbohydrates

Pharmaceutical and biomedical industries are in the process of developing new bioactive products from carbohydrates. The effects of polysaccharides on health, however, are not new. For example, certain medicinal herbs used since ancient times are known to contain bioactive carbohydrates (Liu et al. 2015), and the ingestion of carbohydrate fibres aids with digestion and reduces the risks of certain diseases (Liu et al. 2015; The InterAct Consortium 2015). Indeed, carbohydrates often possess clear advantages over their synthetic counterparts, such as their **biodegradability**, non-toxicity and lower prices. In addition, certain polysaccharides that do not exhibit bioactive properties can also be functionalized for biomedical applications (Kang et al. 2015; Liu et al. 2015). Examples of functionalization reactions are oxidation, etherification, graft copolymerization and esterification (Liu et al. 2015).

Polymers of cellulose, hemicellulose, pectin and starch have the potential to be used in biomedical applications. Starch, for example, has been used in the pharmaceutical industry as component of drug capsules and tablet coatings (Liu et al. 2015), and biomedical and pharmaceutical industries are also developing new bioproducts using carbohydrates as raw materials in the areas of drug therapy and biomedical materials. Tissue engineering can also benefit from the use of carbohydrates since polysaccharides can enhance cell adhesion and cell differentiation or can act as scaffolds for tissue development. Other uses of carbohydrates include the production of wound healing materials and drug delivery. Indeed, plant-based carbohydrates could prove valuable in the area of drug delivery, which is an area of active study due to its potential in terms of improving the effectiveness of many treatments (El-Boubbou et al. 2010; Kang et al. 2015). Oxidized cellulose, for example, has been found to improve the uptake of topical ibuprofen and could result in the need for lower amounts of drugs to yield similar effects, which is especially important when dealing with expensive pharmaceuticals (Celebi et al. 2016). These bioactive products may not have as large a consumption of carbohydrates as other bioproducts, but they are very high-value products.

7.8 Small-Granule Starches

Starch granule size varies among plant sources and impacts its processing and application. For example, small granules of starch tend to have a higher pasting temperature and hydrolyse faster than larger granules (Lindeboom et al. 2004); however, these properties vary depending on the type and structure of the starch chains. Small-granule starches can be found in quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus cruentus*) and cow cockle (*Saponaria vaccaria*).

The seeds of cow cockle plants accumulate around 65% (dry weight basis) small and uniform granules of starch with a diameter that varies from 0.5 to 1.6 μm (Goering and Brelsford 1966) and are rather shear resistant, which contributes to their rupture resistance (Goering and Brelsford 1966; Peng and Yao 2018). The starch from cow cockle seeds forms a solution with a creamy texture, which makes it an ideal replacement for fat in certain products (Lindeboom et al. 2004). The plant is perennial and commonly found in the Northern Great Plains of North America where it is usually considered as weed. Cow cockle seeds also contain flavonoids, cyclopeptides and saponins, which also have industrial and health applications (Willenborg and Johnson 2013). For example, saponins have been shown to exhibit anticholesterol and anticancer activities (Güçlü-Üstündağ and Mazza 2007) and are also used in the pharmaceutical industry as a foaming agent, emulsifier, stabilizing agent or immunological adjuvant (Güçlü-Üstündağ and Mazza 2007; Willenborg and Johnson 2013).

Cow cockle has a high potential to be developed as a new crop for starch production due to its applications and agronomic characteristics. The plant is perennial and commonly found in the Northern Great Plains of North America where it is usually considered as weed. The time to maturity (95–100 days), the amount of fertilizer required and the seeding rate are similar to canola, as are its seed size and shape, which means that the same equipment could be used in the harvesting of canola and cow cockle crops. Cow cockle is also resistant to insects and can tolerate cold, heat and drought (Mazza et al. 1992; Balsevich 2008), which are all positive attributes in terms of potential agronomic performance.

7.9 Closing Comments

Polysaccharides are abundant natural polymers with properties that are suited to a wide range of application where reduced carbon or long, strong or hydrophilic supermolecular assemblies are required. The abundant and renewable supply of polysaccharides makes them competitive with other sources of bioproducts, although the cost of densification and transport of biomass can reduce these advantages. Attempts to substitute polysaccharide-based bioproducts for synthetic products, for example, in plastics or composites, have shown some successes to date. Improving the production and functionality of polysaccharides for bioproducts remains an active area of research.

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Chapter 8
Properties of Plant Proteins

Muhammad Arif and K. Peter Pauls

Chapter Highlights

- Proteins are linear assemblies of different quantities and sequences of amino acids.
- They have structural roles as building blocks of organisms, regulatory roles as transcription factors or metabolic roles as catalysts of biochemical reactions (enzymes) in the cell.
- The functional properties of a protein are defined by the physical and chemical properties of the protein.
- A number of crops including cereals grains, legumes and pulses are rich in plant proteins that can be used directly for manufacturing protein-based bioproducts.
- These plant proteins have a wide range of structures and functions, making them suitable for the manufacturing of a broad variety of bioproducts.

8.1 Introduction

Proteins are linear polymers of amino acids that have a great variety of functional, structural and regulatory roles in organisms. The word "protein" comes from the Greek word "prota", meaning "of primary importance" (http://www.peptideguide.com/proteins.html). Most proteins are assemblages of 20 possible standard amino acids that are directly coded for by DNA codons (Table 8.1, Fig. 2.12) and some nonstandard amino acids in some organisms. A typical amino acid contains an amino group (NH_2 –), a carboxylic group (– CO_2H) and a side chain R group (neutral, acidic or basic) that are all covalently attached to the α-carbon (Fig. 2.11).

M. Arif · K. P. Pauls (✉)
Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada
e-mail: ppauls@uoguelph.ca

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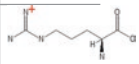
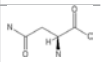
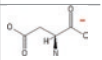
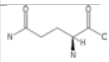
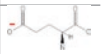
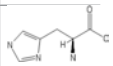
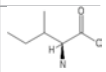
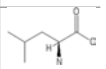
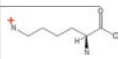
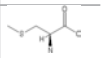
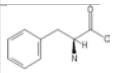
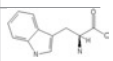
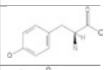
Table 8.1 Primary amino acids: their chemical structures and properties

Amino acid	Symbol		Side-group charge	Side-group polarity	Structure
	Three letter	One letter			
Alanine	Ala	A	Neutral	Non-polar	
Arginine	Arg	R	Positive	Polar	
Asparagine	Asn	N	Neutral	Polar	
Aspartic acid	Asp	D	Negative	Polar	
Cysteine	Cyc	C	Neutral	Non-polar	
Glutamine	Gln	Q	Neutral	Polar	
Glutamic acid	Glu	E	Negative	Polar	
Glycine	Gly	G	Neutral	Non-polar	
Histidine	His	H	Positive (10%) Neutral (90%)	Polar	
Isoleucine	Ile	I	Neutral	Non-polar	
Leucine	Leu	L	Neutral	Non-polar	
Lysine	Lys	K	Positive	Polar	
Methionine	Met	M	Neutral	Non-polar	
Phenylalanine	Phe	F	Neutral	Non-polar	
Proline	Pro	P	Neutral	Non-polar	
Serine	Ser	S	Neutral	Polar	
Threonine	Thr	T	Neutral	Polar	
Tryptophan	Trp	W	Neutral	Non-polar	
Tyrosine	Tyr	Y	Neutral	Polar	
Valine	Val	V	Neutral	Non-polar	

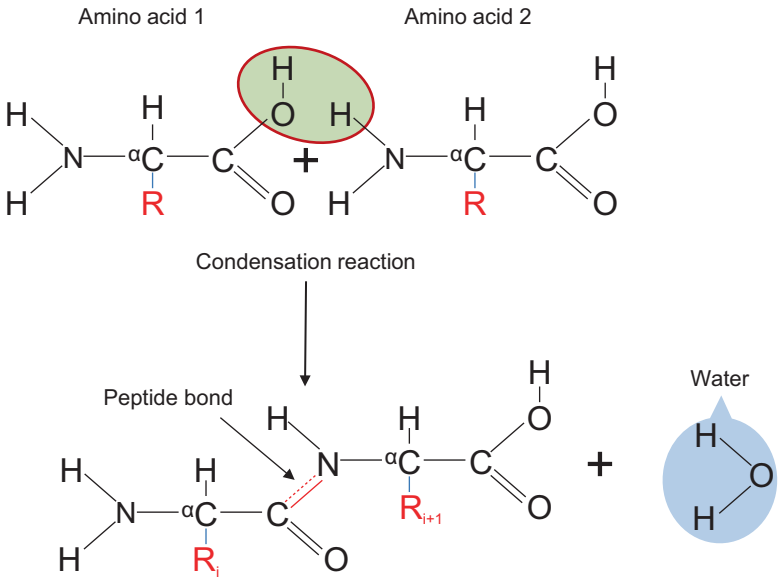


Fig. 8.1 Schematic representation of a peptide (amide) bond in a dipeptide molecule

The side chain R group contains acid, amine, amide, alcohol, alkyl or benzyl groups that determine whether an amino acid is acidic, basic or neutral.

Amino acid monomers can be assembled into a limitless number of sequences and can exhibit a wide range of interactions and chemical reactions (McMurry 1994; Stevens 1999; Pommet et al. 2003). When two amino acids are joined together during protein synthesis on a ribosome of a cell, a partial double covalent peptide (amide) bond (Fig. 2.14) is established between the carboxylic group of the nascent polypeptide or protein and the amino group of the additional amino acid, resulting in the release of a water molecule (Fig. 8.1). Typical proteins are polymers of approximately 350 amino acid residues; therefore, proteins are also called polypeptide chains. The term amino acid residue typically refers to amino acids which are bonded into a polypeptide, or a shorter segment, referred to as a peptide. The ends of polypeptide chains contain free amino and carboxylic groups, referred to as the **N-terminus** and the **C-terminus**, respectively. The ionized N-terminal amino group, C-terminal carboxyl group and groups on side chains determine the net charge, polarity and functionality of the protein polymers.

After its synthesis, the primary polypeptide chain undergoes several chemical and structural modifications that serve to protect the newly synthesized protein from breakdown by the cell's own proteolytic enzymes and functionalize it for various cellular roles. The range of modifications includes interchain folding; phosphorylation; conjugation with sugars, lipids and metals; as well as oligomerization. Proteins have structural roles as building blocks of organisms, regulatory roles as transcription factors or metabolic roles as catalysts of biochemical reactions (enzymes) in the cell.

Functional or structural proteins can be pure or conjugated or globular or filamentous, depending on their chemical and physical nature (Damodaran 1996). This chapter builds upon the information on amino acids and proteins presented in Chap. 2 extending the discussion into the realm of plant proteins, including the major sources and methods of isolation and processing. A considerable amount of the discussion that follows is based on information that can be found in a textbooks used for senior undergraduate courses in biochemistry (e.g. Heldt and Piechulla 2010; Moran et al. 2012; Nelson and Cox 2017).

8.2 Structural and Functional Properties of Proteins

The functional properties of a protein are defined by all the physical and chemical properties of the protein, which affect its behaviour during its interaction with other molecules, processing, storage, isolation, utilization and degradation. Protein structure and function depend on the type, number, order, orientation of its amino acids and interactions among them. **Protein structure** is determined by the basic structure of the peptide bond, the sequence of its amino acids, folding of the primary chain and interactions, as well as intra-protein and inter-protein cross-links among its constituent amino acid side chains (Krochta 2002). The primary polypeptide chain is organized into secondary, tertiary and quaternary structures (Fig. 2.15), based on the kind, number and sequence order of the amino acid residues and their cross-linking interactions within the chain. Hydrogen bonding, van der Waals forces and electrostatic, hydrophobic and covalent disulphide cross-linking bonds organize proteins into their final structures (Krochta 2002).

Discussions about polypeptides or proteins often refer to the terms molecular weight (MW) or relative molecular mass (M_r) which is the mass of the molecule relative to one twelfth the mass of an atom of carbon-12. MW or M_r is dimensionless and has no units. The molecular mass (i.e. absolute molecular mass, m) has the same magnitude as MW (or M_r) but instead is presented in daltons (Da) where 1 Da is an atomic mass unit.

8.2.1 Primary Structure

A protein's primary structure refers to the linear sequence of amino acid residues in the polypeptide chain. The partial double bond nature of the peptide bond, which is the result of the resonance structure created by the delocalization of the lone pair of electrons on the nitrogen, creates a slight positive charge on the amino group (N – Hδ+) and restricts the rotation of the CO–NH bond to a maximum torsion angle (ω) of 6°. This rigidity of the peptide bond keeps its six atoms in a single plane with mostly (1000:1) trans configurations, which reduces steric interactions of the

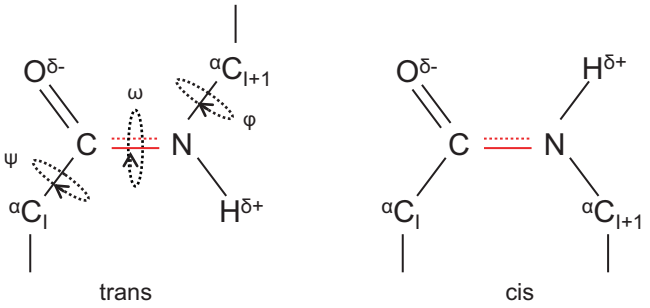


Fig. 8.2 Schematic representation of *tran-cis* orientation of amino acids in polypeptide chain. (Source: Damodaran (2008), Nelson and Cox (2017), Voet et al. (2016))

α-carbons, except for X-proline peptide groups, which have 3:1 (*trans:cis*) ratios. Rotation about the other bonds in the primary chain, namely, between N–Cα (Φ) and Cα–CO (Ψ), occurs freely but is affected by interactions among R groups (Fig. 8.2) (Damodaran 2008).

8.2.2 Secondary Structure

After synthesis, the majority of proteins fold into α-helices and β-sheets, which make up their secondary structures. **α-Helices** are right-handed spirals stabilized by hydrogen bonds between carbonyl oxygens (at positions n) and amide hydrogens of the fourth amino acid (at positions n + 4) towards the C-terminus. Every turn in the α-helix contains 3.6 amino acid residues. Within the α-helix, the side chains of the amino acid residues project outward from the axis of the helix, and its stability is affected by the properties of these side chains. For example, an alanine residue, which has a small uncharged side chain, occurs often in α-helices, whereas tyrosine and asparagine residues, which have large side chains, are less commonly found. Proline residues cannot make up α-helices because their rigid cyclic side chains disrupt the helix conformation and because they do not have hydrogen atoms on their amide nitrogens to form the hydrogen bonds that stabilize helices (Damodaran 1996).

In **β-sheets**, the primary amino acid chain is folded back on itself so that interactions can occur at the sides of the chain for some length to create a planar surface. Hydrogen bonds occur between the carbonyl oxygens of one strand and the amide hydrogens of the next strand, which lie in the sheet in roughly perpendicular positions relative to the long axis of the sheet. Side chains project above and below the plane of the sheet. β-Sheets with primary strands running antiparallel (running in the same N- to C-terminal direction) and parallel (running in opposite N- to C-terminal direction) exist, but the former structure is more stable.

8.2.3 Tertiary Structure

To optimize the intra-chain attractive forces (van der Waals, hydrophobic and electrostatic) and to reduce the free energy and interfacial area, protein secondary structures fold into more compact tertiary structures. The process of folding is complex and often brings hydrophobic side chains, such as those of phenylalanine, alanine, valine, leucine, isoleucine and methionine, into the interior of the molecule, where they are shielded from contact with water, and positions hydrophilic side chains, such as those of lysine, arginine, serine, glutamic acid, histidine and glutamine, to the outside in contact with water to create a hydrate coat. Electrostatic interactions between oppositely charged amino acids residues (such as the amino of side chain of lysine and the carboxylic group of glutamic acid) within close proximity in the three-dimensional space create salt bridges that stabilize tertiary structures. Oxidation of the thiol groups of two cysteine residues (Cys–SH) to form intra-strand disulphide bonds (Cys–S–S–Cys) is another important mechanism for stabilizing tertiary structures.

8.2.4 Quaternary Structure

Some proteins are actually aggregates of monomers arranged into a quaternary structure. These structures are stabilized by the same non-covalent interactions and disulphide bonds as the tertiary structures. For example, most of the plant seed storage proteins, including soy protein (soybean), phaseolin (bean), gluten (wheat) and zein (maize), are oligomers.

8.2.5 Protein Modifications

Post-translational modifications give proteins physical, chemical and functional properties that are used to classify them as glycoproteins (conjugated with sugar), phosphoproteins (phosphorylated), lipoproteins (conjugated with lipids) or metalloproteins (conjugated with metals). These modifications affect the functional and structural properties of proteins as well as their localization, stability and interactions with other molecules.

Glycosylation is the addition of oligosaccharide chains called glycans to proteins during or after their synthesis in the endoplasmic reticulum (ER) and/or Golgi apparatus (GA) of the cell. The oligosaccharide side chain is attached to the amide nitrogen of asparagine (Asn) residues (N-glycosylation) or to the hydroxyl group of serine (Ser), threonine (Thr) or hydroxyproline (Hyp) residues (O-glycosylation) in the protein backbone. N-glycosylation occurs during or after translation in the ER, with the transfer of a preformed lipid-linked oligosaccharide onto the polypeptide.

In contrast, O-glycosylation occurs by the transfer of individual saccharides onto folded proteins in the ER and/or the GA.

Glycosylation represents the most widespread post-translational modification found in biopharmaceutical proteins. Approximately 50% of human proteins are glycosylated, and the functional activities of therapeutic glycoproteins are frequently dependent on the presence and composition of their glycans, since they can affect their plasma half-life and tissue targeting. For foreign proteins synthesized in plant platforms, the glycosylation patterns are of particular importance because plant glycosylation patterns differ in several ways from mammalian N- and O-glycosylation patterns. For example, because plants lack N-acetylglucosaminyltransferase-III, N-acetylglucosaminyltransferase-IV and N-acetylglucosaminyltransferase-V enzymes, which produce branching in glycans, plant N-glycans are bi-antennary structures, instead of the multi-antennary structures found in animal glycoproteins. Also, plant N-glycans contain α (1, 3) fucose and/or β (1, 2) xylose linked to the core $Man_3GIucNAc_2$-Asn of glycans, in contrast to the occurrence of β (1, 4)galactose in mammalian proteins. Furthermore, most oligosaccharides of human glycoproteins are capped by the addition of sialic acid on a penultimate galactose residue, but the pathway for the addition of sialic acids is missing from plants. The absence of the sialic acid modification of galactose causes rapid removal of the protein from circulation through uptake by hepatic galactose-specific receptors. Additional protein engineering is required to produce mammalian compatible proteins in plant-based systems (Webster and Thomas 2012).

8.2.6 Protein Targeting

Higher plants have evolved the ability to accumulate large amounts of proteins in stable forms in their storage organs such as seeds, tubers and roots. The proteins are deposited in specialized protein storage vacuoles or protein bodies in the cell (Herman and Larkins 1999). The storage proteins are transported into the ER during their synthesis. Storage **albumins** and **globulins**, found in most land plants, are trafficked through the normal secretory pathway that includes the GA and accumulate in storage vacuoles of cotyledonary cells, especially in legumes (Otegui et al. 2006). In contrast, **prolamins**, which are found in cereals, form large insoluble polymers within the ER that do not proceed along the secretory pathway and result in the formation of protein bodies in endosperm cells (Herman 2008).

8.3 Physicochemical Properties of Proteins

Knowledge of the physicochemical properties of proteins, including their amino acid composition, structure, net charge, charge distribution, hydrophobicity/hydrophilicity ratio, molecular flexibility/rigidity, MW and ability to interact with other

components, is useful to evaluate their potential utility for manufacturing bioproducts such as adhesives, glues, films, fibres and pharmaceuticals. Collectively, the physicochemical properties of proteins affect their functional properties, such as solubility, hydration, oil miscibility, aroma trapping, viscosity, gelation, elasticity, emulsification and foaming characteristics, which are important parameters for protein-based products. These properties can be grouped into properties related to hydration mechanisms, protein structure and rheology and protein surfaces (Moure et al. 2006).

8.3.1 Protein Hydration Properties

The **hydration properties** of proteins are the result of their amino acid compositions and in particular the ratio of polar versus non-polar and ionic versus neutral amino acids (Table 8.1). Water is a bipolar molecule and interacts easily with other polar molecules, including polar amino acids. Proteins with high polar amino acid fractions have high water binding and holding capacities (Kuntz and Kauzmann 1974).

The locations of the **hydrophobic (non-polar)** and **hydrophilic (polar) amino acids** in protein molecules also influence their water retention properties (Damodaran 1996). To perform their different functions, proteins are usually folded in a way that exposes their hydrophilic amino acids on their surfaces and places their hydrophobic amino acid residues in the centers of their structures (Mierovich and Scheraga 1980). The ratio of hydrophobic to hydrophilic amino acid residues on the surface of the protein molecule and the charge frequency determine the solubility of the protein in various solvents. Higher ratios of surface hydrophobic amino acids increase protein-protein interactions, resulting in lower protein solubilities, while charged amino acids promote protein-solvent interactions, resulting in increased protein solubilities (Damodaran 1996; Moure et al. 2006). Protein concentration, pH, temperature, ionic strength, type of solute ion and atmospheric pressure are the external factors that affect the water-holding capacities and solubilities of proteins (Moure et al. 2006).

8.3.2 Protein Rheology Properties

Viscosity and gelation are the two most studied rheological properties of proteins. **Viscosity** is the resistance to flow of a liquid due to internal friction. A **gel** is an intermediate state between solid and liquid. The viscosity and gelation properties of proteins depend on protein-protein and protein-solvent interactions. These depend on their molecular properties, including MW, molecular size, shape, flexibility and hydration (Damodaran 2008). Proteins containing polar amino acid residues form transparent gels and proteins containing non-polar amino acid residues form opaque gels (Shimada and Matsushita 1980; Totosaus et al. 2002; Moure et al. 2006).

8.3.3 Protein Surface Properties

Proteins procured from different sources have different surface activities, including emulsion and foam properties. Differences in protein surface activities are mostly related to different conformations proteins can assume. Several interdependent factors (including polypeptide chain stability/flexibility, adaptability to environmental change, amounts and distribution patterns of hydrophilic and hydrophobic amino acid residues in their primary polypeptide chain and on the surfaces of the proteins) affect their surface properties. A balance of non-covalent interactions, including electrostatic attractions, hydrogen bonding, covalent disulphide bonding and hydrophobic interactions, determines the emulsion and foaming properties of proteins (Damodaran 1996). However, not only intrinsic factors but also extrinsic factors such as protein concentration, pH, temperature, ionic strength and type of ion and pressure can affect protein-based products (Phillips et al. 1994). A proper balance of attractive, repulsive and hydration forces is required to form strong and stable protein-based products.

The structural and functional properties of proteins make them useful for various industrial applications. For example, proteins can be made into elastic materials that can survive repeated stress-strain cycles, and they can aggregate to form films that provide barriers to gases, moisture and bacteria. Films produced from protein could be used in food packaging, in paper coatings and in bandaging materials. Protein-based materials have become a research focus because of their high performance, low cost and environmentally friendly characteristics (Kumar et al. 2008; Sasmal et al. 2008).

8.4 Plant Proteins

Agricultural crops have been the main source of food and feed for many centuries because they contain high levels and high concentrations of protein, starch and oil. Plants have the potential to function as efficient platforms for pharmaceutical and therapeutic protein manufacturing because their production costs are low, they produce high quantities of proteins and extraction protocols are relatively facile and efficient.

A number of crops including cereals grains, legumes and pulses are rich in plant proteins (Dangaran et al. 2011) that can be used directly for manufacturing protein-based bioproducts. Corn zein, wheat gluten, sorghum kafirin, rice (*Oryza sativa*) bran protein, soy protein, peanut protein and cottonseed protein have all been utilized to manufacture protein bioproducts. Because plant proteins are varied in their chemical, physical, functional and structural properties (Table 8.2), bioproducts made from them have a wide range of uses. In this section, a few common compositions of plant proteins are discussed.

Table 8.2 Amino acid composition (% of protein) of some plant proteins

Amino acids	Corn zein[a]	Wheat gluten[b]	Soybean[c]	Mung bean[d]	Red kidney bean[e]
Glycine	0.0	3.9	1.8	3.6	4.2
Alanine	10.5	3.1	1.8	5.1	4.5
Valine	4.0	5.3	2.5	6.7	4.6
Leucine	21.3	8.3	3.3	9.3	8.1
Isoleucine	5.0	4.9	1.9	5.5	3.9
Phenylalanine	7.3	6.0	2.2	6.8	5.9
Tryptophan	0.2	NR	0.5	NR	1.2
Proline	10.5	14.5	2.0	4.1	4.4
Serine	7.1	5.2	1.9	2.5	6.1
Threonine	3.5	3.5	1.6	1.8	4.5
Tyrosine	5.3	3.8	1.5	3.0	2.9
Methionine	2.4	1.7	0.6	1.3	1.4
Cysteine	0.8	2.3	0.7	–	1.0
Lysine	0.0	2.1	2.7	6.8	7.5
Arginine	4.7	4.5	3.2	7.0	6.3
Histidine	1.3	2.3	1.2	2.5	3.0
Aspartic acid	4.6	3.7	4.8	10.2	13.1
Glutamic acid	26.9	41.0	7.7	19.5	17.3

[a]Golenkov (1985)
[b]Coffman and Garcia (1977)
[c]Mundi and Aluko (2012)
[d]Mosse (1961)
[e]Kovalenko et al. (2006)

8.4.1 Corn Zein

Corn (*Zea mays*) contains 8–12% protein in its kernels (Earle 1977). Gorham first described corn zein in 1821, and Osborn classified it as a prolamin, which is a seed storage, globular, water-ethanol-soluble, protein containing large amounts of proline and glutamine and small amounts of arginine, lysine and histidine (http://www.britannica.com/EBchecked/topic/478591/prolamin), in 1924. Corn **zein** makes up 50% or more of the corn kernel proteins. It is distributed in the outer layer of corn kernels as small, compact bodies embedded in the glutelin protein matrix (Padua and Wang 2002). Because of its low solubility in water, it has a greasy and glossy appearance. It was first commercialized in 1939 for the production of adhesives, plastic films, coatings and fibre applications.

Zein is an aggregate of single polypeptide protein subunits, including α-zein (23–27 kDa), β-zein (15 kDa), γ-zein (27 kDa) and δ-zein (10 kDa). Commercial corn zein is 75–85% **α-zein**. This subunit is rich in non-polar amino acids, including glutamine (21–26%), leucine (20%), proline (10%), alanine (10%) and phenylalanine (8%), but is deficient in basic and acidic amino acids (Table 8.2; Wilson 1988). A higher proportion of non-polar amino acids in α-zein make it soluble in alcohols.

β-Zein is rich in methionine (10%) and tyrosine (8%) and constitutes 10–15% of the total corn zein. **γ-Zein** is rich in proline (25%) and histidine (8%) and accounts for 5–10% of the total corn zein. **δ-Zein** is rich in sulphur-containing amino acids (methionine and cysteine) and constitutes less than 5% of the total zein protein (Thompson and Larkins 1989; Shukla and Cheryan 2001). β-Zein, γ-zein and δ-zein are soluble in aqueous alcohol and a reducing agent (Thompson and Larkins 1989) and are classified as **glutelins**, which are cereal storage proteins soluble in dilute acid or base and not coagulated by heat (http://www.merriam-webster.com/medical/glutelin).

8.4.2 Wheat Gluten

Wheat kernels contain 8–14% protein (Delcour et al. 2012). Wheat proteins (Fig. 8.3) are classified into four groups on the basis of their solubilities and include water-soluble albumins, salt-soluble globulins, alcohol-soluble gliadins and diluted alkaline−/acid-soluble glutenins. Generally, wheat proteins are classified as

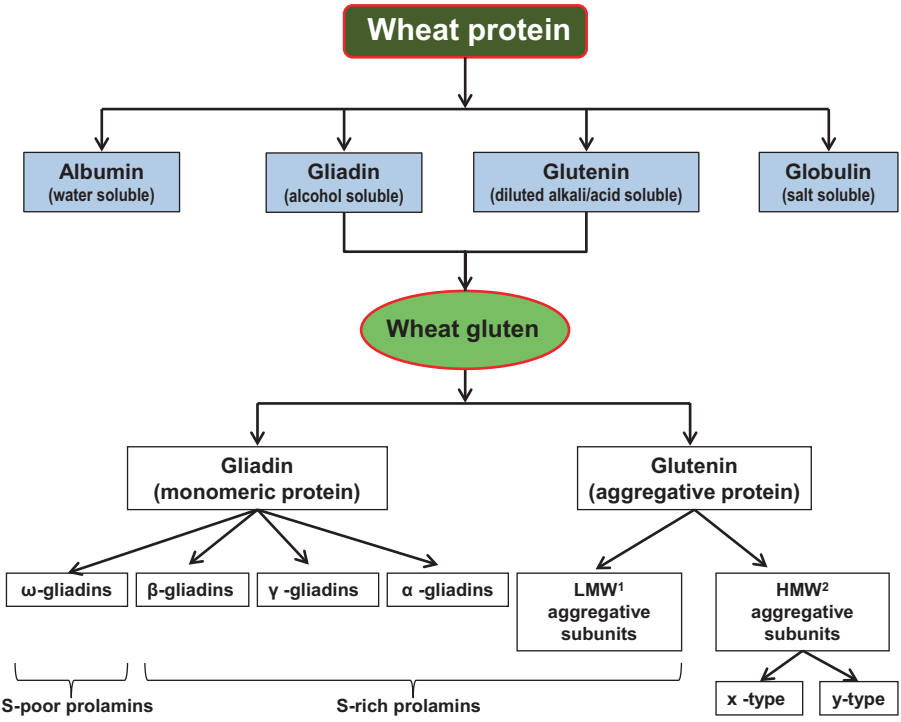


Fig. 8.3 Classification of wheat protein. [1]Lower molecular weight.[2]High molecular weight. S sulphur

glutens, comprising gliadins and glutenins, and **non-glutens**, comprising albumins and globulins. Non-gluten wheat proteins generally constitute 15 to 20% of the total wheat protein (Gupta et al. 1992). They are predominantly monomeric proteins with molecular mass lower than 25 kDa. However, a significant proportion of the chains exist as aggregated polymers with molecular mass between 60 and 70 kDa that are stabilized by intermolecular disulphide bonds. The non-gluten wheat proteins have metabolic (albumins) or structural (globulins) functions.

The addition of water to wheat flour followed by mixing results in the production of a tough, rubbery, elastic substance called gluten. At a microscopic level, gluten is a continuous network of strands composed of gliadin and glutenin proteins stabilized by intermolecular disulphide bonds and non-covalent bonds such as hydrogen bonds and hydrophobic interactions (Figs. 8.4, 8.5). The structure has important viscoelastic properties that make it ideal for bread-making (Veraverbeke and

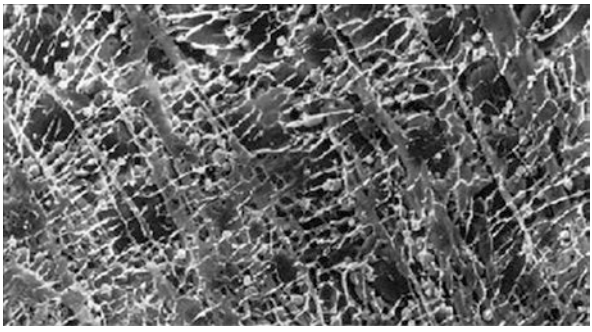


Fig. 8.4 Scanning electron micrograph of wheat gluten. (Source: http://www.bakeinfo.co.nz/Facts/Gluten)

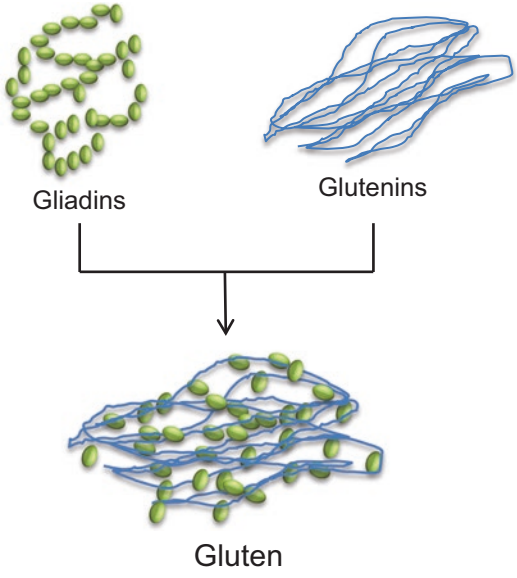


Fig. 8.5 Schematic representation of wheat gluten

Delcour 2002), including its high water absorption capacity (approximately twice its weight), stickiness, extensibility and elasticity. To extract gluten, wheat flour is mixed with water and washed extensively to remove the starch and other water-soluble proteins. The remaining gluten mass is extruded, chopped and dried for packaging. Industrial wheat gluten contains approximately 70–80% protein (33–45% alcohol-soluble gliadin and 40–50% alkaline−/acid-soluble glutenin), 10–14% polysaccharide, 6–8% lipid and 0.8–1.4% minerals. Both gliadin and glutenin are rich in non-polar amino acids, proline and glutamine, and poor in polar amino acid residues (Table 8.2), which contributes to their low solubility in water. Gliadins are compact, globular and viscous in nature (Shewry et al. 1986; Veraverbeke and Delcour 2002). They act as plasticizers in gluten and contribute to its gas retention properties in bread dough.

8.4.3 Soy Protein

Soybean contains up to 50% protein in the dry seed. Soybean protein is a by-product of the soybean oil industry (Rhim et al. 2000) and is available as soy flour, soy protein concentrates and soy protein isolates (SPI) containing 50–59%, 65–72% and ≥90% protein, respectively. Globulins are the most abundant class of proteins in soy protein isolates. Glutamic acid, aspartic acid, arginine, leucine, lysine and valine are the major constituent amino acids in soy protein isolate profiles (Table 8.2). Soy proteins are classified as 2S, 7S, 11S and 15S on the basis of their sedimentation during ultracentrifugation (Fig. 8.6) (larger **Svedberg (S) numbers** indicate smaller protein MW). They account for 22, 37, 31 and 1% of the total protein, respectively (Wolf and Briggs 1956). 11S (**glycinin**) and 7S (**β-conglycinin**) are the principal proteins and account for approximately 70% of the total seed protein in soybean (Thanh and Shibasaki 1976).

The 11S and 7S soy proteins are significantly different in their physical, chemical and functional properties (Wolf and Tamura 1968; Mori et al. 1981). For example, the 11S protein precipitates faster and forms larger aggregates than 7S, and its gels have a higher water-holding capacity than 7S gels. In addition, 11S proteins have higher tensile values and higher hardness and expand more on heating than 7S proteins. The 11S:7S ratio is therefore a predictive indicator of relative protein functional properties (Kwanyuen et al. 1998).

8.4.4 Bean Protein

Dry beans including navy (*Phaseolus vulgaris*), pinto (*Phaseolus vulgaris*), lima (*Phaseolus lunatus*), kidney (*Phaseolus vulgaris*), black (*Phaseolus vulgaris*), white (*Phaseolus vulgaris*), red (*Vigna angularis* and *Vigna umbellata*), pink (*Phaseolus vulgaris*), lentil (*Lens culinaris*, also known as *Lens esculenta)*, black

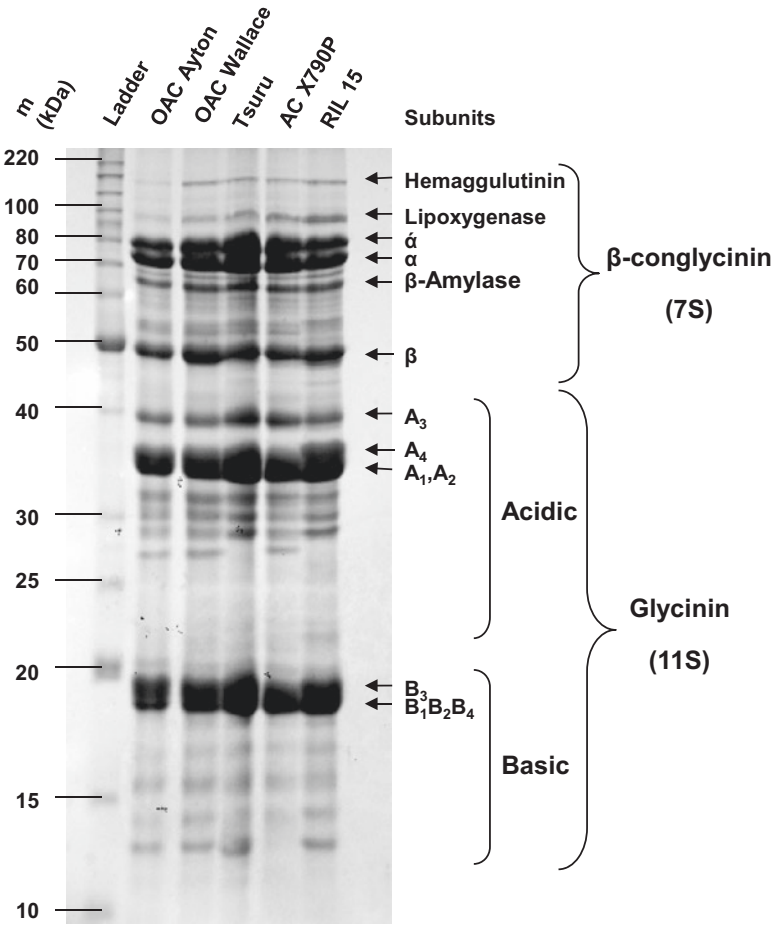


Fig. 8.6 1D SDS-polyacrylamide gel of seed storage proteins in selected soybean genotypes. Regions containing 7S and 11S proteins are shown, and some specific protein bands are identified. m molecular mass

eye (*Vigna unguiculata*), black gram (*Vigna mungo*), garden pea (*Pisum sativum*), chickpea (*Cicer arietinum*), moth bean (*Vigna aconitifolia*), jack bean (*Canavalia ensiformis*)and tepary bean (*Phaseolus acutifolius)* are important food sources in many countries. Common dry bean (*Phaseolus* spp.) seeds contain 15 to 30% protein, on a dry weight basis (Sathe 2002; Yin et al. 2008). The major classes of proteins in dry beans are water-soluble albumin and mildly alkali-soluble globulins. There are also some minor proteins, such as enzyme inhibitors and lectins. Albumin and globulin account for ~80% of the total storage protein with their molecular masses ranging from 10 to 400 kDa. The quantities and ratios of albumins to globulins depend on the species and variety of the dry bean. Generally, dry beans contain 10–30% albumins and 45–70% globulins (Sathe 2002).

Globulins are further grouped by their solubilities at different ionic strengths and by sedimentation in ultracentrifugation. They are classified into **vicilin/phaseolin** and **legumin** with coefficients of sedimentation of 7S and 11S, respectively (Danielsson 1949). Both vicilin and legumin can also be distinguished by their oligomeric organization and polypeptide structures. The 7S vicilin/phaseolin is a glycoprotein of three single peptide chains with molecular mass of 50–75 kDa, while 11S legumin is a hexameric protein linked by disulphide bond(s) between acidic α- and basic β-subunits with molecular mass of ~40 and ~60 kDa, respectively. Six polypeptides are linked together to constitute the legumin molecule with a molecular mass of 320–400 kDa (Lawrence et al. 1990; Sathe 2002). The 7S protein accounts for 50–55% of the total globulin protein content in dry beans on a dry weight basis (Sathe 2002). There is an inverse relationship between 7S and 11S concentrations (e.g. increase in the quantity of 7S causes a proportional decrease in the amount of 11S and vice versa, so the net protein concentrations remain constant) (Ogawa et al. 1989). Dry beans have small amounts of other storage proteins in addition to the globulins, including lectins (a tetrameric glycoprotein of 7S) or phytohaemagglutinin with molecular masses of 27–37 kDa, arcelin (a 2S dimer glycoprotein) with a molecular mass of 35–42 kDa, sulphur-rich protein (a 3S dimer glycoprotein) with a molecular mass of 29–32 kDa and enzyme inhibitor proteins (Sathe 2002).

8.5 Protein Isolation and Purification from Plants

Often, the first step in producing bioproducts from proteins is isolating pure fractions of proteins of interest from mixtures of several other macromolecules, including polysaccharides, lipids and other proteins. However, there is no standard procedure for isolating and purifying proteins from all sources (organisms) because the proteins may vary from source to source in amino acid composition, sequence, structure, size, shape, net charge, isoelectric point, solubility, heat stability, hydrophobicity, ligand/metal binding capacity and post-translational modification.

The level of purity, functionality and quantity of the protein required for a particular application will influence the approach used to purify it. For example, a few micrograms for enzymatic kinetic studies to several kilograms for industrial and pharmaceutical applications can be required. The highest quality and functionality are required for medical uses compared to lower stringencies for proteins used to manufacture commercial films, coatings, fibres and adhesives.

The first step in protein isolation is crushing/milling of the plant source to release the protein from the biological matrix of cells. Generally, the desired protein is released as a mixture with other macromolecules, including other proteins, polysaccharides and lipids. The next step is to separate the targeted proteins from the mixture of molecules through different procedures or a combination of more than one procedure. Protein solubility plays a crucial role in protein isolation and purification. Plant proteins including corn zein, wheat gluten, soy protein, bean protein, peanut protein and cottonseed protein differ in their solubility properties. For

Table 8.3 Protein isolation and purification process[a]

Separation process	Basis of separation
1. Precipitation	
Alkali (ammonium sulphate)	Solubility
Acetone	Solubility
Polyethyleneimine	Charge, size
Isoelectric	Solubility, pI (isoelectric point)
2. Phase partitioning	
Polyethylene glycol	Solubility
3. Chromatography	
Ion exchange (IEX)	Charge, charge distribution
Hydrophobic interaction (HIC)	Hydrophobicity
Reverse-phase HPLC	Hydrophobicity, size
Affinity	Ligand-binding site
DNA affinity	DNA-binding site
Lectin affinity	Carbohydrate content and type
Immobilized metal affinity (IMAC)	Metal binding
Immunoaffinity (IAC)	Specific antigen site
Chromatofocussing	pI (isoelectric point)
Gel filtration/size exclusion (SEC)	Size, shape
4. Electrophoresis	
Gel electrophoresis (PAGE)	Charge, size, shape
Isoelectric focussing (IEF)	pI (isoelectric point)
5. Centrifugation	Size, shape, density
6. Ultrafiltration	Size, shape

[a]Adapted from Burgess (2008)

example, corn zein is soluble in aqueous solution, soy and bean proteins in mild alkali and wheat gluten in mild acid, mild alkali and aqueous ethanol solutions.

A good isolation procedure is simple, reproducible, specific, reliable and cost-effective. Protein extraction procedures, based on their physicochemical properties, are categorized into precipitation, phase partitioning, chromatographic, centrifugation and ultrafiltration procedures (Table 8.3).

8.5.1 Corn Zein Isolation

Commercial zein is a by-product of the corn milling industry. In corn wet-milling, the fine slurry that remains after centrifugal extraction of starch from the endosperm comprises a protein-rich mass called corn gluten meal from which zein is extracted. Corn zein can also be extracted from dried distillers' grains with solubles, a by-product of corn ethanol manufacturing. The commercial method of zein extraction was patented in 1970 by Carter and Reck (Fig. 8.7). Corn gluten meal is solubilized with organic solvent such as isopropyl alcohol a few times to get highly

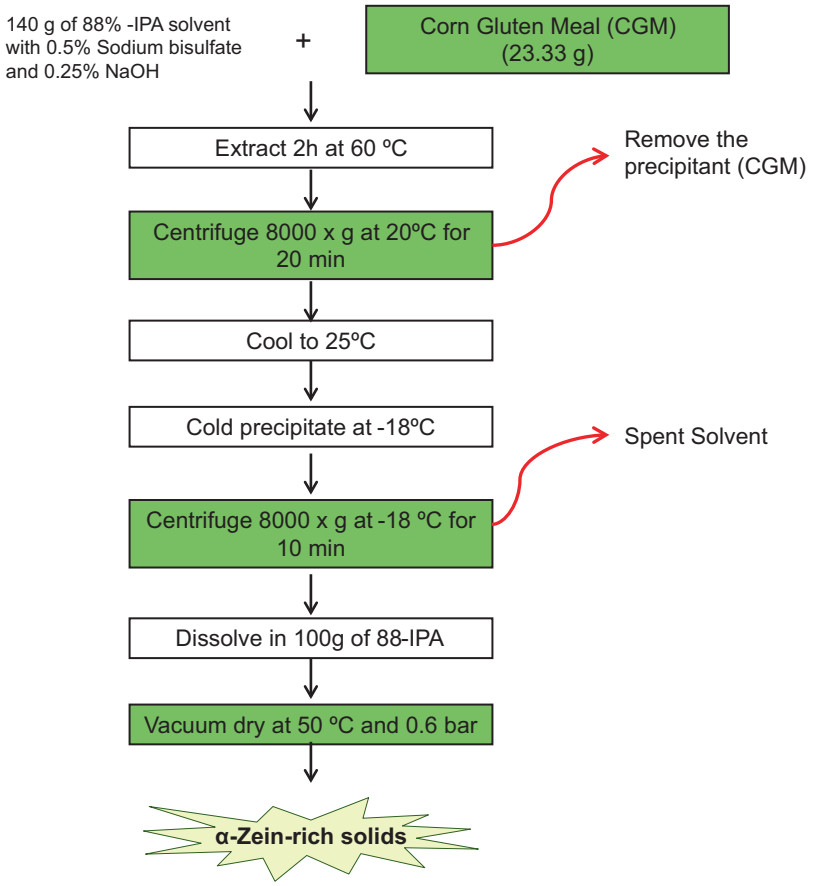


Fig. 8.7 Flow diagram for Carter and Reck (1977) zein extraction procedure. (Adopted from Anderson and Lamsal (2011))

pure corn zein. Precipitation and centrifugation procedures are collectively used to isolate corn zein for industrial applications.

8.5.2 Wheat Gluten Isolation

Commercial wheat gluten is the by-product of the wheat milling industry. After centrifugal removal of most of the starch from wet-milled endosperm, the supernatant is a fine, protein-rich, slurry called **wheat gluten meal**, from which gluten is extracted. The water-insoluble wheat glutens aggregate and form low-density but larger-sized particles than starch. These properties of wheat gluten are utilized to isolate and purify it from the starch. Several factors influence the commercial production of wheat gluten, including the protein content in the raw material,

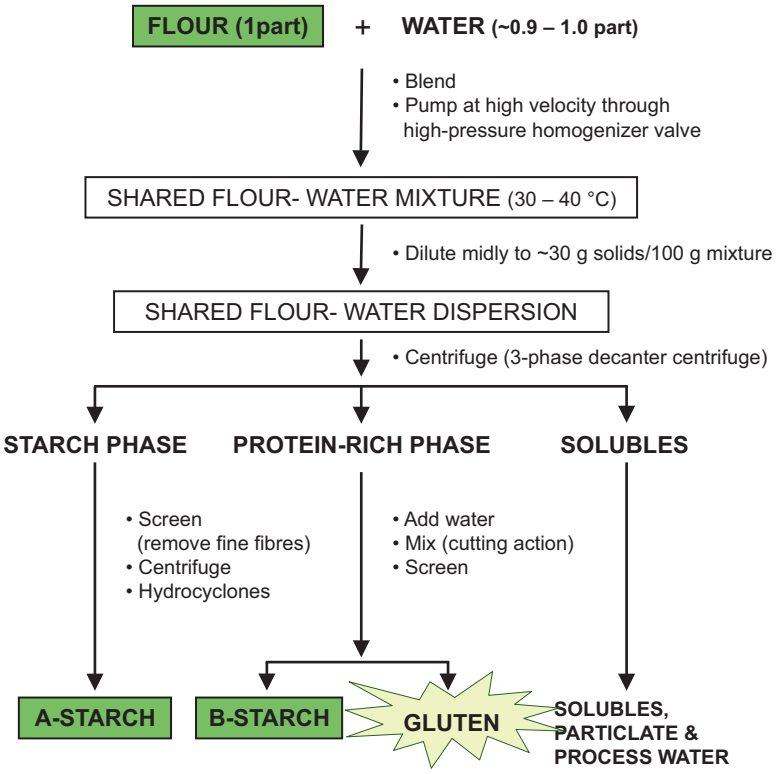


Fig. 8.8 Flow diagram for high-pressure disintegration process on a sheared flour-water dispersion. (Adopted from Sayaslan (2004))

starch-protein production ratio, handling of the processing effluent water, yield and purity of gluten and the cost of production. A number of processes have also been developed which use dry-milled flour as the raw material for these processes. The separation of starch and gluten particles is the initial step in gluten isolation. The extracted gluten granules are further water washed, aggregated, purified and flash dried to yield about 80% proteins.

Extraction processes are typically named after the company or the person who developed or patented the process. Industrially, four processes, including the Martin, Raisio/Alfa-Laval, hydrocyclone and high-pressure disintegration process, are the most popular to produce commercial wheat gluten (Sayaslan 2004). High-pressure disintegration (Fig. 8.8) is the most recent process for starch and gluten isolation from wheat flour. It was initially developed for potato starch extraction and later modified for corn and wheat. Comparatively, this process is the most efficient in terms of water consumption. It consumes 3–4 parts water per part flour compared to 4–5 parts in the HC and 5–7 parts in the modified Martin and Batter processes. This process separates the starch and gluten based on density. Low-gluten flour, such as soft wheat flour, can be used as raw material.

8.5.3 Soy Protein Isolation

Soy proteins are extracted from defatted soy flakes or meal derived from the soybean processing industry. Soy protein concentrates are extracted with 60–80% aqueous alcohol and soy protein isolates with aqueous mild alkali-extraction followed by isoelectric precipitation (Cho et al. 2007). In brief, the defatted soy flakes or meal are dispersed and moderately agitated in heated water at a ratio of between 1:10 and 1:20 and a pH of 7.5–9.0 for 45 min to 1 h. The mixture is centrifuged, and the pulp and extract are collected. The pulp, which contains mostly starch, is rewashed in water for maximum protein extraction. Extracts are filtered to remove any solids, acidified, centrifuged or filter separated. The precipitated curd is collected and washed with water, suspended in a minimum volume of water at neutralized pH and spray-dried in powder form for commercial use (Fig. 8.9).

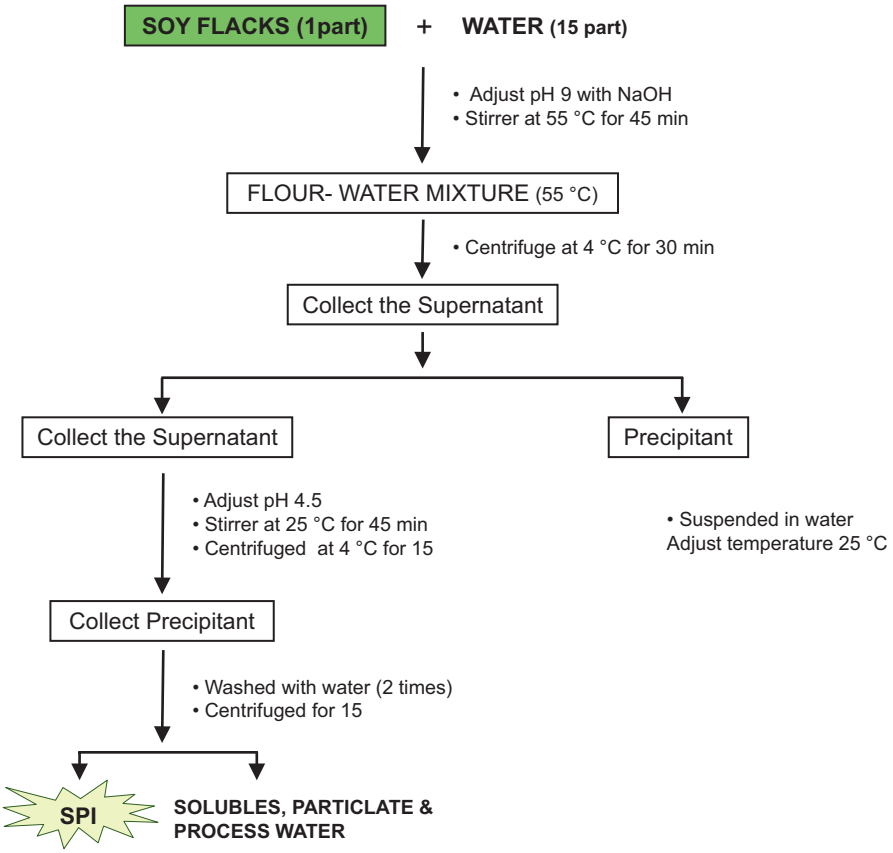


Fig. 8.9 Flow diagram of soy protein isolates extraction conventional procedure from defatted soy flakes meal

8.6 Closing Comments

Plant proteins are very diverse in nature in terms of their physiochemical and structural properties. The combination of different amino acids can generate a huge number of diverse secondary and tertiary structures. In addition, post-translational modifications increase the diversity of proteins. This variation lends them distinct functional characteristics that can be leveraged for the production of a wide range of bioproducts. Major crops could be a source of plant proteins. Zein, gluten and globulin are major proteins in corn, wheat and soybean, respectively.

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Chapter 9
Protein-Based Bioproducts

Muhammad Arif, Loo-Sar Chia, and K. Peter Pauls

Chapter Highlights

- Plant proteins can be used for the production of a variety of bioproducts, including films and coatings, adhesives, fibres and pharmaceuticals.
- Proteins derived from plant production systems have many advantages: they are safe, low-cost and rapidly deployable, allow for simple product storage and result in proteins that are properly folded, assembled and post-translationally modified.
- While plant-derived protein-based products are natural, renewable, biodegradable and environmentally friendly, they tend to be lower in strength and elasticity than their corresponding synthetic products.
- Current research in this area is focused on overcoming challenges in plant production platforms related to yield, purification, regulatory approval and customer acceptance.

9.1 Introduction

The production of protein-based textile fibres, foams for fire extinguishers and plastics started 60–70 years ago (Wormell 1954). Ulrich and Ursula Kölsch, in Essen Germany, assembled a collection of thousands of plastic articles, including items produced from bio-based plastics and composites. The collection includes items that date back to the 1840s, evidence that the manufacture of bio-based materials is not a new phenomenon. In 1855, Francois Charles Lepage patented, in France and England, an extruded plastic composite material manufactured from ebony or rosewood (*Dalbergia latifolia*) sawdust and diluted egg albumin (Lepage, UK patent

M. Arif · L.-S. Chia · K. P. Pauls (✉)
Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada
e-mail: ppauls@uoguelph.ca

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No. 2232). This material was compressed in steel moulds under pressure with steam heat to produce a composite material known as **bois durci** or '**hardened wood**'. Different products were made from bois durci, including portrait plaques, plaques for attaching to furniture and pianos, picture and mirror frames, inkstands, pen trays, blotters and letter racks, barometers, belt buckles and brooches, album and book covers, boxes, clocks, dishes, paper weights, statuettes, purses, caskets and other articles.

The first protein bioproduct patent was based on a mammalian source. It was granted to a German chemist, Adolf Spitteler, and his business partner, Ernst Wilhelm Krische, in 1899, for making plastic from milk casein (protein) and formaldehyde. The process for producing the casein semisynthetic plastic was accidently discovered when Spitteler's cat knocked over a small bottle of formaldehyde one night from the chemist's counter into the cat's milk on the floor. The next morning, the chemist found that the cat's milk had turned into a hard, celluloid-like substance. Spitteler experimented with casein and formaldehyde mixtures and found that casein could be transformed into water-insoluble plastic by letting it sit in formaldehyde for extended periods of time.

His businessman partner, Krische, was the owner of a small book binding and school supplies manufacturing company. He was trying to manufacture washable white writing boards for export to Turkey and was experimenting by coating cardboard with milk curdle, since casein was commonly used as a binding material. In fact, casein has had a long history of nonfood applications. The industrial use of casein goes back to at least 2 centuries BCE in Egypt, where casein was used as an adhesive material for colour pigments in paint manufacturing. Spitteler and Krische found each other and worked together on developing the milk protein plastic. They named it Galalith, a Greek word from gala (milk) and lithos (stone) or milk stone. The other trade names that were used for casein plastic were Aladdinite (in the USA) and Lactoid. In Britain, the trade name Erinoid, derived from the Gaelic word for Ireland, which was the source of most British cheese curds, was used for the milk protein plastics.

When lactic acid is added to skimmed milk, it separates into curds and whey. The curds, after being dried and powdered, can be formed into dough by soaking in water and extruded into rods. When these rods are treated with formaldehyde, they harden into a thermoset plastic. This is a lengthy process, sometimes taking months. One advantage of the casein thermoplastic material is that it is easy to colour.

Casein-based plastics were not utilized in the USA until 1919, and the material had some problems, including moisture absorption, shrinkage after drying, a lengthy and costly manufacturing process and difficulties in disposal of manufacturing waste. In 1929, P.C. Christensen added aluminium stearate to hornlike casein plastic and converted it to a soft plastic for the automotive industry. Worldwide casein production increased from 10,000 tons in 1930 to 30,000 in 1932. In 1937, William S. Murray patented a method for converting the milk sugar in skimmed milk to an aldehyde, thus eliminating the use of formaldehyde in the plastic hardening process

and reducing manufacturing waste (Murray and Utica 1937). The main products made from casein in this era were imitation pearl, tortoiseshell and ivory for buttons, belt buckles, knitting needles and jewellery. World War II resulted in a large reduction in the production and use of casein. Today, casein is still used to manufacture buttons and knitting needles.

Soybean was early plant source for protein-based plastics. This crop was domesticated in China between 1500 and 1027 BCE (Hymowitz and Singh 1987). With the development of sea and land routes, the cultivation of soybean spread to the rest of Asia but remained a minor crop between the first and eleventh century AD (Hymowitz 1990). Samuel Brown, an East India Company employee, introduced soybean from India-Pakistan to North America in 1765, where Henry Young planted it in Savannah, Georgia (Hymowitz and Harlan 1983). However, soybean crops were not developed in North America until World War I, when a shortage of vegetable oil made it an alternative source for this purpose (Ralston and Osswald 2008). Today, soybean is one of the most important sources of oil and protein in the modern world. The dry soybean seed contains approximately 40% protein by weight (Liu et al. 2007). The first patent on soybean protein plastic was granted in Europe (France and UK) in 1913 and in the USA in 1916 to a Japanese researcher named Sadakichi Satow. Unfortunately, soy protein plastics had similar drawbacks to casein, including shrinkage, porosity and moisture absorption after drying in formaldehyde.

Manufacturing products from agricultural production was a major interest of Henry Ford, who was the owner of the Ford Company, and the inventor Thomas Edison also became involved in the development of these products. Ford prepared moulded plastics from soybean meal and hardened them with formaldehyde. By 1936, one million Ford vehicles were on the road containing 15 pounds of soymeal plastic parts. However, the auto parts made from this extruded soymeal were moisture-sensitive. Prior to World War II, some progress was made to produce slightly hydrophobic soy-based materials, but the war impaired the opportunity. In any case, these developments in the production of bio-based materials in the 1800s and early 1900s played significant roles in shaping the modern materials industry.

The idea of utilizing renewable, biodegradable and/or edible materials to manufacture industrial goods received significant attention in the 1980s, when the cost of fossil fuel-derived raw materials rose dramatically and people became newly interested in preserving the global environment. New interest evolved in the scientific community to use bio-based technologies in the context of the knowledge and resources available today. Customer willingness, health and environmental concerns and the efficient utilization of agricultural production have been the key driving factors for the re-emergence of protein-based products. Plant proteins from soybean, bean, wheat and corn are now being widely tested for their utility in producing bioproducts. In addition, plant systems, especially tobacco, are being used as platforms for producing proteins from a wide range of species for novel applications, including pharmaceuticals.

9.2 Protein-Based Products

In addition to their importance in human nutrition, proteins are increasingly utilized to produce bioproducts of various sorts, including fibres, films and coatings, adhesives and glues and pharmaceuticals. These protein-based products have a large number of applications in food packaging, pharmaceutical encapsulation, agricultural mulching, novel textile production, medical suturing, protective coating, bonding materials and medicine. In the following sections, the major protein-based bioproducts will be discussed. The major focus, however, will be on the use of proteins from plants.

9.2.1 Films and Coatings

The production of films and coatings is the most studied use of proteins for bioproduct manufacturing. A **protein film** is an independently produced sheet or membrane formed from a protein isolate and a plasticizer by solvent casting or extrusion methods, which have known physicochemical properties that are suitable for a particular use. **Coatings** are films formed from proteins directly on the surfaces of objects and provide some separation from the environment. In some cases, these coatings may be edible, especially if they are deposited on food products. For example, edible coatings are applied commercially to citrus fruit, apples and pears to improve gloss and control weight loss.

Protein-based films and coatings are generally manufactured from native proteins dissolved in different solvents, depending on their solubilities (Table 9.1). For example, corn zein, wheat gluten and sorghum (*Sorghum bicolor*) kafirin are soluble only in aqueous ethanol. Soy, peanut, common bean, cottonseed and rice bran proteins are soluble in water and alkaline water. Like all other physical and chemical properties, the solubilities of native proteins also depend on their constituent amino acid residues.

Table 9.1 Native protein solubility in protein solvents

			Protein solvents	
Protein	Water	Acidic water	Alkaline water	Aqueous ethanol
Corn zein				X
Sorghum kafirin				X
Wheat gluten		X	X	X
Rice bran protein		X	X	
Soy protein	X		X	
Peanut protein			X	
Cottonseed protein			X	
Common bean protein			X	

Adapted from Krochta (2002)

Table 9.2 Plasticizers used in protein-based films

Plasticizer	Physical property	Solubility	Chemical formula
Stearic acid	Viscous solid	Alkaline water	$CH_3(CH_2)_{16}CO_2H$
Glycerol	Viscous liquid	Water	$C_3H_8O_3$
Sorbitol	Solid	Water	$C_6H_{11}O_6$
Polyethylene glycol	Viscous liquid	Water	$(CH_2\text{-}O\text{-}CH_2)_n$
Propylene glycol	Liquid	Water	$C_3H_8O_2$
Triethylene glycol	Liquid	Water	$C_6H_{14}O_6$
Sucrose	Solid	Water	$C_{12}H_{22}O_{11}$

Plant proteins, such as soybean protein and corn zein, need a small quantity of plasticizer to weaken the intra- and inter-peptide cross-linking and attractive forces and to reduce the brittleness and stiffness in the films and coatings. **Plasticizers** are low molecular weight and low volatility substances that work as spacers to reduce the strength of intermolecular attractive forces and lower the glass transition temperature of amorphous or partially crystalline protein films. Generally, plasticizers increase the molecular flexibility and extensibility but decrease elasticity, mechanical resistance and barrier properties of protein films and coatings (Gounga et al. 2007). Water is the most effective plasticizer in biopolymer materials, enabling them to undergo glass transition at a lower temperature as well as facilitating deformation and processability of the biopolymer matrix (Hernandez-Izquirdo and Krochta 2008). Besides water, common plasticizers for films include monosaccharides, oligosaccharides (sucrose), polyols (glycerol, sorbitol, propylene glycol and polyethylene glycol or polyethylene oxide), lipids (stearic acid) and their derivatives (Table 9.2) (Sothornvit and Krochta 2005). Plasticizer composition, size, shape and ability to attract water are important for the mechanical and barrier properties of protein films (Sothornvit and Krochta 2000).

Glycerol is the most widely used plasticizer in protein films (Cuq et al. 1997; Sothornvit and Krochta 2001; Cho and Rhee 2002). Its high plasticizing effects are attributed to the ease with which the glycerol molecule inserts and positions itself within the three-dimensional protein network (di Gioia and Guilbert 1999). The critical factors for a good protein plasticizer are that it has a low melting point, low volatility and compatibility (Pommet et al. 2005). In addition to these characteristics, the retention of the plasticizer by the film and amount needed should be taken into account when choosing a plasticizer (di Gioia and Guilbert 1999; Sothornvit and Krochta 2001). The relative effects on the mechanical and barrier properties of films can vary a great deal among different plasticizers, tested in different testing conditions (temperature and relative humidity).

9.2.1.1 Film Preparation Methods

The main ingredients of protein-based films and coatings are proteins, solvents, plasticizers and additives. Native proteins exist as folded structures that need to be unfolded for film and coating formation. Generally, higher temperatures,

high pH and water are used to unfold native protein structures. Other ingredients such as plasticizers and additives such as antioxidants, antimicrobials, nutraceuticals, flavours and colourants are also added to film formulations (Han 2003; Suppakul et al. 2003).

9.2.1.2 Physicochemical Properties of Protein-Based Films and Coatings

Protein films have great potential to be used for producing environmentally friendly food and drug packaging (Janjarasskul and Krochta 2010). Films and coatings made from renewable resources, such as plant proteins, could create new uses for agricultural products and byproducts that could protect, extend the shelf life and add value to food and drug products. To provide physical protection, the films require strength and elasticity, and to extend shelf life, they must act as barriers to water, oxygen, oil, aromas and microbes. If they are used as food coatings, they could add value by incorporating antioxidants, antimicrobial agents, nutrients, colours and flavours. In general, protein films have acceptable strength and elasticity and they are good barriers to oxygen, oil and aromas, but they are poor barriers to moisture at high humidity. However, at low to medium humidity, protein films are acceptable water vapour barriers. Generally, the mechanical and barrier properties are evaluated in a laboratory before industrial production.

Mechanical Properties of the Protein Films

The **mechanical properties** that are commonly measured for films include their strength, elasticity and plasticity. These measurements are made by clamping the film between the jaws of a **tensometer** and applying a strain at a fixed linear rate to the sample and measuring the stress. The resulting stress-strain curves can be used to calculate the **tensile strength** (TS), **elastic modulus** (EM), **yield point** and **break point** of the film (Fig. 9.1). Tensile strength and EM are usually expressed in pascals (Pa); one pascal is equal to one newton (1 N) of force applied over one metre squared (1 m^2). **Strain**, which is the geometrical measure of deformation, is expressed in percent elongation (E) and represents the relative displacement between particles in the material body (Jacob 2008). The EM measures the stiffness of the film (Banker 1966). It is calculated by drawing a tangent to the initial linear portion of the stress-strain curve (Fig. 9.1), selecting any point on the tangent curve and dividing the tensile stress by the corresponding strain. Yield strength is the amount of stress at which the film starts to plastically deform. Prior to the yield point, the film deforms elastically and will return to its original shape and size when the stress is released (Dieter et al. 2003). Tensile strength at break point is the amount of force per unit of the original cross-sectional area to pull the film to point where it breaks (Banker 1966). The distance between the yield point and the tensile strength point along the stress-strain curve indicates the degree of plasticity of the film.

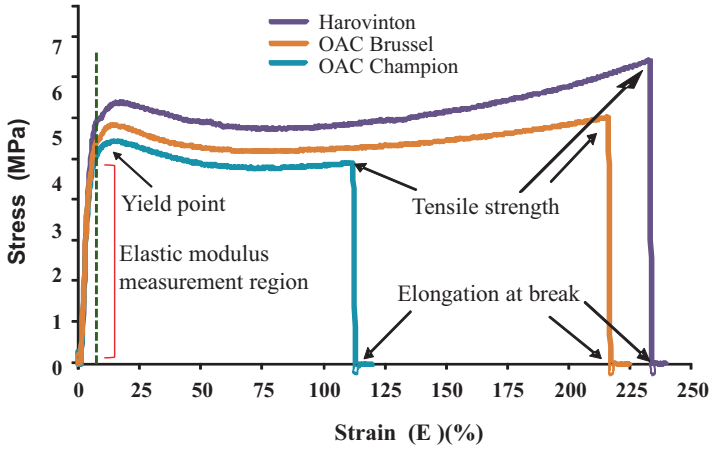

Fig. 9.1 Stress-strain curve of six soybean variety (Ontario, Canada) protein film show tensile strength, elastic modulus and elongation at break point (ASTM 2012)

Table 9.3 Protein-based films plasticized with glycerol

Protein film[a] (protein: plasticizer)	TS (MPa)	E (%)	Reference
Corn zein (2:0.6)	7	2.6	Parris and Coffin (1997)
Wheat gluten (2.7:1)	4.4	142	Park et al. (1994)
Wheat gluten (3:1.1)	1.9–4.4	170–208	Gennadios et al. (1993)
Soy protein isolate (2:1.2)	3.1–5.2	66–86	Brandenburg et al. (1993)
Peanut protein (1:0.67)	4.35	105	Jangchud and Chinnan (1999)
LDPE[b]	8.6–17	500	Salame (1986)
HDPE[c]	17–35	300	Smith (1986)
Pp[d]	38	400	Loo and Sudesh (2007)

E, elongation at break point; TS, tensile strength
[a]Test condition: temperature ~25 °C, relative humidity ~50%
[b]Low-density polyethylene; [c]high-density polyethylene. [d]Polypropylene

To produce films, proteins are initially denatured (Gennadios et al. 1994), which exposes their functional groups and allows them to interact with each other to form three-dimensional intermolecular networks when the temperature returns to ambient (Wang and Damodaran 1991; Subirade et al. 1998). The tensile properties of films are affected by protein composition (Table 9.3), protein concentration, amount of plasticizer, pH, ionic strength and heating temperature (Sze et al. 2007). Attractive forces between proteins in the protein film matrix, including hydrogen bonds between backbone amino and carbonyl groups to stabilize α-helix and β-sheet secondary structures within the proteins and to form links between protein molecules, or with the plasticizer (Choi et al. 2003), **van der Waals forces** or electrostatic forces among polymer chains (Takashi et al. 2007), ionic interactions or salt linkages or bridges between oppositely charged functional groups of amino acids in protein side chains and disulphide (S – S) bonds within and between different protein chains (Subirade

Table 9.4 Soy protein secondary structure

Protein secondary structures	7S subunit (%)	11S subunit (%)
α-helices	~ 12	~ 10
β-sheets	~ 37	~ 39
Random coils	~ 22	~ 20
Unordered	~ 28	~ 31

Adapted from Sze et al. (2007)

et al. 1998; Sang et al. 2000), allow the development of a film matrix from denatured protein. During the film drying period, water is progressively eliminated, and protein conformations change, including the degree of protein unfolding, which determines the types and numbers of bonds that establish interactions between proteins (Denavi et al. 2009; Mauri and Anon 2006). The cohesion of the film network is a function of all these interactive forces, which determine the properties of the film.

Proteins with different physical properties result in films with different properties (Table 9.4; Wang and Damodaran 1991). For example, higher β-sheet content in the film matrix increases the tensile properties of protein films, and strong protein cross-linking increases film stiffness and strength but decreases the ability of the film to elongate.

Barrier Properties

Films and coatings can be used to protect the objects they surround from various organic and inorganic materials including moisture, oil and microbes. Sometimes the barrier properties of the protein films and coatings have to meet certain standards in order to be used for particular applications, such as packaging foods or drugs. Generally, protein films are permeable to polar substances, such as water, but less permeable to nonpolar substances such as oxygen, oil, aroma and microorganisms compared to low-density polyethylene films (Lim et al. 1999; Krochta 2002; Table 9.5). The high permeability to polar substances reflects the fact that its two major ingredients, namely, proteins and plasticizers, are generally polar in nature. Nevertheless, protein films manufactured from different proteins have different moisture and oxygen permeabilities (Table 9.5).

Similarly, plasticizer type and concentration also affect film properties. High levels of plasticizer weaken the attractive forces in film networks and dramatically reduce film stiffness but elevate elongation properties (Tables 9.6 and 9.7). Different types and amounts of plasticizers interact differently even within a single polypeptide chain. For example, studies of soy protein films plasticized with glycerol revealed that there were two glass transition temperatures, indicating that the films contained two microdomains that interacted differently with glycerol (Chen and Zhang 2005). The presence of these domains suggests that protein and glycerol are not uniformly compatible across the polymer chains, but there is a preferential linking between protein polymer regions and glycerol molecules. Usually, higher

Table 9.5 Water vapour permeability and oxygen permeability of selected protein films plasticized with glycerol

Protein film (Protein: plasticizer)	WVP[a] (g.mm/m^2.d.kPa)	OP[b] (cc^3.µm/m^2.d.kPa)	Reference
Corn zein (4.9:1)	7.69–11.49 (21 °C, 85% RH)[c]	13.0–44.9 (30 °C, 0%RH)	Park and Chinnan (1995)
Corn zein (2.3:1)	32.52 (25 °C, 100% RH)	–	Parris and Coffin(1997)
SPI(1.7:1)	154 (25 °C, 50/100% RH)	4.75 (25 °C, 0% RH)	Brandenburg et al. (1993)
Wheat gluten (2.5:1)	–	3.82 (23 °C, 0% RH)	Gennadios et al. (1993)
Wheat gluten (2.5:1)	108.4 (26 °C, 50%/100% RH)	6.7 (38 °C, 0% RH)	Aydt et al. (1991)
Peanut protein (1:0.67)	9.03 (37.8 °C, 50%RH)	0.46 (30 °C, 0% RH)	Jangchud and Chinnan (1999)
LDPE	–	1870	Salame (1986)
HDPE	0.02	427	Smith(1986)

[a]WVR, water vapour permeability; [b]OP, oxygen permeability
[c]Test condition: temperature ~25 °C, relative humidity ~50%

Table 9.6 Selected protein films as affected by plasticizer types and amounts

Protein film (protein: plasticizer)	WVP (g.mm/m^2.d.kPa)	OP (cc^3.µm/m^2.d.kPa)	TS (MPa)	E (%)	Reference
WG:EG (2:1)	–	–	2.7	393	Sánchez et al. (1998)
WG:DEG (2.7:1)	–	–	2.5	479	
WG:TEG (3.2:1)	–	–	3	423	
WG:G (3.8:1)	–	–	1.8	562	
WPI:G (5.7:1)	–	18.5	29.1	4.1	McHugh and Krochta (1994)
WPI:G (2.3:1)	–	76.1	13.9	30.8	
WPI:S (2.3:1)	–	4.3	14	1.6	
WPI:S (1:1)	–	8.3	14.7	8.7	
PPI:G(1:0.67)(g/g)	9.03	0.46	4.4	105	Jangchud and Chinnan (1999)
PPI:G (1:1.67)(g/g)	8.97	1.20	4.1	164	
PPI:G(1:1.71) (g/g)	10.64	0.11	5.1	125	
Polypropylene			38	400	Loo and Sudesh (2007)
LDPE	0.02	1870	10	620	

DEG, Diethylene glycol; E, elongation at break point; EG, ethylene glycol; G, glycerol; OP, oxygen permeability; PEG, polyethylene glycol; PPI, peanut protein isolates; S, sorbitol; TEG, tetra ethylene glycol; TS, tensile strength; WG, wheat gliadin; WVR, water vapour permeability
Test condition: temperature ~25 °C, relative humidity ~50%

quantities of plasticizers reduce mechanical and barrier properties (Cuq et al. 1997). Extensive research efforts have been focused on modifying the properties of protein-based films to improve their mechanical and barrier properties for industrial applications (Rhim 2004; Rhim and Weller 2000; Rhim et al. 1999, 1998, 2000; Micard et al. 2000; Gennadios et al. 1993, 1998; Ghorpade et al. 1995; Park et al. 1993).

Table 9.7 Effect of plasticizer types and quantity on mechanical and water barrier properties of egg white protein films

Plasticizer	WVP(g.mm/m^2.d.kPa)	TS (MPa)	E (%)
30% G	210.48	4.12	12,4
40% G	246.48	2.23	18.7
50% G	256.32	1.26	32.2
50% PEG	149.28	3.84	59.7
60% PEG	149.04	3.37	88.1
50% S	117.6	3.71	15.0
60% S	136.56	2.22	18.6

E, elongation at break point; G, glycerol; PEG, polyethylene glycol; S, sorbitol; TS, tensile strength; WVR, water vapour permeability
Test condition: temperature ~25 °C, relative humidity ~50%
Adapted from Gennadios et al. (1996)

Film age also affects its properties. Over a period of time, protein films can change chemically and/or physically. Chemical changes such as oxidation degrade the protein chains, while glycerol plasticizer has the tendency to migrate to the film surface (Anker et al. 2001) with the passage of time and water in the film also evaporates. These changes reduce the intermolecular spaces between proteins, which facilitate attractive forces to increase cross-linking and make the film harder and also more brittle (Kim et al. 2002).

For commercial applications, it is desirable that protein films meet industry standards set for petroleum-based plastics, particularly polypropylene and low-density polyethylene films. However, generally, protein-based films have lower mechanical and water barrier properties than synthetic plastics. For example, protein-based films have tensile strengths of 2–24 MPa, elongation at break points of 3–210% and water vapour permeabilities of 6–300 g.mm/m^2.d.kPa, compared to tensile strengths of 8–38 MPa, elongation at break points of 300–500% and water vapour permeabilities of 0–0.02 g.mm/m^2.d.kPa measured for polypropylene and low-density polyethylene (Tables 9.6, 9.8, and 9.9). Protein films, however, have better oxygen barrier properties than synthetic films. For example, protein-based films have oxygen permeabilities of 2–45 cc^3.μm/m^2.d.kPa, compared to 427–1870 cc^3.μm/m^2.d.kPa for polyethylene (Tables 9.5 and 9.6).

Although the mechanical properties of protein films are sufficient for a number of industrial applications including food wraps, pouches, medical capsules and bandages (Krochta 2002), research efforts have been focused on modifying protein properties to enable the manufacture of films that have properties that are closer to standard industry mechanical and barrier properties (Rhim 2004; Rhim and Weller 2000; Rhim et al. 1998, 1999, 2000; Micard et al. 2000; Gennadios et al. 1993, 1998; Ghorpade et al. 1995; Park et al. 1993). In order to improve emulsification, gelation, water-holding capacity, foaming and solubility properties of films produced from proteins, various treatments have been used, including acylation, alkylation, phosphorylation, enzymatic modifications and conjugation with polysaccharides (starch) and lipids (Achouri et al. 2005). In addition, various film

Table 9.8 Physical properties of protein-based fibres

Fibre	Denier[a]	Breaking tenacity (MPa)	Elongation at break (%)	Tensile modulus (GPa)	Moisture regain (%)	Reference
Soy protein	–	37–104	0.4–5.9	–	–	Reddy and Yang (2007)
Zein	–	36–60	1.8–5.0	–	–	Yang et al. (1996)
Gluten	34	115	23	5	18	Reddy and Yang (2007)
Wool	8–15	174–260	30–40	4.3–6.5	16	Huang et al. (1995)

[a]A den is a unit of measure for the linear mass density of fibres. Mass in gram per 9000 metres.

Table 9.9 Properties of soy protein fibres affected by plasticizers (salts were used as plasticizers), post-spinning chemical reagent treatments

Treatment	Fibre process	Tenacity (g/tex)	Elongation at break %	Flexibility (mm)	Moisture uptake %
Common plasticizers					
0% glycerol	Extruded	1.49	0.5	45	1.59
15% glycerol	Extruded	1.57	1.6	21	1.61
15% sorbitol	Extruded	0.38	0.7	45	1.20
7.5% glycerol, 7.5% sorbitol	Extruded	1.23	1.3	21	1.24
Salts used as plasticizers					
Control (15% glycerol)	Extruded	1.57	1.6	21	1.61
$ZnCl_2$ 4%	Extruded	1.12	2.1	5	1.37
$CaCl_2$ 4%	Extruded	0.81	1.3	11	1.36
$ZnCl_2$, $CaCl_2$ 2% each	Extruded	0.74	1.2	11	1.20
Na_2HPO_4 4%	Extruded	0.75	1.8	11	1.53
$NaCl_2$ 10%	Wet-spun	0.68	0.5	45	1.06
$ZnCl_2$ 10%	Wet-spun	0.26	0.7	16	1.47
$CaCl_2$ 10%	Wet-spun	1.06	0.6	45	2.58
$ZnCl_2$-$CaCl_2$-$NaCl_3$. 3% each	Wet-spun	1.84	0.5	45	1.61
Post-spinning chemical reagents treatments					
Acetaldehyde 25%	Extruded	2.19	0.9	11	0.76
Acetic anhydride/ Acetic acid (9:1)	Extruded	2.31	4.7	2.0	0.77

Adapted from Huang et al. (1995)

additives and production modifications have been tested to improve the mechanical and barrier properties of the protein films. These include (1) various plasticizer types and protein/plasticizer concentrations and ratios; (2) various additives such as cysteine, propylene glycol alginate, methylcellulose, bee wax, gossypol, fatty acids, mineral oil and different casting solvents; (3) adjustments in pH and drying conditions; and (4) post-casting film treatments with mild acid and alkali or exposure to UV radiation (Krochta 2002).

9.2.1.3 Protein Film and Coating Applications

Protein films have a wide range of potential applications, including incorporation into food covers, wraps, separation layers, casings, pouches, bags, capsules, microcapsules, labels, trash bags, water-soluble fertilizer and pesticide bags and agricultural mulches. They can be used as coatings for drugs, paper and paper products (such as disposable plates) and disposable laboratory items (such as gloves, gowns and disposable diapers) (Krochta 2002). Soy proteins are very useful for papermaking and paper coatings. Their film-forming characteristics improve the strength and heat resistance of paper, allowing it to be used at higher production speeds in print applications. Their amphoteric nature (possessing both positive and negative charges) and high water-holding capabilities also improve ink receptivity and printability (Brentin 2014).

For some applications, edibility and biodegradability are two important properties. For example, most of the food and drug coatings manufactured from biomaterials are edible, and this property is determined by its formulation, method of manufacture and modification treatments that were used (Krochta 2002). The biodegradability of films by microorganisms in composting environments at the end of their life cycle, by naturally occurring microorganisms into water, carbon dioxide, methane, biomass and mineral residues, is an important attribute of many protein-based bioproducts, as it helps to reduce environmental pollution due to packaging. ASTM International (American Section of the International Association for Testing Materials) standard methods for aerobic compositing (D6400–12) and aerobic biodegradation (D5338) have been developed to measure the biodegradability of materials. In order for a plastic product to be labelled compostable, it must meet the US Standard ASTM D6400 and/or the European Norm EN 13432. Both specifications require that materials be completely biodegraded during composting at a rate similar to other known compostable materials (90% of organic carbon to CO_2 within 180 days) and should not leave visual or toxic residue.

Opportunities for utilizing protein films are increasing because of the application of new technologies to improve their mechanical and barrier properties while retaining their biodegradability and edibility. In addition, the food, pharmaceutical and biofuel industries are producing large amounts of protein meals, concentrates and isolates as byproducts, which are available for industrial-scale protein-based product manufacturing. Therefore, this bioindustry is moving from a pilot project stage to a commercial stage. For example, industrial-scale corn zein protein-coated

confectionaries, nuts and drug tablets are already on the market (http://www.zein-products.com/zeinapplications.html), and these films provide a relatively effective water vapour barrier compared to other edible films. Other potential uses of plant proteins include coatings for fresh vegetables, fruits and fried foods (Trezza and Krochta 2002; Shukla and Cheryan 2001). The use of protein coatings decreases water loss, reduces pigment degradation, prevents undesirable pigment development, delays ripening, improves gloss, intensifies peel colouration and reduces oxygen, aroma and oil transfer.

In addition, an interesting property of edible coatings is their ability to incorporate active ingredients that can enhance their functionality, including antimicrobials (such as organic acids, fatty acid esters, polypeptides and plant essential oils, nitrites and sulphites), texture enhancers (such as calcium salts) and nutraceuticals (such as certain fatty acids and vitamins). All of these effects can improve quality, shelf life and safety and reduce postharvest losses and additional packaging costs. Some of the problems and limitations that are associated with these coatings include anaerobic respiration in fruits and vegetables when the coating is too thick and restricts exchange of CO_2 and O_2, undesirable tastes associated with particular compounds like essential oils and food safety issues such as allergic reactions (Dhall 2013).

9.2.2 Protein Adhesives

Adhesives are nonmetallic liquids or gels that bind the surfaces of materials together and resist separation (https://www.britannica.com/technology/adhesive). Adhesives (or glues) hold objects together through adhesive forces between adhesive materials and the surfaces of materials (called **adherents**) and cohesive forces within the glue (Fig. 9.2). The physical and chemical properties of the adhesive, the type of adherents and the nature of the surface pretreatments are important factors in glue performance, in the short and long terms. To make initial molecular contact, adhesives have the ability to wet and spread evenly on the surfaces of the materials one wishes to joint. Once that is achieved, intrinsic attractive forces are generated across interfaces through a number of mechanisms including **adsorption** (occurs when adhesive molecules are attracted to a specific site on a solid surface through weak van der Waals forces or chemisorption through covalent bonding), **mechanical interlocking** (occurs when the adhesive flows into pores or solidifies around the projections), **interdiffusion** (occurs when the adhesive dissolves and diffuses into the substrate material) and **electrostatic attractions** (occur when electrons are transferred across the interface, thus creating positive and negative charges that attract one another). Generally, several mechanisms contribute to the performance of adhesives with various types of adherents.

In the formation of an adhesive joint, a transitional region arises in the interface between the joint surface and adhesive. In this transitional region, the chemical and physical properties of the adhesive may be considerably different from those in the noncontact portions or cohesive region (Fig. 9.2). It is generally believed that the

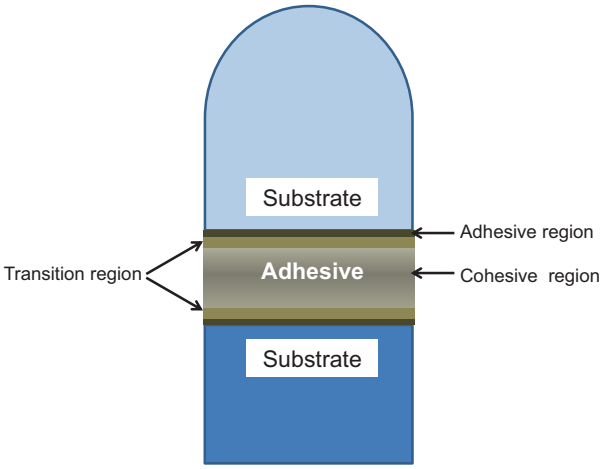

Fig. 9.2 Schematic presentation of adhesives and substrate interaction

interphase composition controls the durability and strength of an adhesive joint and is primarily responsible for the transference of stress from one surface to the other. This transitional region is frequently the site of environmental attack, leading to joint failure (Encyclopaedia Britannica).

Adhesives are used in every sphere of human life, including aerospace, automotive, electronics, construction, furniture construction, carpet manufacturing, musical instrument building, packaging, plywood manufacturing and agriculture. In 1998, the share of natural adhesives was 0.03% of the total North American wood industry (Seller 2001). In Canada, about 80% of adhesives and sealants are used in industrial applications including packaging, automotive, construction and the furniture industry and the remainder by individual consumers for home maintenance and renovation.

Adhesives are formulated by mixing base materials such as proteins, starch and lignin with fillers, pigments, stabilizers, plasticizers and other additives to yield products with desirable characteristics. Adhesives may be synthetic or natural, depending on their base materials. Many synthetic adhesives are formaldehyde-based resins derived from petrochemicals including phenol formaldehyde and urea formaldehyde resins. Although synthetic adhesives have high-performing characteristics, including excellent bond strength, environmental resistance and durability, their base chemical, formaldehyde, is a human carcinogen (International Agency for Research on Cancer, https://www.iarc.fr), and they are derived from nonrenewable resources. Alternatively, natural adhesives are thus being developed to replace these formaldehyde-based adhesives currently on the market because of these concerns.

Natural adhesives and sealants are derived from natural biopolymers obtained from plants, animals and microbes (Lambuth 2003; Imam et al. 2013). Proteins have been used to formulate commercial adhesives, and sealants, for many years, but initially, animal proteins were used for glues. Protein adhesives are used in antique

furniture and old religious texts. These adhesives have excellent flexibility and non-warp characteristics, as well as permanent and tenacious adhesion. They are water-soluble, easy to clean up, nontoxic, eco-friendly, biodegradable, recyclable and repulpable. Protein adhesives have some limitations, however, such as a lack of specific adhesion on coatings and nonporous surfaces and sensitivity to temperature and humidity changes. The use of plant proteins as adhesives is more recent with soy protein-based adhesives widely used between 1930 and 1960. However, they were completely replaced thereafter by cheaper and stronger synthetic adhesives.

Today, soy protein alone, or in combination with animal proteins such as casein, gelatin and blood proteins, is used to produce adhesives that are widely used as glues in paper, book binding, packaging, furniture and wood industries (Frihart 2009; Lambuth 2003). Generally, protein adhesives have sufficient strength in dry conditions but are susceptible to moisture and mould (Lambuth 2003). The nature of the protein determines the formulation, mixing and application of the adhesive. The manufacturing steps, however, are common and include grinding dry protein extracts to glue particle sizes (typically with surface areas between 3000 and 6000 cm^2 per gram), sufficiently dispersing the ground proteins in alkaline water for maximum binding efficiency and addition of fungicide (such as sodium ortho-phenylphenate, sodium pentachlorophenate, copper-8-quinolinate or copper naphthenate) to prevent mould.

Adhesive durability has been a problem for protein glues. Several protein modifications including physical, chemical and enzymatic treatment are used to enhance the functional properties of protein adhesives such as bonding strengths and environmental and moisture resistance. Treatment of proteins with organic or inorganic alkali, such as sodium hydroxide or trisodium phosphate, breaks the internal hydrogen bonds of protein molecules, unfolds the protein structure and exposes the polar functional groups of the amino acid residues for adhesion to binding surfaces such as wood (Brother et al. 1940; Lambuth 2003). The combination of alkali treatment and mild heating of the protein in deionized water breaks the inter- and intramolecular protein hydrogen and disulphide bonds and unfolds the protein structure to improve the adhesive and viscosity and hydrophobic properties of the glues (Graham and Krinski 1983). For example, soy protein heated at 50 °C and a pH of 10.0 improved adhesive strength by 118% and the hydrophobic properties of soy protein glues by 92% (Hettiarachchy et al. 1995). The amount of alkali in the protein adhesives depends on the usage in the final product. For example, high-alkali (Table 9.10) soy protein adhesives prevent glue swelling, maintain glue viscosity and improve moisture resistance by forming insoluble proteinates (Laucks and Davidson 1928). However, it also burns wood cellulose and causes reddish-brown stains on wood surfaces (Truax 1929). On the other hand, low-alkali soy protein adhesives are less dispersive and have lower bonding strengths. This makes them good for paper and softboard lamination, but not for structural usage such as sheathing plywood (Sheeran 1957; Lambuth 2003). Furthermore, salt treatment of disulphide bond-containing proteins, such as soy protein, results in cleavage of the disulphide bonds and unfolded protein structures, which improves the viscosity of glues without

Table 9.10 Soybean protein adhesives: ingredients and mixing procedure

Ingredients and mixing procedure	High-alkali amount (kg)	Low-alkali amount (kg)
Water at 16–21 °C	87.5	112.5
Adhesive-grade soybean flour[a]	48.5[b]	48.5[b]
Pine oil or diesel oil defoamer: Mix 3 min or until smooth	1.5[b]	1.5[b]
Water at 16–20 °C: Mix 2 min or until smooth	72.5	75.0
Fresh hydrated lime: (as a slurry in)	6.0	15.0
Water at 16–21 °C: Mix 2 min or until smooth	12.0	25.0
50% sodium hydroxide solution: Mix 1 min	7.0	–
Sodium silicate solution: Mix 1 min	12.5[c]	–
Orthophenyl phenol or other preservative: Mix 10 min	2.5	2.5

Adapted from Lambuth (2003)
[a]44% protein, specific surface 3000–6000 cm^2/g
[b]Normally dry-blended for easier handling and dust control
[c]8.90% Na_2O, 28.70% SiO_2, 41 ° Baume´

reducing adhesive strengths and water resistance at certain concentrations. High concentrations of salts, however, reduce viscosity, adhesive strengths and water resistance (Kalapathy et al. 1996).

The addition of chemicals such as urea, guanidine hydrochloride, sodium dodecyl sulphate, maleic anhydride, polyethylenimine and polyamidoamine-epichlorohydrin, which react with the carboxylic acid and amino groups in proteins, results in cross-linking of protein molecules and the formation of three-dimensional networks. These networks improve the adhesive and moisture resistance properties of protein-based glues. For example, soy protein modified with urea and guanidine hydrochloride increases the average shear strengths in walnut (*Juglans* spp.), cherry (*Prunus* spp.) and pine plywoods by 34 and 37%, respectively. In addition, both urea and guanidine hydrochloride-modified soy protein exhibited 100% moisture resistance. These chemicals are known to increase the production of secondary structures in globular proteins, which may be responsible for enhancing adhesive strength and also expose hydrophobic amino acids, which might enhance water resistance (Huang and Sun 2000). Furthermore, the addition of polyamidoamine-epichlorohydrin to maleic anhydride-grafted soy protein isolates improves the adhesive properties of glues to such an extent that they exceed those of commercial phenol formaldehyde glues (Liu and Li 2007).

Enzymatic hydrolysis of soy protein with proteases such as trypsin is another method for improving its adhesive properties. Glue strength increases of 58–119% have been observed with these treatments (Kalapathy et al. 1995; Hettiarachchy et al. 1995). Other soy protein modifying enzymes, including urease, pepsin and transglutaminase, also improve the adhesive and water resistance properties of soy protein glues (Thames et al. 2010; Imam et al. 2013).

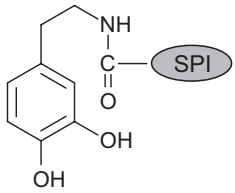

Fig. 9.3 Dopamine-grafted soy protein. (Adapted from Liu and Li (2002))

Marine mussel adhesive proteins allow the adhesion of objects in seawater (Waite 1987) and contain a substantial quantity of 3,4-dihydroxyphenylalanine (DOPA). DOPA incorporated into synthetic polypeptides mimics marine adhesives and plays an important role in moisture-resistant adhesion (Liu and Li 2002; Yu and Deming 1998). Above and beyond its effectiveness on wet surfaces, this adhesive protein has several other advantages, such as strong adhesive strengths and resistance to biological degradation. However, marine adhesive proteins are difficult to produce at reasonable costs. For example, DOPA content in soy proteins can be increased through genetic engineering, and dopamine-grafted (Fig. 9.3) soy protein showed significant increases in adhesive strengths and water resistance in wood glues (Liu and Li 2002).

Blending together different proteins is another way to enhance the functional properties of glues. For example, soybean proteins have good adhesive properties but weak water resistance, while casein proteins have good water resistance but poor adhesion. A blend of these proteins results in an adhesive with better properties than those derived from the either single protein. However, for this approach to be successful, the proteins in the mixture must be compatible and have similar processing requirements to convert them into glues. Soy-blood and soy-casein blends have successfully been used in interior plywood and softwood manufacturing during the 1930s to 1960s and again during the oil embargo in 1973. Generally, a soy-casein mixture provides an excellent adhesive for softwood and millwork assembly (Lambuth 2003).

9.2.3 Protein Fibre

Fibres have extensive uses in textile and clothing manufacture; they are used for protection and have medicinal and aesthetic applications. A fibre is a continuous filament or discrete, elongated, piece of material. The word ‘fibre’ comes from the Latin word *fibra* or *fillum*, meaning thread. Fibres can have micro (10^{-6} m) or 10^{-9} nano- (10^{-9} m) diameters and almost limitless lengths (Castano et al. 2012).

Synthetic fibres are made from petroleum-derived plastics, such as polyester, nylon and rayon. Plants produce natural fibres such as cotton and bast fibres (e.g. derived from flax, hemp, jute [*Corchorus* spp.], ramie [*Boehmeria nivea*], kenaf [*Hibiscus cannabinus*] and abaca [*Musa textilis*]), and fibres can also be synthesized from natural sources such as **alginate** (a natural polymer that exists widely in many

species of brown seaweed.), cellulose (to produce lyocell fibres from cellulose), polylactic acid (from sugars extracted from crops like corn and sugar beet), polyhydroxyalkanoate (from bacterial sources) and protein. Natural fibres are generally biodegradable; they are mostly hydrophilic and made up of short, flexible chains with low levels of crystallization. They often have chain backbones with oxygen or nitrogen links and/or pendant groups containing oxygen or nitrogen atoms. Biodegradable fibres are suitable for all applications, including knitwear, intimate apparel, shirts, trousers, dress material, bath linen, floor coverings, bed linens, furnishing and industrial yarns. Biodegradable fibres impart colour brilliance to fabrics and garments, which remain bright and true even after repeated washes. The fibres often give fabrics a soft and bouncy feel.

Wool and silk are two natural protein fibres that have been used for centuries in textile manufacturing. These natural protein fibres are made from filamentous animal proteins. Generally, they have good physical properties, but they also have some limitations, such as variable fibre diameters, propensity to shrink and high cost. Fibres produced from plant globular proteins, especially seed storage proteins, are alternatives to wool and silk (Wormell 1954). Kajita and Inoue in Japan and Boyer in the USA first patented fibre development from soybean protein in 1940. Fibres from soybean protein and corn zein called **prolons** were investigated extensively in the 1930s and 1940s, but they were not commercialized because of their high cost and poor/inconsistent physical properties. The cheaper and excellent physical properties of synthetic fibres called **synthons**, manufactured from petroleum, at that time led to a rapid commercialization of synthetic textiles (Huang et al. 1995). Renewed interest in eco-friendly and renewable protein fibre materials has led to the commercialization of soy protein fibres and garments manufactured from these textiles by Chinese companies around the globe (Zhang et al. 2003). Plant protein fibres have excellent properties, including natural lustre and smooth surfaces, as well as good physical and dyeing properties. Garments manufactured from soybean fibre textiles have good breathability and comfort, and they also have a fine appearance with excellent drape (Fig. 9.4).

Fig. 9.4 Men's and ladies' soybean cotton spandex jersey shirts [52% cotton, 43% azlon (protein from soybean), 5% spandex] (http://www.nyfifth.com/ash-city-e-c-o-knits-88622-mens-soybean-cotton-spanddex-jersey-polo-p-34520.html, used with permission, searched on Feb. 27. 2018)

9.2.3.1 Properties of Protein Fibres

The morphological and mechanical properties of protein-based synthetic fibres are important determinants of their commercial utility. The morphological properties of importance include surface texture, fibre diameter, length and circularity. Scanning electron microscopy (SEM) is a useful tool to examine and measure the morphological properties of fibres, including surface properties, cross-sectional area and circularity. SEM and atomic force microscopy can also be used to measure some mechanical properties such as the strength of the electrospun nanofibre. Mechanical properties of fibres, including intra- and intermolecular alignments and crystallinity, can also be determined by X-ray diffraction and differential scanning calorimetry, respectively. Tensile tests determine the strength, elongation at break point and flexibility of the fibre (Table 9.8).

Several prior- and post-spin factors can affect the physical properties of protein-based fibres (Table 9.9). The prior-spinning factors include source, type and concentration of protein, additives and solvents, blends with other polymers, pH, temperature and viscosity of the protein solution and extrusion/spinning instrument set-up. Post-spinning factors include washing, drying, drawing, chemical treatments, thermal and conditioning treatments, as well as annealing and testing conditions. The variations among proteins from different sources described above necessitate that specific formulations, with different additives, blends and electrospinning conditions need to be investigated and optimized to produce fibres with specific properties.

9.2.3.2 Protein Fibre Applications

Plant protein fibres have been on the market for decades with different trade names, including Vicara, Zycon and Wavecrape for corn zein fibres (Lawton 2002), Prolon and Alysol for soy protein fibres and Ardil for peanut protein fibres. Today, **Azlon** is the common generic name for all fibres regenerated from plant proteins (http://info.fabrics.net/meet-the-azlons-from-a-to-z-regenerated-rejuvenated/). Azlon blended textile fabrics are commercially available (Fig. 9.4), and a considerable amount of research is currently being carried out to improve the technology and properties of the plant protein-based fibres in public and private sectors. Some proteins such as soybean protein, corn zein, wheat gluten and peanut protein have greater potential for use in producing fibres than others because they are readily available for industrial availability (Xu et al. 2012). Some of the ongoing limitations of protein fibres involve performance characteristics, such as moisture sensitivity and mechanical properties. However, like any field of material research, improvements in protein-based fibres are being explored. In particular, blends with other polymers, including polyethylene oxide, polylactic acid, polyvinyl alcohol, polycaprolactone, polyacrylonitrile, hydroxyapatite and polysaccharides, are the wave of the future and considered as the next generation of materials.

In addition to the general usage of protein-based fibres for textiles and clothing, they have other speciality uses in (1) medicine as medical sutures and for drug delivery, bandaging and enzyme mobilization; (2) cosmetics and skin care; (3) tissue engineering of blood vessels, bone tissues, heart tissues and cartilage tissues; (4) electronics including nano-sensors; and (5) military protective clothing and body armour (http://news.bbc.co.uk/2/hi/science/nature/379338.stm). Historically, silk and animal gut were widely used as surgical sutures because they are eventually degraded by human proteolytic enzymes, but recently they have been replaced by synthetic sutures (made of polyglycolic, polylactic acid, polydioxanone and caprolactone) because of concerns about possible contamination of gut sutures with prions. Plant protein fibres are an ideal alternative to synthetic sutures because they are renewable and absorbable.

The ideal wound dressing is one that is sterile, breathable and supports a moist healing environment. Such a dressing will reduce the risk of infection, help the wound heal more quickly and reduce scarring. Conventional dressings do not efficiently induce haemostasis (the mechanism that stops bleeding) or adhere in moist environments around wounds. With the advances in nanotechnology seen in the last two decades, it is now possible to design and produce **nanofibre-based wound dressings** that contain an electrospun nanofibrous layer applied to a basic support fabric material. These wound dressings have very high surface area to volume ratios. They are able to control the release of drugs such as antibiotics and analgesics co-spun with protein nanofibre; prevent haemostasis, high filtration and liquid absorption efficiencies; and stimulate the growth of live cells. Thus, the combination of nanotechnology with electrospinning and the development of new wound dressing materials from plant proteins with highly desirable properties may lead to bioproducts that can could enhance the healing of wounds significantly compared to the conventional fibrous dressing materials.

Nanofibres have also been used for drug delivery because their very small sizes and extraordinarily large surface areas make them highly efficient delivery and carrier systems. Some nanofibres can also control the release of active ingredients and protect the chemical integrity of drugs. For example, protein (gelatin)-polyvinyl alcohol nanofibres containing a model drug have been produced, and their encapsulation and delivery efficiencies have been demonstrated (Yang et al. 2007). The development of nanofibres into efficient drug delivery systems is attracting much attention, and in particular, the use of electrospun nanofibers manufactured from biodegradable polymers, such as proteins, for drug delivery systems is being actively studied. Variables that affect their efficiencies and drug release rates include the physical properties of the drug and the protein microfibre.

9.3 Plant Crops as Platforms for Speciality Protein Products

Proteins play crucial roles in living organisms, including humans, to enable a large number of fundamental processes, such as cell signalling, immune responses, cell adhesion, cell division and cell growth and differentiation. The continuous progress

in biotechnology, including genetic and protein engineering, in the last few decades has made it possible to manipulate different platforms for the commercial-scale production of proteins in transgenic bacteria, yeast, filamentous fungi, insects, mammalian and plant cell cultures and transgenic animals and plants. These biotechnological advances have significantly affected many industries, including food, pharmaceutical, nutraceutical, enzyme, hormone, textile, leather, paper, pulp, polymer, plastics and agriculture industries. For example, there are more than 200 approved peptide and protein pharmaceuticals in the US Food and Drug Administration list, including human insulin, serum albumin, human growth hormone, various antibodies, edible vaccines, collagen, human epidermal growth factor and blood coagulating protein (Factor VIII), among many others.

Of the different recombinant proteins that are produced on a commercial scale, 39% are made in *Escherichia coli*, 35% in Chinese hamster ovary (CHO) cells, 15% in yeasts, 10% by other mammalian systems and 1% by other bacteria and systems (Rader 2008). Microorganisms and cell cultures are robust recombinant protein synthesis production systems. They possess certain challenges, however, such as high culture development costs, high cell culture maintenance costs, cell culture variability and limitations concerning the production of large molecular weight proteins.

In principle, DNA from any source can be manipulated in any living system. Genetically engineered animals have been created that produce recombinant proteins in their tissues, milk, blood or urine (http://www.youtube.com/watch?v=q0WCjX8jUE4). By the late 1980s, it was shown that transgenic plants could be used as alternative, commercial-scale, recombinant protein production platforms, after immunoglobulins and the assembly of functional antibodies were successfully achieved at 1.3% of the total leaf protein in tobacco leaves (Hiatt et al. 1989). This opened many new windows of opportunity to use genetically engineered plants for the production of recombinant proteins in whole plants or in their tissues, seeds and cell culture. Some transgenic plants carrying human protein genes are given in Table 9.11. Further progress made in biotechnological fields in the 1990s and early 2000s prompted interest in the production of pharmaceuticals in plants, known colloquially as ‘pharming’ (Hunter 2011).

Plant crop protein production platforms have certain advantages over animal and microbial systems. Mammalian cell culture systems are complicated and expensive processes; they require large bioreactors and high energy inputs for commercial-scale production. In contrast, plant systems are cost-effective, quicker to scale up, easy to propagate and simple to distribute. In addition, there is no risk of contamination by human pathogens (such as viruses and prions), and relatively cheap systems exist for purification and concentration of the therapeutic proteins. Plant platforms can synthesize and accumulate valuable proteins to high levels. These proteins are properly assembled and folded and can be post-transcriptionally modified to yield complex protein molecules. In addition, if the plants are engineered to accumulate the proteins in storage tissues and cellular compartments, they may be stably stored without refrigeration.

Table 9.11 Plant platforms for the production of human and animal recombinant proteins

Product	Plant platform	Level	Application	Reference
Human protein				
Protein C	Tobacco	<0.01% TSP[a]	Anticoagulant (human)	Cramer et al. (1999)
	Canola	0.30% seed protein	Thrombin inhibitor	
Epidermal growth	Tobacco	<0.01% TSP	Wound repair and control of cell proliferations	
Interferon-α	Rice; turnip	–	Hepatitis C and B treatment	
Haemoglobin α,β	Tobacco	0.05% seed protein	Blood substitute	
Somatotropin	Tobacco	<0.01–7.00% TSP	Growth hormone	Staub et al. (2000)
Erythropoietin	Tobacco	<0.01% TSP	Anaemia	Kusnadi et al. (1997)
Enkephalins	*Arabidopsis*	0.10% seed protein	Anti-hyper analgesic	
Interferon-β	Tobacco	0.01% FW[b]	Hepatitis C and B treatment	
Lactoferrin	Potato	0.10%tsp	Antimicrobial	Chong and Langridge (2000)
Homotrimeric collagen	Tobacco	<0.01% FW	Collagen	Ruggiero et al. (2000)
Non-human proteins				
α-Trichosanthin from TMV-U1 subgenomic coat protein	Tobacco	2.00% TSP	HIV therapies	Giddings et al. (2000)
Glucocerebrosidase	Tobacco	1.00–10.00% TSP	Gaucher disease	Cramer et al. (1999)

[a]Total soluble protein
[b]Fresh weight

Plants are capable of assembling two or more subunits of proteins into complex three-dimensional structures. For example, spider dragline silk genes were successfully expressed in tobacco (*Nicotiana* spp.) and potato (*Solanum tuberosum*) plants, and spider silk proteins accumulated in transgenic tobacco leaves and potato tubers up to at least 2% of total soluble proteins with >90% homology to *Nephila clavipes* native proteins (Scheller et al. 2001; Menassa et al. 2004). Even more dramatic was the production of spider silk protein in transgenic *Arabidopsis thaliana*, which accumulated to 18% of total soluble proteins (Yang et al. 2005).

Plant platforms, including major crops such as alfalfa (*Medicago sativa*), potato, wheat, rice, tobacco, soybean, carrot (*Daucus carota* subsp. *sativus*) and turnip (*Brassica rapa* subsp. *rapa*), have been extensively tested for their ability to produce human and animal antibodies and vaccines (Table 9.12). For example, the hepatitis B surface antigen has been produced in transgenic tobacco plants, and

Table 9.12 Plant platforms for the production of antibodies and vaccines

Product	Plant platform	Level	Application	Reference
Antibodies				
ZMapp	Tobacco	–	Ebola virus	Qiu et al.(2014)
FVIII	Tobacco	370 µg/g[a]	Haemophilia A	Sherman et al. (2014)
Influenza HA	Tobacco	400–1300 mg/kg leaves	Influenza (humans)	Shoji et al. (2011)
ScFvT84.66 (ScFv)	Wheat	900.0 ng/g leaves; 1.5 µg/g seed	Cancer treatment; carcinoembryonic antigen	Stoger et al. (2000)
	Rice	29.0 µg/g leaves 32.0 µg/g; seed 3.8 µg/g callus 27.0 µg/g leaves		Stoger et al. (2000); Torres et al. (1999)
T84.66 (lgG)	Tobacco	1.0 µg/g leaves	Diagnostic; antihuman lgG	Vaquero et al. (1999)
Guy's 13 (SlgA)	Tobacco	500 µg/g FW[a] leaves	Dental caries; streptococcal antigen I or II	Ma et al. (1998); (1995)
Anti-HSV-2 (lgG)	Soybean	–	Herpes simplex virus 2	Zeitlin et al. (1998)
Vaccine				
Heat-labile toxin B-subunit	Maize	–	Enterotoxigenic *E. coli* (humans)	Streatfield et al. (2000)
	Tobacco	<0.01% TSP	Enterotoxigenic *E. coli* (humans)	Haq et al. (1995)
	Potato	0.19% TSP	Enterotoxigenic *E. coli* (humans)	Haq et al. (1995); Mason et al. (1998); Tacket et al. (1998)
Cholera toxin B-subunit	Potato	0.30%tsp	*Vibrio cholerae* (human)	Puchta (2000); Arakawa et al. (1998)
Envelope surface protein	Potato	<0.01% FW	Hepatitis B virus (humans)	Richter et al. (2000)
	Lettuce; lupin	<0.01% FW	Hepatitis B virus (humans)	Kapusta et al. (1999)
Capsid protein	Tobacco	0.23% TSP	Norwalk virus (humans)	Mason et al. (1996)
Capsid protein	Potato	0.37% TSP	Norwalk virus (humans)	Mason et al. (1996); Tacket et al. (2000)
Rabies virus glycoprotein	Tomato	1.00% TSP	Rabies virus	McGarvey et al. (1995)

(continued)

Table 9.12 (continued)

Product	Plant platform	Level	Application	Reference
Glycoprotein S	*Arabidopsis*	0.06% TSP	Transmissible gastroenteritis corona virus (pigs)	Gómez et al. (1998)
	Tobacco	0.20% TSP	Transmissible gastroenteritis corona virus (pigs)	Tuboly et al. (2000)
	Maize	<0.01% FW	Transmissible gastroenteritis corona virus (pigs)	Streatfield et al. (2000)

[a]Fresh weight
[b]Total soluble protein

human insulin has been produced in transgenic *A. thaliana* seeds at levels of 0.13% of the total soluble seed protein (Nykiforuk et al. 2006). The quality of these antibodies and vaccines was equivalent to the present commercially produced proteins in microorganism-based systems. For example, a 44 kDa fragment of human collagen Iα1 (CIα1) expressed in corn grains was molecularly equivalent to that produced in recombinant yeast (*Pichia pastoris*).

Commercial enzyme production is another area where transgenic plants can play an important role. The global industrial enzyme market will be as high as $7 billion by 2015 (http://www.prweb.com/releases/industrial_enzymes/proteases_carbohydrases/prweb8121185.htm), according to Global Industry Analysts (Hood and Requesens 2012). These commercial enzymes, including proteases, amylases, cellulases, xylanases, lipases and reduction/oxidation enzymes are utilized by many industries, such as the manufactures of detergent, pulp and paper, textile, chemical, feed, food, biofuels and bio-based products (Hood and Requesens 2012). Currently, the largest markets for technical enzymes are the pulp and paper, food and beverage and animal feed industries (https://www.freedoniagroup.com/World-Enzymes.html). These may be replaced, however, by the lignocellulosic-supported biofuel and bio-based product industry market in the near future since large quantities of enzymes, including cellulases, hemicellulases and ligninases, will be required to deconstruct feedstock materials (Hood and Requesens 2012).

In 2011, the US Department of Agriculture approved the first commercial-scale production of **Enogen**, a transgenic corn plant developed by Syngenta US to express α-amylase. Enogen eliminates the need to use liquid α -amylase in dry-grind ethanol production (Table 9.13). Another industrial enzyme, bovine trypsin (a protease) is widely used for commercial purposes to digest other pharmaceutical proteins. This enzyme can be expressed in the corn grain (Woodard et al. 2003) and has been marketed by Sigma Chemicals, USA, with the trade name TrypZean. **TrypZean** produced in plant-based systems could replace other production systems such as animal cell cultures to eliminate the chances of human pathogen contamination of the enzyme preparation. Several plant crops (Table 9.13) including tobacco, *Arabidopsis*, potato, rice, alfalfa, canola, pea, barley, soybean, wheat and corn are

Table 9.13 Transgenic plant platform expressing commercially significant enzymes

Enzyme	Plant platform	Level	Reference
α-Amylase (archaea)	Corn	0.08–0.16% DW[a]	Urbanchuk et al. (2009)
	Tobacco	0.3%TSP[b]	Pen et al. (1992)
	Tobacco	5.0% TSP	Kumagai et al. (2000)
Xylanase	*Arabidopsis*	1.4–3.2% TSP	Bae et al. (2008)
	Tobacco	4.1TSP	Herbers et al. (1995)
	Rapeseed	2kU/kg seed	Liu et al. (1997)
Glucanase	Tobacco	0.22–0.38% TSP	Bae et al. (2010)
	Tobacco	0.3% TSP	Lebel et al. (1998)
	Barley	–	Jensen et al. (1996)
	Arabidopsis	26% TSP	Ziegler et al. (2000)
Phytase	Tobacco	1% TSP (seed)	Pen et al. (1993)
	Tobacco	14.4% seed	Verwoerd et al. (1995)
	Soybean	–	Denbow et al. (1998)
Bovine chymosin (rennin)	Brassica	0.5% total seed protein	van Rooije et al. (2008)
	Flax	0.5% total seed proteins	van Rooije et al. (2008)
Bovine trypsin	Corn	58 mg/kg seed	Woodard et al. (2003)

[a]Dry weight
[b]Total soluble protein

being investigated to produce commercial enzymes such as amylase, glucanase, xylanase, phyrase and chymosin (Hood and Requesens 2012; Biesgen et al. 2002). Besides enzyme purity, the commercial viability of these products depends on several factors including the cost and demand for the product.

Like any other system, plant platforms also display certain challenges, such as low accumulation of some proteins, difficult purification, biological equivalence, regulatory status and consumer acceptance. Other possible challenges are the potential for horizontal dissemination of transgene(s) to other plants through pollen grains, especially for open-pollinated crops, such as corn, and the contamination of plant tissues and protein products with pesticides, herbicides and toxic plant metabolites (Fitzgerald 2003).

Plant-based systems require methods of purification that are different from other systems, such as mammalian cells. **Protein fusion technology** is being tested to overcome two significant challenges of plant-based systems, namely, low accumulation of the protein and difficulty in terms of purification. In this approach, the DNA encoding the target protein is fused with DNA sequences encoding targeting peptides, stabilizing sequences, elastin-like proteins, hydrophobins and prolamine seed storage proteins (γ-zein), amino acid affinity tags or oil bodies (Conley et al. 2011). Both SemBioSys (no longer trading) and Plant Farm Corp. have developed technologies that combine the high-capacity, low-cost production of therapeutic proteins in seeds with a novel technology that simplifies downstream purification. In this instance, the genes encoding the target proteins are fused with a sequence encoding a small protein called oleosin. The resulting fusion protein accumulates on oil bodies within the seed. These oil bodies and the oleosin/protein fusion are then

Table 9.14 Plant platform-based pharmaceuticals in clinical development

Product	Product type	Developer	Platform	Developmental stage
ELELYSO (taliglucerase alfa)	Enzyme (type 1 Gaucher disease)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Licenced USFDA (http://www.protalix.com)
ZMapp	Antibodies	Mapp/KBP	Transgenic tobacco	Preclinical
Oral PRX-106	Antibodies (immune-mediated hepatitis)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Phase II
PRX-107	Enzyme (human alpha-1-antitrypsin for emphysema)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Phase II
PRX-110	Therapeutic protein DNase 1(cystic fibrosis)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Phase II
Influenza virus VLP	Subunit vaccine (avian influenza H5)	Medicago Inc.	*N. benthamiana* transient expression by agroinfiltration	Phase II complete
Influenza virus HA	Subunit vaccine (avian and swine influenza)	Fraunhofer CMB	*N. benthamiana* transient expression by agroviral	Phase I complete
2G12	Antibody (indicated as HIV microbicide)	Pharma-planta	Transgenic tobacco	Phase I completed
Biosimilar trastuzumab or Herceptin®	Antibody (cancer)	PlantForm Corp	Transgenic safflower	Phase II completed
MAPP66	Antibodies (HSV/HIV)	Bayer/ICON	*N. benthamiana* transient expression with MagnICON virus-based vectors	Phase II completed
CaroRx	Antibody (dental caries bacteria prevention)	Planet biotechnology	Launch vector	Phase II in the USA; product licenced in EU as a medical device
RhinoRx	Antibody (cold-causing rhinovirus)	Planet biotechnology	Transgenic tobacco	Phase I completed
DoxoRx	Antibody (drug-induced alopecia)	Planet biotechnology	Transgenic tobacco	Preclinical stage

simply purified from other components in ground seeds by centrifugation because they float. The protein is released from the oil bodies into an aqueous extraction solution by enzymatic digestion of the oleosin/protein linker, and initial purification is accomplished without expensive chromatography.

Research in the last decade has overcome many of the technical challenges that have delayed the application of molecular pharming. Good manufacturing practices for plant-derived proteins are in place, with an emphasis on the containment of therapeutic protein in a particular tissue/organ of the plant such as seed, sustainability in production and similarity to mammalian cell-derived therapeutic protein. Indeed, some plant-derived therapeutic proteins are already on their way to commercialization as described in the Table 9.14.

9.4 Closing Comments

Environmental concerns and consumer awareness have been driving forces for the reintroduction of protein-based bioproducts to the market. Advances in genetics, protein chemistry and the availability of superior tools and technologies have accelerated the utilization of proteins for manufacturing consumers' goods. Continuous efforts are ongoing to make these new products economical, durable and sustainable, and the commercialization of such products is gaining momentum. A few, such as protein-coated nuts and fruit, soybean cotton spandex shirts, fabricated nano-spun dressings and plant platform-multiplied vaccines and enzymes, are already on the market, and many more are close to commercialization.

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Chapter 10
Biomass-Derived Building Block Chemicals

Lucas J. Falarz, Stacy D. Singer, and Guanqun Chen

Chapter Highlights

- The chemical industry is currently based on simple molecules derived from petroleum.
- These simple molecules, called building block chemicals, can also be produced from plant biomass.
- Building block chemicals can be used to produce a wide range of intermediates or end-products.
- Twelve biomass-based chemicals have been identified for their potential usefulness in the chemical industry.
- These biomass-based chemicals can enter the market via a "drop-in" strategy relying on a well-established route or an "emerging" strategy to create new routes and products.
- Further research is needed to improve the production and use of building block chemicals from plants.

L. J. Falarz
Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

S. D. Singer
Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

G. Chen (✉)
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
e-mail: guanqun.chen@ualberta.ca

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10.1 Introduction

The chemical industry produces a broad range of raw materials and uses them to make various consumer goods. Most of these raw materials and final products are made using a limited number of low-value chemicals with simple structures, which are also known as **building block chemicals** or **platform chemicals**. The petrochemical industry is presently the major source of these simple molecules (Biddy et al. 2016), many of which are derived from either naphtha, a light fraction of refined petroleum, through a process called steam cracking (Matar and Hatch 2001; Bamufleh et al. 2016), or from the ethane fraction of natural gas (Bamufleh et al. 2016).

Using a combination of different reactions and catalysts, a small number of building block chemicals such as ethylene, propylene, butadiene, benzene, toluene, and xylene (Fig. 10.1) can be used to synthesize a myriad of different chemicals which are raw materials for the production of various end-products, including bottles, paints, cell phones, cars, and buildings. In general, one type of building block chemical can produce a broad range of derivatives that can be used together to generate an even larger diversity of compounds. Some sample derivatives of benzene and their applications are shown in Fig. 10.2.

Despite the advantages of these platform chemicals, the need for new materials and the finite nature of petrochemical supplies are leading the chemical industry to look for alternative and **renewable** sources of these building blocks, such as **plant biomass**. In addition, many consumers are keen to buy products that are sustainable, which has created a new market that necessitates biomass-derived feedstocks (Alam et al. 2017). The fact that oil and natural gas prices can vary substantially depending on supply and demand (prices increase due to low supply or high demand) and/or the stability of the geopolitical environment also creates a need for alternative sources of feedstocks (Bamufleh et al. 2016; Biddy et al. 2016). Furthermore, the development of a plant-based chemical industry is encouraged by countries that prefer to decrease their dependency on imported petroleum/petrochemicals.

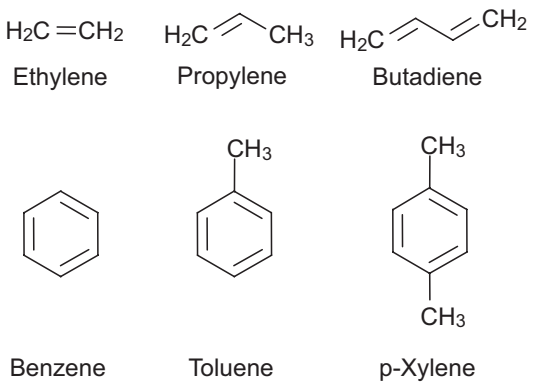

Fig. 10.1 Chemical structures of important building block chemicals. (Adapted from NCBI (2004a, b, c, d, 2005a, b))

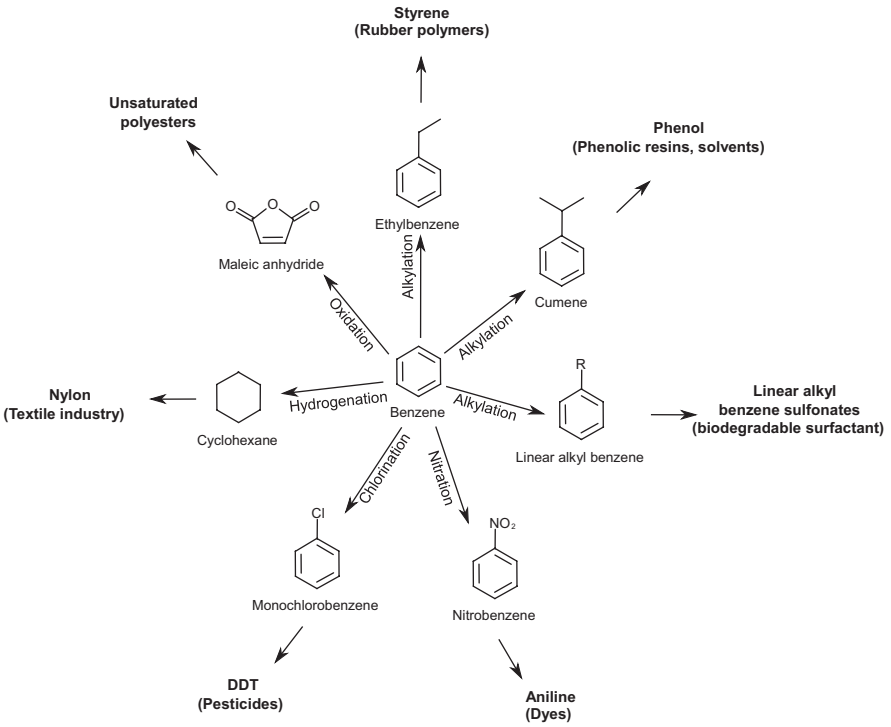

Fig. 10.2 Benzene and its derivatives and their applications. (Source: Frank and Stadelhofer (1988))

Plants have complex metabolic pathways and can synthesize many different chemicals (Nikolau et al. 2008). For example, plant compounds such as sugars can be broken down by microorganisms via fermentation to produce a number of simple building block chemicals (Corma Canos et al. 2007; Biddy et al. 2016). Such diversity in terms of the molecules produced means that plant-based products could meet demands for commonly used precursors, as well as those that are not easily obtained from petroleum (Gallezot 2012; Shanks and Keeling 2017). In this chapter, we will discuss plant-derived building block chemicals.

10.2 The Most Used Petrochemicals and Their Derivatives

It is useful to begin by considering the use of petrochemicals. Part of the success of the petrochemical industry is based on the fact that a few simple compounds can generate a large number of derivatives. The most used starting chemicals are divided into olefins and aromatics. The olefins include ethylene, propylene, and butadiene, while the aromatics comprise benzene, toluene, and xylene (Bamufleh et al. 2016).

They have a broad range of derivatives and applications. Ethylene, for example, is used as a feedstock for the production of ethylbenzene, which can be used to produce styrene (Petrochemicals Europe 2015). Styrene is an organic chemical that can be used to produce various types of plastics and rubbers, like polystyrene, which is a raw material in the manufacturing of food packaging and electronic goods (Petrochemicals Europe 2015). Another feedstock for the production of ethylbenzene is benzene. Benzene can also be used to synthesize intermediate chemicals which can be used in the production of rubbers, synthetic fabrics, plastic polymers, resins, dyes, pesticides, and surfactants (Frank and Stadelhofer 1988; Matar and Hatch 2001).

Butadiene is used in the production of **styrene-butadiene rubber** (SBR), which is a raw material in the manufacturing of tires. It can also be used in the synthesis of polybutylene (a plastic that is impermeable to gases), methyl tert-butyl ether (an additive of gasoline that helps reduce the emission of pollutants), and higher olefins (used in the production of fatty alcohols; Petrochemicals Europe 2015). Furthermore, butadiene can be combined with propylene derivatives to produce widely used compounds. Propylene-derived acrylonitrile, for example, is combined with butadiene in the manufacturing of nitrile rubber, which is a rubber with a high resistance to oil. Acrylonitrile butadiene styrene is a thermoplastic derived from the polymerization of acrylonitrile, styrene, and polybutadiene. It has a high water and impact resistance and is ideal for electric insulation (Petrochemicals Europe 2015).

Plastic polymers can also be produced from derivatives of aromatic petrochemicals. The nitration of toluene produces precursors of toluene diisocyanates, which are used in the manufacturing of polyurethanes (Matar and Hatch 2001). Toluene is also a solvent for adhesives, inks, paints, and coatings. Xylene is the precursor of terephthalic acid, which is the feedstock for the production of polyethylene terephthalate (PET), a polymer used in plastic bottles (Matar and Hatch 2001).

10.3 Fermentation as a Means to Produce Building Block Biochemicals

Fermentation processes represent a large contribution to the production of bioproducts. Plants produce complex molecules that cannot be used directly in most industrial processes because they must be broken down into simple compounds via biological or chemical conversion. These biological processes rely on enzymes and microorganisms to perform the conversions. Enzymes are used in biotransformations to catalyze chemical reactions. Starch, for example, can be broken down into monomers of glucose through the action of amylases and debranching enzyme, which catalyze the hydrolysis of chemical bonds. Microorganisms are used in fermentation processes, and they can use plant-based feedstock to produce simple compounds. Ethanol and lactic acid are examples of simple molecules that are produced by the fermentation of carbohydrates by certain microorganisms.

Fermentation is an intracellular process whereby some microorganisms use pyruvate or other organic compounds as the final electron acceptor in a process to produce energy (in the form of adenosine triphosphate [ATP]) in the absence of oxygen. Products of fermentation are usually easier and cheaper to produce than those obtained via chemical conversion (Valli et al. 2006). Indeed, this process is a very effective method to produce simple starting chemicals using plant biomass as raw material. *Saccharomyces cerevisiae*, for example, is able to use sucrose from sugarcane to produce ethanol. However, sucrose cannot be used directly by the yeast cell. *S. cerevisiae* uses an extracellular enzyme called invertase to catalyze the breakdown of sucrose into glucose and fructose, which can then be transported into the cells (Marques et al. 2015). These monosaccharides are used to produce ethanol in alcoholic fermentation. As a building block chemical, ethanol can be used to produce ethylene, which is a widely used precursor in the petrochemical industry (Harmsen et al. 2014).

10.4 Biomass-Derived Building Block Chemicals

Building block chemicals derived from plant biomass, or its fermentation, can also act as precursors or intermediates in the production of a broad range of complex materials and chemicals (Bio-Tic 2014; Biddy et al. 2016). A primary source of biomass is crops such as corn, soybean, and rapeseed and their by-products (husks, bagasse), which are rich in carbohydrates, proteins, lipids, and other organic compounds (Tuck et al. 2012; Sheldon 2014). These compounds can be converted to building block chemicals via chemical reactions (e.g., hydrolysis, dehydrogenation, and oxidation), microbial fermentation, or a combination of both processes (Tuck et al. 2012; Sheldon 2014).

For instance, cellulose can be broken down into glucose molecules through a **hydrolysis reaction** (chemical reaction whereby chemical bonds are broken down by the addition of a molecule of water), which can be then effectively converted to lactic acid via bacterial fermentation (Sheldon 2014). Some other representative glucose derivatives include sorbitol, ethylene glycol, ethanol, and 5-hydroxymethylfurfural. Glycerol, obtained mainly as a by-product of biodiesel production, is another building block with a great diversity of derivatives with broad applications in pharmaceutical, textile, chemical, energy, and food industries (Fig. 10.3).

The use of plant biomass is advantageous for several reasons, one of which derives from the fact that resources are abundant (Sheldon 2014). In certain cases, plants can grow very quickly, which means that a lot of biomass can be produced in a relatively short period of time. In addition, some plant components that are produced in vast amounts during the harvesting and processing of crops are considered waste (e.g., lignocellulose; Tuck et al. 2012; Sheldon 2014) but have the potential to be used for bioproduct production. Indeed, several molecules derived from biomass

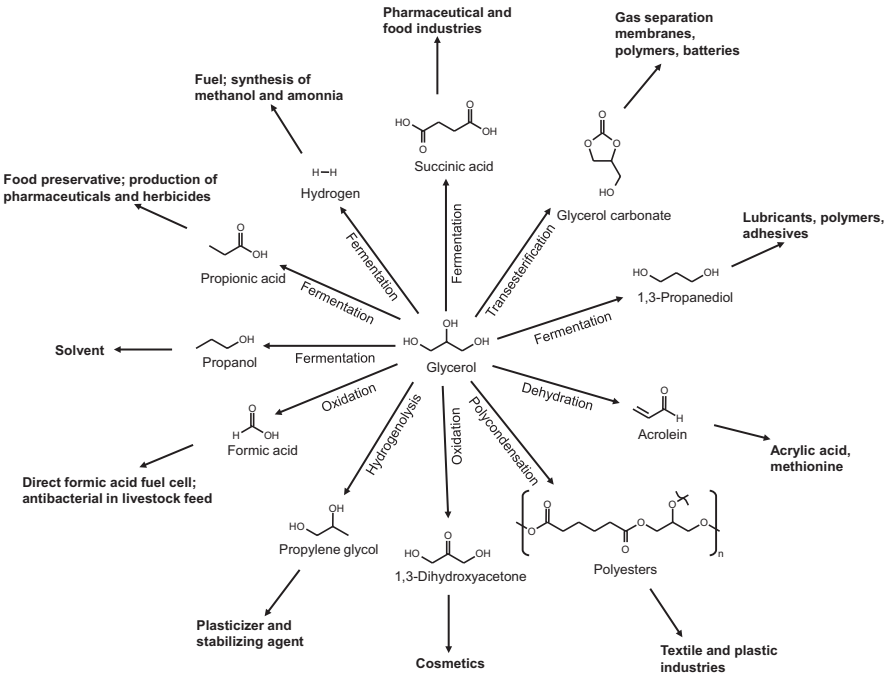

Fig. 10.3 Glycerol and its derivatives and their applications. (Adapted from Anitha et al. (2016))

are as cost-competitive as those derived from petrochemicals, and some can be even more competitive (Harmsen et al. 2014).

Many of the building block chemicals typically manufactured from petrochemical sources can also be obtained from plants. For example, many plastics derived from biomass-based feedstock have the same properties as those manufactured using petrochemicals (Harmsen et al. 2014). It is also possible, however, to obtain additional building block chemicals from biomass to produce new chemicals and materials (Werpy and Petersen 2004) since some plant-derived simple molecules have novel configurations and functional groups that are not easily obtained from petrochemicals. These novel molecules have the potential to be used to create new products with special qualities (Werpy and Petersen 2004; Nikolau et al. 2008; Shanks and Keeling 2017). Furthermore, some biomass-derived chemicals have more oxygen in their composition than those from petrochemicals, which can be beneficial during oxidation processes (Werpy and Petersen 2004; Nikolau et al. 2008).

On the other hand, biomass-derived chemicals can also have some disadvantages compared to those derived from petrochemicals. For example, while the presence of oxygen is beneficial under certain circumstances, it is unfavorable when its removal is necessary and can lead to an associated increase in processing costs (Werpy and Petersen 2004; Nikolau et al. 2008). In addition, some processes used to produce

building block chemicals from biomass are not yet as efficient or well-developed as those used in the petrochemical industry. With further research and development, these compounds have the potential, however, to be more competitive than their oil-derived counterparts in the future (Nikolau et al. 2008).

Biomass-derived building block chemicals can enter the marketplace via two main strategies. The first is known as a **drop-in strategy**. With this approach, intermediates produced from biomass are the same as those produced using petrochemicals; thus, they already have a well-established market and chemical route for their use as feedstocks (Vennestrøm et al. 2011; Harmsen et al. 2014). The advantage of this strategy is that a mature market and an optimized route of synthesis are already in existence (Vennestrøm et al. 2011). One example of this is ethylene, which can be obtained from the dehydration of ethanol and can act as a substitute for fossil fuel-derived ethylene (Vennestrøm et al. 2011; Harmsen et al. 2014). The ethanol used in this reaction is the product of the fermentation of sugars from plant biomass (e.g., sugarcane) by yeasts. The second main strategy is called an emerging strategy, which involves the use of biomass-derived building block chemicals in new products via novel routes of synthesis (Vennestrøm et al. 2011). This strategy could potentially yield benefits through the development of unique products but demands more investment in research, as well as the creation of a market for the new products.

Many building block chemicals have great market potential. In 2004, the Department of Energy of the United States listed 12 biomass-derived building block chemicals with a high potential of being developed in the near future. Some of the compounds listed include succinic acid, fumaric acid, malic acid, 2,5-furandicarboxylic acid, 3-hydroxypropionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, levulinic acid, 3-hydroxybutyrolactone, glycerol, sorbitol, and xylitol (Werpy and Petersen 2004). This list was recently updated according to market changes and new criteria (Biddy et al. 2016). Some of the new building block chemicals mentioned in this publication include para-xylene, succinic acid, propylene glycol, 1,3-propanediol, lactic acid, isoprene, glycerol, furfural, fatty alcohols, 1,3-butadiene, 1,4-butanediol, and ethyl lactate (Fig. 10.4).

10.5 The Twelve Most Important Biomass-Based Building Block Chemicals: Production and Application

One of the criteria for choosing relevant biomass-based chemicals is their potential to be developed and used in the near term. Also, the technology needed to obtain those compounds from biomass must be at an advanced stage. These technological processes involve the use of chemical, biological, and thermochemical conversions (Biddy et al. 2016). In this section, the twelve biomass-derived building block chemicals that are currently the most valuable and relevant are discussed, along with their means of production and downstream applications.

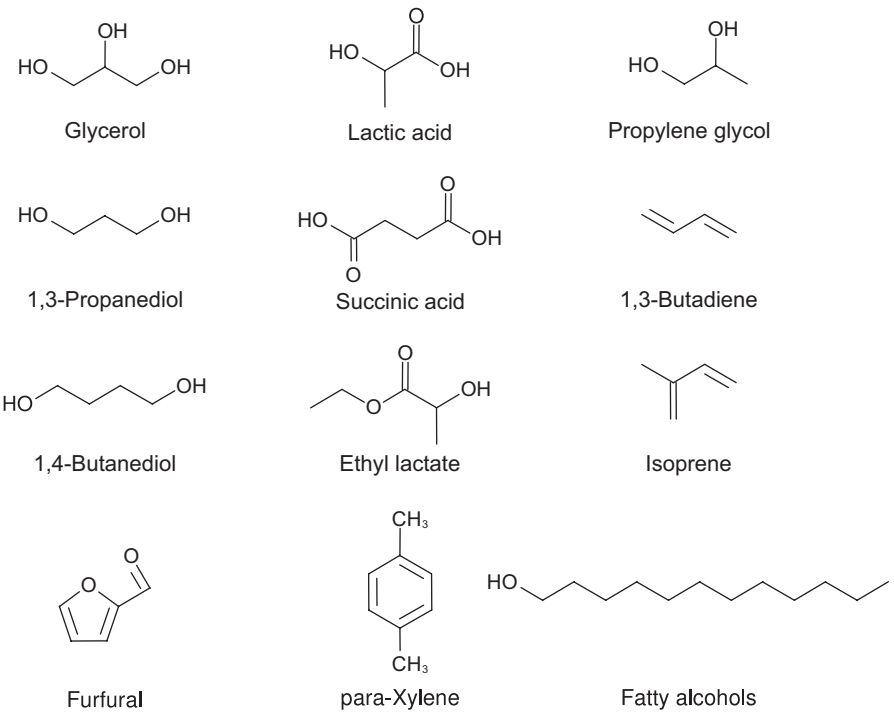

Fig. 10.4 The 12 high-potential building block chemicals identified by the US Department of Energy. (Source: Biddy et al. (2016))

10.5.1 Lactic Acid

Lactic acid is mainly produced via fermentation by some bacteria (e.g., *Lactobacillus* spp.). In the fermentation process, these microorganisms consume monomers of carbohydrates, such as glucose, and convert them to pyruvate, which is further reduced to lactic acid (Biddy et al. 2016). These carbohydrate monomers are usually obtained from more complex sugars and starch. The chosen feedstock source (e.g., sugarcane, corn, beet) depends mainly on the price of each crop type in the region where production will take place. Also, certain companies have developed a lactic acid production process using plant-derived cellulose as a feedstock (Biddy et al. 2016).

Lactic acid has broad applications (Fig. 10.5), but it is mainly used as an **acidulant** or flavoring agent in the food industry. The production of polylactic acid (PLA), however, will likely demand a great amount of lactic acid in the near future (Biddy et al. 2016). PLA is a biodegradable plastic that can be synthesized through the polycondensation of lactic acid or via the polymerization of lactide produced from lactic acid. The first route produces low-molecular-weight polymers, while the latter is used for the synthesis of high-molecular-weight PLA (Jamshidian et al. 2010;

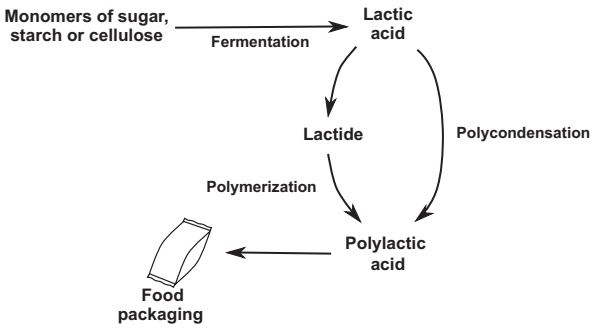

Fig. 10.5 A value chain for lactic acid. (Adapted from Jamshidian et al. (2010) and Biddy et al. (2016))

Biddy et al. 2016). PLA can be used for the production of food packaging, but because it has a higher oxygen permeability than other plastics, PLA-derived packaging is mainly used for foods not sensitive to the presence of oxygen (Jamshidian et al. 2010).

10.5.2 Glycerol

Glycerol, also known as 1,2,3-propanetriol, has historically been produced mainly from petrochemicals but is now largely obtained from renewable sources as it is a by-product of biodiesel and soap production (Anitha et al. 2016). During biodiesel production, all kinds of lipids, including animal fat, vegetable oils, and algal oils, can be used as a feedstock (Biddy et al. 2016). These lipids are mainly composed of triacylglycerol, which is composed of one glycerol backbone and three long carbon acyl chains (see Fig. 2.4). During a transesterification reaction with methanol or ethanol, methyl fatty esters are produced, which are used in the generation of biodiesel (see Fig. 4.1). Glycerol is generated as a by-product and remains mixed with impurities, which can be removed through purification (Quispe et al. 2013). There is currently no lack of low-price glycerol on the market since recent increases in biodiesel production have flooded the market with this chemical by-product (Silva et al. 2009b; Biddy et al. 2016).

The main markets for this building block chemical are the cosmetics and food industries. Glycerol is also used in detergents, explosives, and tobacco products (Biddy et al. 2016). Indeed, a very diverse number of chemicals can be obtained through chemical and biological conversions of glycerol (Fig. 10.3). For example, a polycondensation reaction with glycerol and adipic acid as substrates can yield polyesters that can be used in the textile industry (Bueno et al. 2015), while the microbial fermentation of glycerol can generate propanol (Anitha et al. 2016). The dehydration of glycerol can also form acrolein, which is a precursor for the synthesis of methionine (Fig. 10.6) (Katryniok et al. 2009).

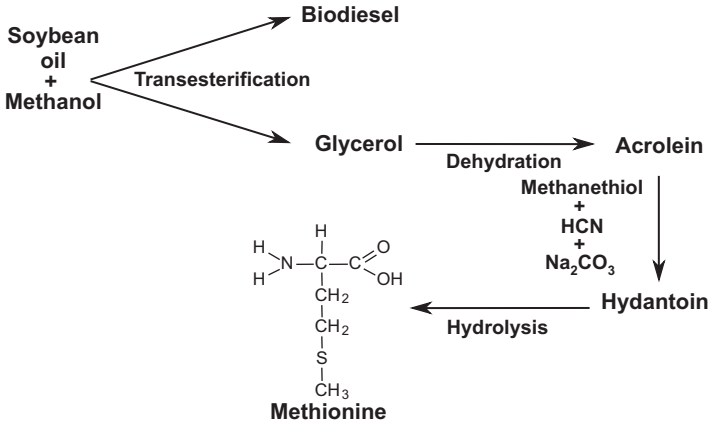

Fig. 10.6 A value chain for glycerol. (Adapted from Katryniok et al. (2009, 2010))

10.5.2.1 Production of Methionine from Glycerol

Methionine is an essential amino acid (see Fig. 2.12) that is not synthesized by animals and therefore must be included in their diet (Katryniok et al. 2010). Extraction from plant biomass does not yield high amounts of this amino acid; an alternative pathway for its production is catalytic synthesis. Biomass-based glycerol can be the precursor for the synthesis of methionine, which makes the manufacturing of this compound more sustainable. The first step in this process is the dehydration of glycerol to produce acrolein. This reaction takes place in the presence of sulfuric acid under high pressure and high temperature (Katryniok et al. 2009). In the next step, acrolein and methanethiol react to form the intermediate 3-methylthiopropionaldehyde, which reacts with hydrogen cyanide and sodium carbonate to produce hydantoin. Hydantoin is then hydrolyzed to generate methionine (Katryniok et al. 2010). This process produces a **racemic mixture** of D-methionine and L-methionine. Because some animals are able to convert D-methionine to L-methionine, a separation of the racemic mixture into its D and L forms is usually not necessary (Ülgen 2009).

10.5.3 Succinic Acid

In the chemical industry, succinic acid is a derivative of butane and can also be obtained through fermentation using genetically engineered microorganisms, such as *Escherichia coli* (see Sect. 10.5.3.1; Biddy et al. 2016). In the case of fermentation, glycerol and carbohydrates from plants (e.g., sugars, starches, and cellulose)

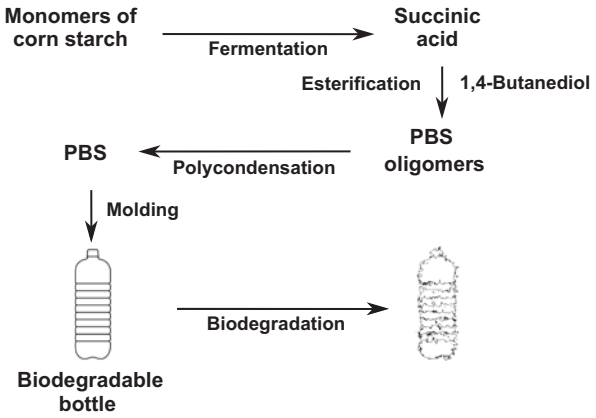

Fig. 10.7 A value chain for succinic acid. PBS polybutylene succinate. (Adapted from Xu and Guo (2010), Showa Denko (2015), and Biddy et al. (2016))

are used as feedstock for the process. Following fermentation, purification steps are necessary to obtain pure succinic acid (Gallezot 2012).

Derivatives of succinic acid, including the succinic acid esters, γ-butyrolactone, 1,4-butanediol, and tetrahydrofuran (Gallezot 2012), are utilized by pharmaceutical, food, and chemical industries to produce a broad range of products (Biddy et al. 2016). **Polybutylene succinate** (PBS) can also be manufactured using succinic acid as a feedstock and has a strong environmental appeal since it is a bioplastic that has properties comparable to those of polypropylene but can be degraded by microorganisms in compost or fresh water (Xu and Guo 2010; Harmsen et al. 2014; Showa Denko 2015). The production of PBS requires succinic acid and 1,4-butanediol, which react through an esterification reaction to form oligomers of PBS that provide the reactants in a polycondensation reaction to yield the high-molecular-weight polymer (Fig. 10.7). As an alternative to this chemical process, the second step can also be catalyzed by a lipase enzyme, which can be reused following a separation step (Ren et al. 2015).

10.5.3.1 The Use of Genetically Engineered Microorganisms to Produce Biochemical Building Blocks

The use of microbial fermentation is common for the production of certain compounds because it can be more economical or simpler than the chemical route (Valli et al. 2006). The use of certain microorganisms is preferred, however, due to their characteristics, such as robustness to harsh conditions, fast growth, and the possibility to be genetically engineered, such as *S. cerevisiae* and *E. coli*. Furthermore, there are well-established protocols for genetic engineering of these microorganisms.

The production of succinic acid by *E. coli* is an example of genetic engineering to produce a desired product. Besides being part of the tricarboxylic acid cycle (see Fig. 2.17) in these bacteria, succinic acid is produced in a mixture of ethanol, lactic acid, and acetic acid under anaerobic conditions (Thakker et al. 2012). The amount

of succinic acid must be increased for industrial production. The strategy adopted to achieve a higher production involved the use of metabolic engineering and metabolic evolution (Jantama et al. 2008). Metabolic engineering uses genetic engineering to modify the metabolism of an organism to increase or allow the production of desired metabolites (see Chap. 3). In the case of increasing succinic acid production in *E. coli*, genes encoding enzymes required for the production of ethanol, acetic acid, formic acid, lactic acid, and acetyl-CoA were deleted. This means that carbon would instead flow preferentially through the succinic acid production pathway, since the competing pathways are eliminated. **Metabolic evolution** is the consecutive growth of a microorganism strain in different culture conditions to select desirable characteristics. This technique was necessary in order to remedy certain limitations of the genetically engineered *E. coli* strain as it was not able to grow well in simple media and required acetate for its growth. Following metabolic evolution, however, the resulting strain was able to grow well, without the addition of complex nutrients to the media, while at the same time producing high amounts of succinic acid (Jantama et al. 2008).

10.5.4 1,3-Propanediol

1,3-Propanediol is a building block chemical which is useful for the production of polyesters. It is mainly derived from a bio-based process which expends 40% less energy than the traditional chemical route (Biddy et al. 2016). The bio-based production of this platform chemical requires glycerol as the main substrate in the presence of bacteria or fungi generally belonging to the genera *Klebsiella*, *Clostridium*, *Lactobacillus*, or *Aspergillus* (Liu et al. 2010). A genetically engineered form of *E. coli*, which is able to use glucose as a substrate in this reaction, has been developed recently by DuPont and Genencor (Liu et al. 2010). Corn makes up the major source of sugars used for the production of 1,3-propanediol, but cellulose also has potential to be used in this biological route (Kurian 2005; Biddy et al. 2016).

Bio-based 1,3-propanediol is used in the production of polymers including polyurethane and unsaturated polyester, as well as antifreeze, heat transfer fluids, and pharmaceutical applications in the form of a humectant and solvent. One of the major applications of 1,3-propanediol is the manufacturing of **polytrimethylene terephthalate** (PTT), a polymer with better stain and ultraviolet resistance than other polymers such as PET (Liu et al. 2010; Biddy et al. 2016), which is used in the generation of fabric for clothing. For the synthesis of PTT, 1,3-propanediol is reacted with terephthalic acid in a two-step process: an esterification step to produce oligomers followed by a polycondensation step to produce PTT. Interestingly, the production of PTT releases 63% less CO_2 than nylon 6 manufacturing (DuPont 2012). A possible route for the synthesis of 1,3-propanediol is depicted in Fig. 10.8.

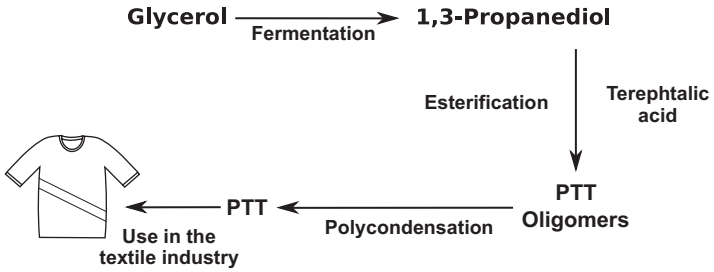

Fig. 10.8 A value chain for 1,3-propanediol. PTT polytrimethylene terephthalate. (Adapted from Liu et al. (2010), DuPont (2012), and Biddy et al. (2016))

10.5.5 1,4-Butanediol

In the petrochemical industry, there are two routes for the synthesis of 1,4-butanediol: one that uses formaldehyde and acetylene as feedstock and the other that uses maleic anhydride and ethanol (Biddy et al. 2016). There are also two routes to produce this compound using plant biomass as a source of raw materials. The first occurs via fermentation using an *E. coli* strain that is able to use both glucose and sucrose from sugarcane, corn, or the hydrolysis of cellulosic material as feedstock (Yim et al. 2011). The second route of synthesis occurs through the reduction of biomass-derived succinic acid (Harmsen et al. 2014).

As shown in Fig. 10.7, 1,4-butanediol is mainly used in the production of PBS, although it is also utilized in the synthesis of tetrahydrofuran (Biddy et al. 2016) and **polybutylene terephthalate** (PBT). PBT is better than PET for injection molding due to its fast crystallization properties (Devroede et al. 2009) and thus has many applications in the electronic and automotive industries, including the production of windshield wipers (Darda et al. 2005; BASF 2013). The chemical synthesis of this material is similar to that of PTT and PBS. There is an initial esterification reaction between 1,4-butanediol and terephthalic acid to form oligomers, and a subsequent polycondensation is used to polymerize these oligomers to form PBT (Darda et al. 2005). A value chain of 1,4-butanediol is shown in Fig. 10.9.

10.5.6 1,3-Butadiene

Although 1,3-butadiene cannot be directly produced via fermentation, ongoing research is focused on the development of this process (Biddy et al. 2016). To obtain this compound, it is instead necessary to first produce ethanol, butanol, butene, isobutene, 1,4-butanediol, or 2,3-butanediol, via fermentation, and then use chemical methods to convert one of these fermentation products to 1,3-butadiene. For the fermentation step, glucose from cornstarch or waste cellulose can be used as feedstock in the reaction (Baek et al. 2014).

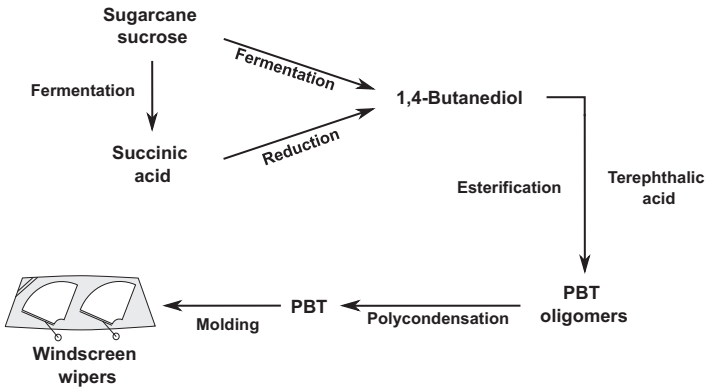

Fig. 10.9 A value chain for 1,4-butanediol. PBT polybutylene terephthalate. (Adapted from Darda et al. (2005), Yim et al. (2011), BASF (2013), and Harmsen et al. (2014))

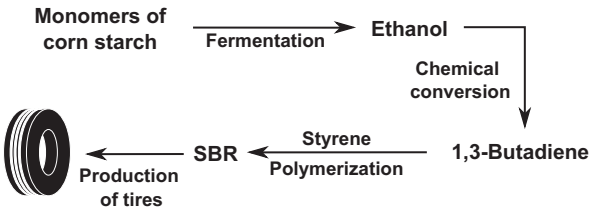

Fig. 10.10 A sample value chain of 1,3-butadiene. SBR styrene-butadiene rubber. (Adapted from IISRP (2012a), Baek et al. (2014), and Biddy et al. (2016))

This building block chemical is mainly used for the production of **styrene-butadiene rubber** (SBR), one of the main components of tires (IISRP 2012a). The synthesis of SBR occurs through a controlled polymerization reaction between 1,3-butadiene and styrene, which will be present in a 3:1 ratio (w/w) in the finished polymer (IISRP 2012a). Although manufacturing of tires is the major industrial application for this product, SBR is also used in the generation of products such as chewing gum, shoe soles, food container sealants, and rubber toys (IISRP 2012a). Figure 10.10 shows the value chain of biomass-based 1,3-butadiene.

10.5.7 Fatty Alcohols

A broad range of renewable sources including plants, animals, and algae can be used as biomass-based sources of oil/fat feedstocks for the production of fatty alcohols (Corma et al. 2007; Biddy et al. 2016). Storage lipids composed of triacylglycerol are initially transesterified with an excess of methanol catalyzed by a strong base or acid, which results in the release of fatty acids from glycerol and their conversion to fatty acid methyl esters (also used in the production of biodiesel; see Fig. 4.1). The use of lipases to release fatty acids is an alternative method to

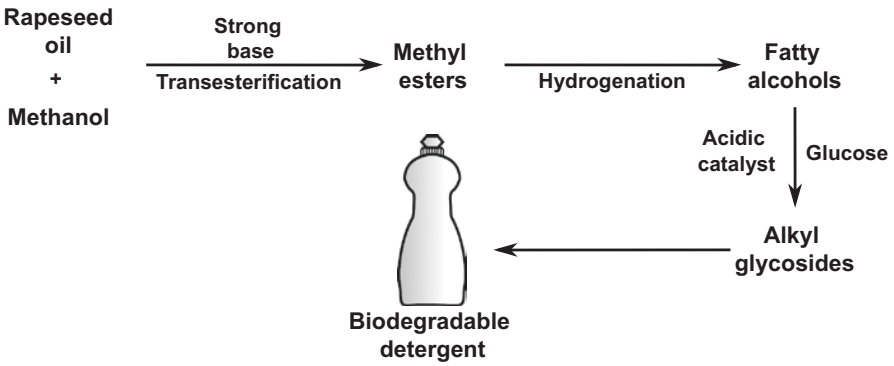

Fig. 10.11 A value chain for fatty alcohols. (Adapted from Schmid and Tessman (2001), Corma et al. (2007) and Biddy et al. (2016))

transesterification (Corma et al. 2007). The resulting methyl esters are further hydrogenated to produce fatty alcohols through chemical means (Biddy et al. 2016). The production of fatty alcohols can also be achieved via the aerobic fermentation of carbohydrates with a genetically engineered bacteria strain, in a bioprocess that requires high amounts of oxygen and thus increases the operational and investment costs (Zheng et al. 2012). The production of fatty alcohols in transgenic plants is also under study [e.g., Miklaszewska et al. (2017)] but has not been commercialized as of yet.

Fatty alcohols are mainly utilized by the detergent industry to generate surfactants. A small portion is also used as feedstock in the manufacturing of lubricants and plasticizers (Corma et al. 2007; Biddy et al. 2016). Typically, fatty alcohols with 12–18 carbon atoms are preferred for the production of detergents, while C8–C10 fatty alcohols are used in the synthesis of plasticizers (Zheng et al. 2012). **Alkyl glycosides** (or alkyl polyglycosides) are one class of surfactants derived from fatty alcohols that are used in the production of biodegradable detergents (Fig. 10.11). One route for their production is the direct reaction of glucose and fatty alcohols using an acidic catalyst (Schmid and Tessman 2001; Corma et al. 2007).

10.5.8 Propylene Glycol

Propylene glycol is a compound that can be obtained from both petrochemical and renewable sources. Glycerol is the main feedstock for the synthesis of biomass-based propylene glycol (Biddy et al. 2016), which is produced through hydrogenolysis. This building block chemical has a diverse range of applications in pharmaceutical and food industries, at least in part because it is safe for human consumption. It is also used as a feedstock in the synthesis of unsaturated polyester resins, which are applied in the manufacturing of fiber panels for construction, boat

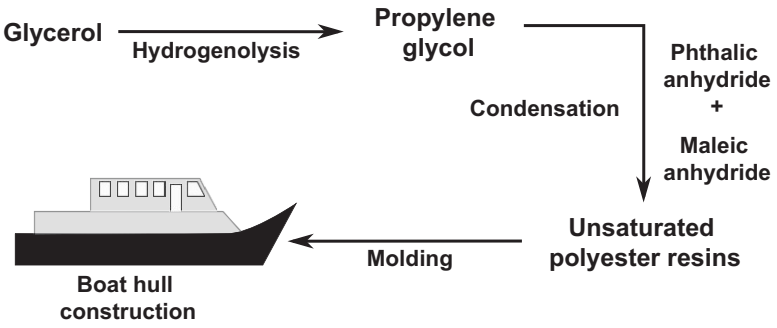

Fig. 10.12 A value chain for propylene glycol. (Adapted from Ul Haq (2007), Dholakiya (2012) and Biddy et al. (2016))

hulls, containers, and other applications (Ul Haq 2007; Dholakiya 2012). A condensation reaction among phthalic anhydride, maleic anhydride, and propylene glycol results in these unsaturated polyester resins, which can be further modified (Fig. 10.12). For example, they can be blended with styrene to generate thermoset products (Dholakiya 2012).

10.5.9 Para-xylene

Para-xylene is important for the production of PET and is currently derived mainly from the processing of fossil fuel (Biddy et al. 2016). A number of multistep processes, however, are under development that would also enable the production of biomass-derived para-xylene. For example, using monomers derived from sugar, starch, or cellulose as the carbon source, a fermentation process is being developed to produce isobutanol, which can be further converted to para-xylene (Tuck et al. 2012). A handful of companies are also trying to develop a process involving the chemical conversion of plant-derived lignin monomers into para-xylene. In this instance, they are using chemical and physical processes to obtain a stream of aromatics and mixed xylenes, which are then used in the synthesis of para-xylene via the typical petrochemical-based route (Biddy et al. 2016; Van Uytvanck et al. 2017). It is also possible to use acrolein (derived from glycerol) and isoprene (obtained from the fermentation of sugars) to synthesize an intermediate which can then be used to produce para-xylene with a reduction and an aromatization step (Wang and Tong 2016).

The oxidation of para-xylene results in the production of **terephthalic acid**, which can in turn be used as a feedstock in the production of diverse polyesters, such as PTT, PBT, and PET (Matar and Hatch 2001; Biddy et al. 2016). For example, PET, which can be used to produce environmentally friendly bottles, can be synthesized from terephthalic acid and ethylene glycol in a two-step reaction including esterification and polycondensation (Al-Sabagh et al. 2016). A value chain for para-xylene is depicted in Fig. 10.13.

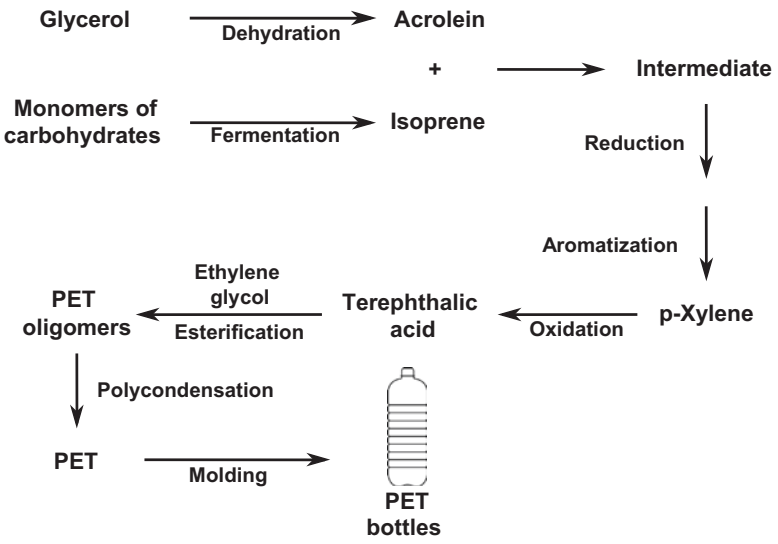

Fig. 10.13 A value chain for biomass-based para-xylene. PET polyethylene terephthalate. (Adapted from Matar and Hatch (2001), Al-Sabagh et al. (2016), Biddy et al. (2016) and Wang and Tong (2016))

10.5.10 Isoprene

Isoprene is the building block of synthetic rubber and is mainly produced from petroleum sources; however, the biomass-based production of this biochemical is actively being pursued. Indeed, a small number of aerobic fermentation bioprocesses have been developed that convert biomass-derived carbohydrates to isoprene using genetically engineered yeasts and bacteria (Morais et al. 2015; Biddy et al. 2016). Isoprene is the precursor of polyisoprene (synthetic rubber), which is used in the manufacture of tires, surgical gloves, and shoes. The polymerization of isoprene molecules in the presence of specific catalysts produces polyisoprene rubber, which can be further modified into desired products (IISRP 2012b). A value chain for biomass-based isoprene is shown in Fig. 10.14.

10.5.11 Furfural

Furfural is mainly produced from lignocellulosic materials through chemical conversions. Pentoses (e.g., xylose and arabinose), which are the major precursors for the synthesis of furfural, can be obtained from pentosans in the hemicellulose present in lignocellulosic biomass but must first be subjected to hydrolysis to release pentose monomers. The dehydration of these compounds produces furfural

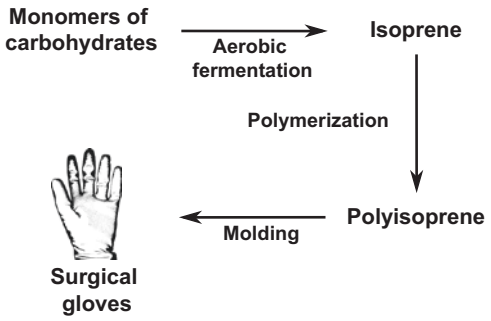

Fig. 10.14 A value chain for biomass-based isoprene. (Adapted from IISRP, (2012b) and Biddy et al. (2016))

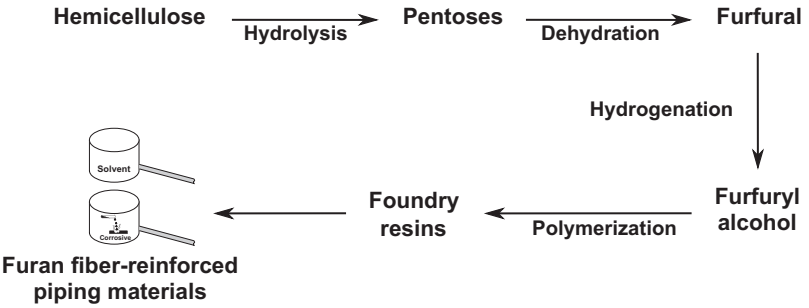

Fig. 10.15 A value chain for furfural. (Adapted from Yan et al. (2014) and Biddy et al. (2016))

molecules (Yan et al. 2014; Biddy et al. 2016). Corncobs and sugarcane bagasse are often used as a source of pentoses for the production of furfural.

Furfural can lead to many derivatives, thus acting as a precursor for the production of chemicals that can be used as solvents in printing inks, agricultural applications, and chromatography, as well as precursors in pharmaceutical drugs and insecticides (Yan et al. 2014). In addition, one furfural derivative, 2-methylfuran, has the potential to be used as a component of biofuels. Furfural can also be hydrogenated with specific catalysts to produce furfuryl alcohol, which can undergo a polymerization reaction to produce **foundry resins** (Yan et al. 2014). These resins can be used in the manufacturing of furan fiber-reinforced plastics, which are ideal for piping materials that must be resistant to corrosives and solvents. A value chain for furfural is shown in Fig. 10.15.

10.5.12 Ethyl Lactate

Unlike other biomass-based chemicals, ethyl lactate is not used as an intermediate in the production of other compounds. This building block chemical itself is a final product and is mainly used as a solvent (suitable as a coating component). Ethyl lactate has enhanced chemical properties compared to most petroleum-derived

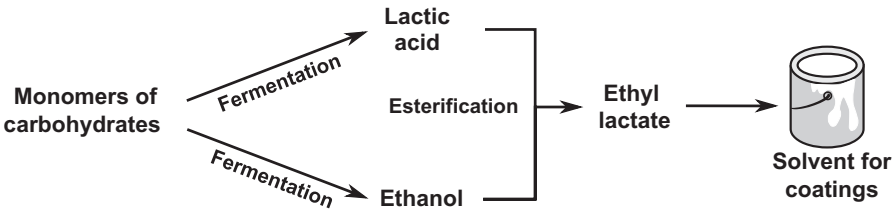

Fig. 10.16 A value chain for ethyl lactate. (Adapted from Pereira et al. (2011) and Biddy et al. (2016))

solvents such as toluene (Pereira et al. 2011), and it is biodegradable, nontoxic, recyclable, and even approved by the US Food and Drug Administration to be used as a flavor additive (Clark and Tavener 2007; Pereira et al. 2011). Its manufacturing can also be environmentally friendly as it can be produced through the esterification of lactic acid and ethanol, which can be obtained through the fermentation of sugars. Additionally, lignocellulosic materials have a high potential to be used in these processes (Biddy et al. 2016). A possible value chain for ethyl lactate is shown in Fig. 10.16.

10.6 Lignin as a Source of Aromatic Compounds

Lignin is a major component of **lignocellulose**, which is composed of carbohydrate polymers (cellulose, hemicellulose) and the most abundant form of biomass on earth and often considered waste (Tuck et al. 2012; Isikgor and Becer 2015). Lignocellulose is composed of cellulose, hemicellulose, and lignin, the latter of which can represent up to one third of some biomass materials. This compound is responsible for the strength and rigidity of plant tissues, aids in water transport, and functions to protect against attack by insects and infection by microorganisms (Holladay et al. 2007; Isikgor and Becer 2015). The use of lignin as a feedstock in the production of biomass-derived building block chemicals is a very promising avenue since it has the potential to contribute to a reduction in the emission of greenhouse gases and could replace fossil fuel sources in many cases.

Lignin is made up of phenylpropanoids called **monolignols** (Isikgor and Becer 2015), including *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The amount and structures of the particular monolignol subunits vary among different biomass sources (Fig. 10.17; Holladay et al. 2007; Isikgor and Becer 2015). While lignin can be an excellent source of aromatic compounds, the major drawback to spread the use of this polymer is that an efficient process to break lignin into its monomer components has not been developed as of yet. A standardized process by which to obtain these monomers is also challenging due to the random structure of lignin and its variability among biomass sources (Zhang et al. 2011; Isikgor and Becer 2015). Currently, a few industrial processes rely on existing technologies to

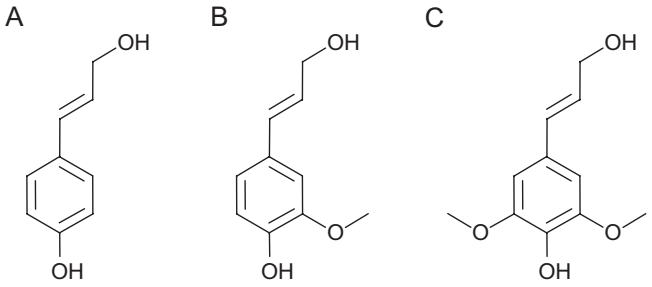

Fig. 10.17 Monolignols. (**a**) p-coumaryl alcohol, (**b**) coniferyl alcohol, (**c**) sinapyl alcohol. (Adapted from NCBI (2005c, 2017a, b))

break lignin, such as the production of vanillin and dimethyl sulfoxide (Holladay et al. 2007). Considerable research is under way to improve this technology, which may allow the use of lignin in the production of chemicals, drugs, and other applications. Indeed, lignin has the potential to be used to generate a diverse number of derivatives, including phenol, toluene, styrene, benzene, xylene, and terephthalic acid, which can be used directly as drop-in chemicals in already existing organic synthesis processes (Isikgor and Becer 2015).

Vanillin is a derivative of the degradation of lignin that has high commercial value (see Fig. 2.20) and is mainly used in food applications because it is an important component of vanilla flavor. It can also be used as an intermediate in the synthesis of pharmaceuticals and herbicides (Walton et al. 2003; Silva et al. 2009). Currently, the majority of vanillin is obtained via organic synthesis from petroleum-derived guaiacol, because natural vanillin extracted from *Vanilla planifolia* orchids is limited. Natural vanillin is mainly produced in Madagascar (around 80%) which supplies less than 1% of the market (Bomgardner 2016). Despite this scenario, some food companies are avoiding or restricting the use of vanillin and other artificial components because consumers prefer products that are derived from natural and sustainable sources. These market conditions create great potential for lignin-derived vanillin as a means to increase its market share. This process occurs in the presence of an oxygen or air stream in a very alkaline medium under elevated temperature and high pressure (Mathias 1993; Silva et al. 2009a). These conditions permit the breakdown of lignin and further oxidation into vanillin and other products. Following this reaction, some purification steps are necessary to obtain pure vanillin.

10.7 Challenges and Future Directions

Chemicals and materials derived from petrochemicals have a large market due to the fact that their entire value chain is efficient, which leads to products with high quality and low price (Nikolau et al. 2008). In order to achieve comparable

competitiveness with plant-derived chemicals, two challenges will first need to be solved. First, the biomass-derived building block chemicals will require an optimized route for their production and an efficient process for their application in the manufacturing of other products. Secondly, in order to be used as a source of feedstock, biomass must yield inexpensive intermediate chemicals. For example, an immense amount of low-cost lignocellulosic material is generated as a by-product of many agricultural and industrial processes, which is often burned to produce electricity (Tuck et al. 2012). These by-products have the potential, however, to be used in the production of low-cost and high-quality building block chemicals.

Compared to the petrochemical industry, research in biomass utilization is a relatively new topic, and enhanced efforts will be necessary to find more efficient routes for the production of biomass-based chemicals (Chatterjee et al. 2015). Currently, many routes to produce biomass-based chemicals involve fermentation, but more research is needed to achieve higher yields. Regarding lignocellulosic materials, the components are often difficult to break down, and the yield of monomers produced from this type of biomass is low and must be remedied (Isikgor and Becer 2015). Also, routes for the consumption of these types of materials must be redesigned to accept different sources. All of these optimizations will lead to a reduction in costs. Similarly, it is going to be imperative that we improve the means by which these building block chemicals are used as starting materials for the production of bioproducts. In many cases, existing processes will require modification since plant-based precursors might not have the same characteristics as those derived from petrochemicals. New technologies combined with existing ones, such as genetic engineering, will aid in the development of a biomass-based industry.

10.8 Closing Comments

In this chapter, the potential of biomass as a source of building block chemicals for industrial purposes was discussed. Since society is increasingly concerned about the environment and there is a need for sustainable, environmentally friendly and renewable processes and products to reduce our dependence on fossil fuels (Jong et al. 2012), biomass-derived chemicals could provide an answer. In most cases, biomass-derived chemicals have similar or improved performance when compared to petroleum-derived chemicals and a better carbon footprint (Erickson et al. 2012). As such, many companies and governments are investing in the development of biomass as a feedstock for the manufacturing of these products (Biddy et al. 2016), and it is therefore expected that in the near future, not only will fuels be generated from biomass but also the large-scale production of valuable chemicals. Indeed, it is conceivable that these new bioproducts could contribute to a new and sustainable economy.

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Chapter 11
Biorefinery of Plant-Based Products

Youn Young Shim, Shahram Emami, Kornsulee Ratanapariyanuch, and Martin J.T. Reaney

Chapter Highlights

- Biorefining is defined as the sustainable processing of biomass into a spectrum of marketable products and energy.
- Similar to the value of refinery to fossil fuel resources, biorefinery is important in the utilization of plant biomass/seeds for the production of bioproducts.
- The process of biorefining crop seeds varies according to seed type but displays some common factors.
- Biorefinery is a key step in ethanol fermentation and can be further developed for the utilization of fermentation by-products.
- The development of biorefinery systems is a key factor in the production of plant bioproducts with high efficiency and low cost.

11.1 Introduction

Throughout most of humankind's existence, humans have used plant materials to meet the technological needs of society. Wood has been used to generate heat and to make weapons, farming tools, and dwellings for thousands of years. Similarly, vegetable oil and animal fats have been used to generate light for over 40,000 years. Early civilizations harvested grasses and grew grains to feed animals that provided

Y. Y. Shim · M. J. Reaney (✉)
Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Prairie Tide Chemicals Inc., Saskatoon, SK, Canada

Guangdong Saskatchewan Oilseed Joint Laboratory, Department of Food Science and Engineering, Jinan University, Guangzhou, Guangdong, China
e-mail: martin.reaney@usask.ca

S. Emami · K. Ratanapariyanuch
Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

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power and transportation. However, with the introduction of coal as a source of energy, a shift began away from the use of bioproducts as a major supply of materials and moved instead toward the use of fossil resources. Initially, technological advancements with regard to bioproducts progressed in parallel with those attained relating to the utilization of fossil fuels. For example, the production of alcohol and key chemicals from wood was a significant advancement, as was the development of technology allowing the separation of cellulose fiber from wood for the production of paper and cardboard. Similarly, advances in the fermentation of natural products enabled the conversion of biomass to alcohol, ketones, acids, and other products (Brown 2003; Zhou et al. 2014). Despite this technological progress, the low cost of fossil fuel-derived carbon has led to the prevalence of its use for societal needs, but with increasing demand for renewable products from a growing world population, an interest in the utilization of renewable materials is flourishing once more. The rising price of fossil fuel and its destructive impact on the environment are further driving this newfound interest.

Part of the success of fossil fuels and natural gas derives from our ability to convert them into a small number of intermediate products that are easily transformed or incorporated into a wide range of final products. Indeed, the reactions or unit processes in a petroleum refinery enable the refiner to shift the ratio between several output products so that waste may be minimized or even eliminated. It has been proposed that similar concepts, if employed in the processing of biomaterials, would lead to a more efficient use of these materials and greater competition with fossil-based resources. A refinery that utilizes biological material in a similar manner to a refinery that utilizes fossil-based material would be termed a biorefinery and would produce products that compete directly with petroleum products, such as platform chemicals, fuel, and fiber, and also potentially materials such as food, fertilizer, and feed for animals. When processing crops, waste is typically discarded. For instance, the processing of pulse grains results in a hull fraction that is not readily used. Increasingly, however, such materials are being processed to make fuel, electricity, chemicals, and other products. The transition of a traditional process to biorefinery occurs with the conversion of waste to coproducts of manufacturing.

According to the International Energy Agency (IEA) Bioenergy, Task 42, **biorefining** has been defined as "the sustainable processing of biomass into a spectrum of marketable products and energy" (IEA Bioenergy 2009). Much of the literature on biorefinery focuses on the processing of dedicated energy crops, which are typically fast-growing plants that produce the maximum possible potential biomass. These biomass crops tend to be burned to produce energy with little diversity of manufactured products. However, the refining of materials used for food and animal feed can also provide the raw materials for producing chemicals and energy while simultaneously improving food quality. In this chapter, the similarities and differences between plant biomass- and fossil fuel-derived resources will be briefly discussed. With this basic information, the concept of biorefining will be discussed, and specific biorefinery processes, including the processing and treatment of seeds (oilseeds and starch seeds) for ethanol fuel fermentation, will be considered. The concept of biorefining can also be applied to the extraction of proteins from plants, such as zein and gluten from corn and wheat, respectively. This topic is discussed in Chap. 9.

11.2 Biorefinery, Petroleum Refinery, and Plant Biomass- and Fossil Fuel-Derived Materials: Similarities and Differences

A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, heat, and value-added chemicals from biomass. Food and feed could also be considered as products of biorefinery processing (Fig. 11.1). The petroleum refinery is a flexible system that allows the operators to change input products to lower the costs of production and to change operating conditions to control the ratio of output products. The latter capability allows the refiner to respond to variance in supply as well as market demand for products. Similarly, the biorefinery should, where possible, have the flexibility and control to meet the demand for manufactured materials.

Fresh plant biomass and fossil-based resources have a common origin in living materials. Their elemental composition, however, is quite different. The elemental composition of most biomass is rich in the elements hydrogen, oxygen, carbon, nitrogen, sulfur, phosphorous, and essential minerals. Fossil oils and bitumen products, on the other hand, are composed mostly of carbon and hydrogen. The amount of oxygen, nitrogen, phosphorous, and other minerals is typically quite low. Depending on the fossil source, the amount of sulfur present can be several percentages of the product.

The carbon in both biomass and fossil carbon was largely converted from atmospheric carbon dioxide to plant and algal carbon. Photosynthetic organisms use the energy in light for photosynthesis. Carbon and some of the oxygen from the carbon dioxide are combined with other plant nutrients to make plant biomass. The remaining oxygen in plant biomass mostly arises from water. Other elements in biomass such as nitrogen, phosphorous, and sulfur are present in soil and water. Nitrogen can come from atmospheric sources where symbiotic nitrogen-fixing organisms convert the nitrogen gas (N_2) to useful nutrients.

Energy production is a major application of both fossil carbon and biomass. Biomass varies in its energy content per gram. Generally, biomass that is rich in oxygen has less energy than biomass that has little oxygen. Carbohydrates (CH_2O)

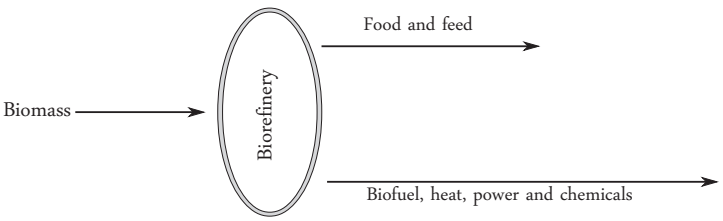

Fig. 11.1 Schematic depicting the sustainable processing of biomass into different value-added products in a biorefinery

have much less energy per gram than hydrocarbons (CH_2). The energy-embodied biomass is largely due to the substantial energy contained in bonds between carbon atoms and the energy in bonds that join carbon and hydrogen. This energy may be released by combustion with oxygen. The bonds between oxygen and carbon or oxygen and hydrogen have little energy and release comparatively less energy when reacted with oxygen. The most common biomass materials are the cell walls of plants and algae, which are rich in carbohydrate polymers including cellulose and hemicellulose. Lignin-rich materials are also present in many plant biomass sources. This material has a higher ratio of carbon to oxygen than cellulose and, therefore, a greater energy density. Hydrocarbons are among the highest-energy biomass materials. They are not common in most biomass crops. While most biomass also contain protein and a wide range of small molecules, these components are usually present at comparatively lower concentrations.

11.3 Processing of Seed Materials

11.3.1 Oilseeds

Vegetable oils are broadly used as a source of dietary lipids, renewable biomaterials, and biofuels. The three most widely grown oilseed crops are soybean, *Brassica* oilseed species (*Brassica* spp., including canola-type, *B. napus*), and sunflower. In this section, canola will be used as an example to discuss the processing of seed oil. *Brassica* spp. oilseeds are grown throughout the world as a source of vegetable oil and protein-rich animal feed (Kimber and McGregor 1995). According to statistical data from the Canola Council of Canada (2017), the average annual production of Canadian canola over the period 2013–2016 was 18.2 million tonnes. The Canadian oilseed-crushing industry crushed an average of 8.0 million tonnes of canola and produced an average of 3.5 and 4.6 million tonnes of canola oil and canola meal annually, respectively, in the same period. Commercial oilseed extraction may include solvent extraction, mechanical expeller-press extraction, or combinations of mechanical and solvent extraction to produce oil and meal (Kimber and McGregor 1995).

Seed crushing is required to separate oil and meal components from seed oil. Nevertheless, pretreatments including seed cleaning, flaking, and conditioning need to be accomplished prior to mechanical extraction. Seed cleaning is an important process to obtain high-quality finished products. A rotary screener is one example of a machine used to clean *Brassica* seeds. The waste associated with seed cleaning can amount to several percent of the total seed mass, which is a substantial amount of biomass and is therefore typically used as an ingredient in animal feeds due to its fat and protein content. Dehulling, which is necessary for some limited markets, makes use of aspiration and/or a fluidized bed sorter. After cleaning, the seeds are conditioned via heat treatment and mechanically flaked by passing through two

cast-iron rollers to increase the efficiency of oil extraction. Conditioning and flaking allow oil bodies to be broken open, reduce oil viscosity, and increase the diffusion rate of prepared oil cake. In addition, hydrolytic enzymes, especially myrosinase, are destroyed by heat. Consequently, glucosinolates do not break down and thus do not release sulfur-containing products into the oil but remain in the meal (Carr 1995). Typically, crude vegetable oil can then be extracted by several means, including mechanical pressing and/or solvent extraction, which are known as conventional methods (Strop and Perry 1994). A series of low-pressure continuous screw presses or expellers are generally utilized to remove as much oil as possible from flaked seeds, and for industrial purposes, this is usually followed by solvent extraction (Carr 1995).

Crude vegetable oil is generally unacceptable for most usages due to high levels of phosphorus compounds (i.e., phospholipids, phosphatides, phosphoglycerides), gum, and minerals (calcium, magnesium, and iron) (Strop and Perry 1994). In addition, it tends to be composed of approximately 3% solid matter termed "foot," which requires roughly 3 h of gravity-induced settling in a screening tank for its removal prior to further refining processes. Oil remaining in the foot may be re-extracted and recycled back to the screening tank using a separate foot screw press or screening and centrifugal separation. The foot remaining in suspension in the screening tank is called "fines," and it is removed using filtration or centrifugation. Oil remaining in the meal/cake from the expeller is accessed using extraction with a solvent (most often hexane). Solvent remaining in the meal/cake is then desolventized (Carr 1995).

After removing foot and fines, the oil goes through several refining processes, including degumming, neutralization (alkali refining), bleaching, and deodorization (Nawar 1996). When soluble phospholipids are present in the oil, alkaline-refining method is sometimes used to remove them to give a good final oil quality, but the treatment results in oil losses. In this case, water degumming is performed to extract and harvest phosphatidylcholine (lecithin) because this polar lipid fraction is a valuable compound used for multiple applications. If nonpolar phospholipids are present in the oil, acid degumming is preferred to precipitate them out. Free fatty acids, odor, flavor, and some color from phospholipids remaining after the degumming process are removed by alkali refining, which involves mixing the oil with refining agents. Carotenoids and chlorophyll pigments are eliminated from the oil by mixing with bleaching clay, which is called a bleaching process. Steam distillation is utilized to remove natural flavor and odor components (Carr 1995).

Refined vegetable oils can be utilized as edible oils, but they can also be used to produce biodiesel (see Chap. 4) and other bioproducts (see Chap. 5). Oilseed meal is the main by-product of the oil extraction process (Bell 1995), and in the case of canola, it is widely used as a protein source in poultry, swine, beef, fish, and dairy cattle feeds because of its excellent amino acid profile (Canola Council of Canada 2015). However, even though defatted canola meal has a high protein content, it also contains various anti-nutritional factors such as glucosinolates, tannins, phytates, other phenolic compounds, and crude fiber, which require removal prior to its use in some nonruminant animal and fish rations (Bell 1993; Khajali and Slominski 2012; Wickramasuriya et al. 2015; Mejicanos et al. 2016; Higgs et al. 1995).

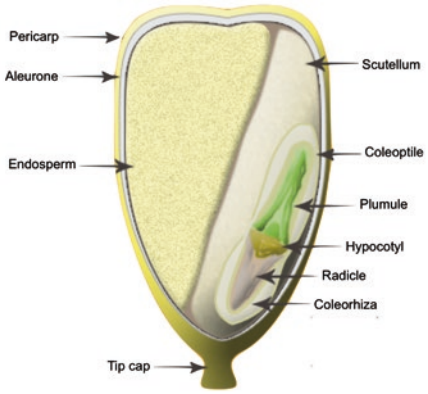

Fig. 11.2 Diagrammatic illustration of corn anatomy (This figure was reproduced with permission from geochembio.com)

11.3.2 Starch-Enriched Seeds

Cereals are rich in starch, which is useful in food, animal feed, hygiene, and pharmaceutical, paper, cosmetic, and textile industries. The five most extensively grown cereal crops worldwide are maize, rice, wheat, barley, and sorghum. Due to their overwhelming prevalence and consumption, the production of these five cereals has a major impact on governments' economies, human nutrition, and related processing industries. In this section, we will discuss the biorefining process utilized for cereal seeds, with maize as an example.

Maize or corn (*Z. mays*) is the most important cereal in North America. Its seed, or kernel, is made up of an embryo, endosperm, aleurone, and pericarp (Fig. 11.2). The endosperm, which is the major constituent of the kernel, constitutes 82–84% of the kernel's dry weight and 86–89% starch by weight (Riahi and Ramaswamy 2003). Indeed, it is made up of 98–99% starch and is low in fat, while the germ, which comprises the embryo and scutellum (an organ that provides nutrition to the embryo during the initial stages of germination), contains 81–85% fat on a dry weight (Lakner et al. 1993). Corn is used as feed, as well as in the production of ethanol, starch, food products, and various other bioproducts.

Corn processing is carried out either by **dry milling** or **wet milling**. Dry milling processing yields germ and endosperm fractions and consists of dehulling, conditioning, removal of the germ, grinding, sifting, purifying and aspirating of grits, and packaging. The endosperm fraction is classified according to size as flaking grits (particle size of 5.8–3.4 mm), coarse grits (2.0–1.4 mm), medium grits (1.4–1.0 mm), fine grits (1.0–0.65 mm), coarse meal (0.65–0.3 mm), fine meal (0.3–0.17 mm), and flour (<0.17 mm). To carry out this process, the kernels are first cleaned and external objects removed, and then the moisture content is adjusted to approximately 16%, after which time the kernels are stored in a bin for 6–8 h to equilibrate moisture levels. The germ is then removed using a Bella-type de-germination device (also called turbo-crushers in Europe), and the endosperm fraction is subsequently ground using a roller mill and separated into products with different size particles using sieves (Gyori 2010). Medium- and large-sized fractions are obtained from primary

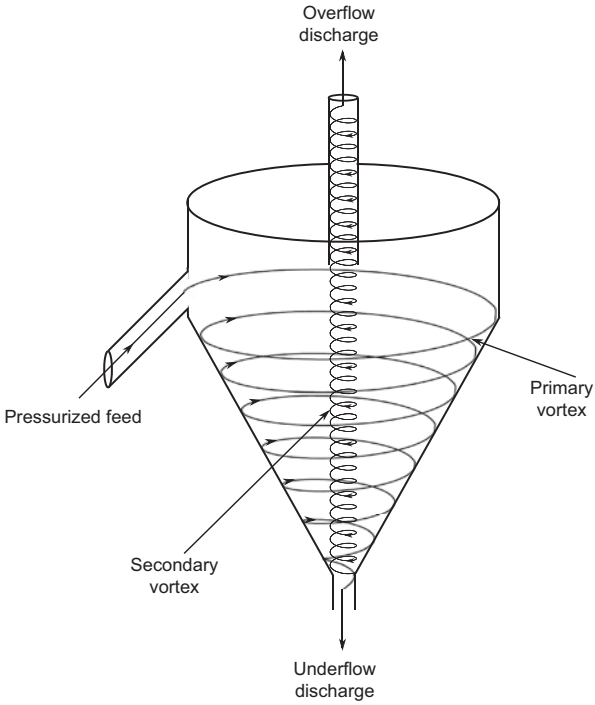

Fig. 11.3 Schematic representation of the spiral flow in a hydrocyclone (This figure was modified from Rijkswaterstaat Leefomgeving http://rwsenvironment.eu/)

grinding, while the small fraction results from secondary grinding. It is very important to separate germ prior to grinding because oil extraction from clean germ is more efficient and the endosperm would suffer from rancidity due to the presence of oil in the germ.

Wet milling begins with dehulling followed by steeping of the seeds in water containing 0.2% sulfur dioxide at a temperature of 48–52 °C until the corn moisture content reaches 45%. The purpose of this process is to soften the seed and facilitate separation of hull, germ, and endosperm. On an industrial scale, steeping is typically carried out in ten tanks with the corn moving from tank one to ten and water moving in the opposite direction. The bisulfite ion generated by the sulfur dioxide reacts with S-S bonds in proteins and breaks the protein matrix into two hydrophilic molecules. As a result, starch-protein separation is facilitated and the starch yield is increased. At the end of this process, the steeping water contains 5–7% dry matter, which is concentrated by reverse osmosis filtration to about 55% and then mixed with bran to be used as feedstuff (Hoseney 1998; Gyori 2010). The resulting soft grain is ground coarsely using an attrition mill in order to release the rubbery germ. This ground slurry is passed through a hydrocyclone to collect germ, which makes up the lower-density fraction (overflow), and the starch, which makes up the higher-density fraction (underflow) (Fig. 11.3). The underflow fraction is then sieved to separate the coarse fraction, which is reground to release starch, protein, and fiber. The fiber fraction is separated by sieving followed by several washes to remove

adhering starch, while starch-protein separation is carried out using a continuous centrifuge or hydrocyclone. The resulting starch and protein fractions are dewatered by centrifugation and then dried (Hoseney 1998).

11.4 Industrial Production of Ethanol from Plant Carbohydrates

The United States (USA) is the world leader in ethanol fuel production from cereal grains, and it is predicted that world ethanol production will reach approximately 155 billion liters by the end of 2020 (OECD/FAO 2011). In the sections below, we will discuss the process of obtaining ethanol from agricultural feedstocks. Both corn (starch) and sugarcane (sugar) will be examined as they make up the most widely used crops for ethanol production. Although carbohydrate-enriched biomass as source starting material for ethanol production was discussed in Chap. 6, the information below emphasizes the actual processes involved in the context of the biorefinery.

11.4.1 Production of Ethanol from Corn

Corn is the preferred feedstock for the production of ethanol in the USA and as discussed earlier, there are two main industrial processes through which it is obtained, termed dry milling and wet milling. The main difference between the two processes concerns whether the starch is separated from the other components of the kernel before hydrolysis; this is the case with wet milling, but not dry milling. The aim of dry milling plants is mainly the production of ethanol, which requires less investment in equipment units and increases the return per unit of ethanol compared to wet milling (Bothast and Schlicher 2005; Vohra et al. 2014). Conversely, while wet milling plants require higher investments because of the additional separation steps, the intent of this process is to not only produce ethanol but also many other corn derivatives, such as corn oil, gluten meal, gluten feed, high-fructose corn syrup, and dextrose (Bothast and Schlicher 2005; Vohra et al. 2014). The main steps and products of dry milling and wet milling are depicted in Fig. 11.4.

In both cases, the starch is broken down using enzymes since yeasts are unable to use starch as a substrate for ethanol production. A thermostable α-amylase is added initially, and the temperature is increased to above 100 °C using a jet cooker (Bothast and Schlicher 2005). This reduces the size of starch chains by breaking α-1,4 glycosidic bonds via the enzyme's catalytic action as well as the mechanical shear provided by the jet cooker. This consequently lowers the viscosity of the solution. The dextrins, which are low molecular mass products of starch digestion, are then broken into Glc monomers by the action of glucoamylases, which catalyze the hydrolysis of both α-1,4 and α-1,6 glycosidic bonds. Dextrin formation from

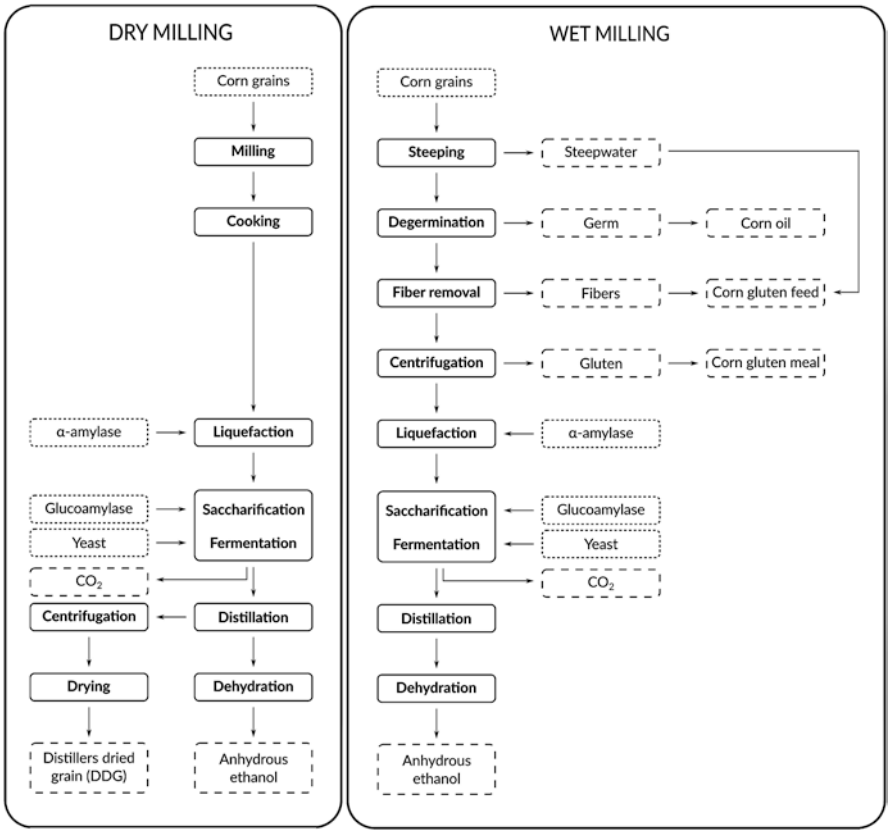

Fig. 11.4 Schematic representation of dry milling and wet milling processes for the production of corn-derived ethanol

starch is sometimes referred to as **liquefaction**, whereas production of Glc monomers from dextrins is referred to as **saccharification**. Both of these enzymes are produced on an industrial scale by microorganisms such as bacteria or fungi (Sauer et al. 2000; Souza and Magalhães 2010); however, new technologies regarding the production of amylases are being developed to reduce industrial production costs. Among the innovations, a variety of corn expressing a thermostable α-amylase has recently been developed by Syngenta (Sainz 2009). Dry milling using grains from these varieties of corn does not require additional microbial enzymes, which reduces processing costs.

Another new technology is "cold hydrolysis," which is the hydrolysis of starch granules at lower temperatures (Cinelli et al. 2015). In this process, the enzymes (mainly α-amylase) catalyze the degradation of starch initially in the outer surface of the granules and then continue catalyzing the reaction radially. The lower temperatures reduce operational costs, eliminate the need to deal with viscous solutions, and reduce the number of undesirable reactions.

Yeasts are added to the fermenter in the fermentation step, which often occurs simultaneously with the saccharification step and allows the conversion of Glc into ethanol and carbon dioxide. This reaction occurs at room temperature in the presence of a nitrogen source (such as urea or ammonium), which is added to help the yeasts grow (Bothast and Schlicher 2005). Certain processing plants also add proteases to break down corn proteins, resulting in the release of amino acids that the yeasts can then consume. During fermentation, the concentration of ethanol in the broth can reach 12%, which limits further fermentation (Schobert 2013). Since carbon dioxide is a by-product of fermentation, certain corn-processing plants capture this gas and sell it for the manufacturing of dry ice or carbonated beverages (Bothast and Schlicher 2005).

In the distillation step, the fermented broth is transferred to distillation columns, where the ethanol is separated from the mixture. This process, however, does not remove all of the water from ethanol (Bothast and Schlicher 2005). The hydrated ethanol is passed through a molecular sieve for dehydration. The resulting anhydrous ethanol can be blended with gasoline, and a denaturant is often also added to discourage human consumption. The remaining broth contains fibers, oils, and proteins; it is centrifuged to remove excess water and then dried to produce DDG (Bothast and Schlicher 2005; Vohra et al. 2014) following the dry milling process. The wet milling process yields only minor amounts of solids after fermentation because most parts of the kernel were removed in previous steps (Vohra et al. 2014).

The unevaporated residue of distillation, called **stillage**, is passed through screens and/or a centrifuge to collect the suspended solids (Wall et al. 1983) as a sludge called wet cake (Wilkins et al. 2006). The remaining turbid liquid is called thin stillage (Wall et al. 1983). Usually, the water in thin stillage is evaporated to form thick syrup, which is then mixed with wet cake to make **wet distillers' grains with solubles** (WDGS). WDGS has a high moisture content (65%) and consequently a shelf life of only 1–2 weeks (Bothast and Schlicher 2005). To increase the shelf life and decrease transportation costs, WDGS may be dried to 10–12% moisture to generate a material of commerce called **dried grains with solubles** (DDGS) (Bothast and Schlicher 2005). DDGS is often used in animal feeds (Wilkins et al. 2006). Over 3.8×10^6 tonnes of DDGS is produced annually from ethanol plants in the USA (Bothast and Schlicher 2005).

Drying thin stillage, however, is not energy efficient. The energy required to evaporate the large amount of water entrapped in thin stillage is a major cost in ethanol production. For example, evaporation of water from stillage consumes about 40–45% of the thermal energy and 30–40% of the electrical energy utilized in a dry-grind facility (Wilkins et al. 2006). According to Meredith, (2003), evaporation of 100,000 lb./h of water requires 1,000 kW of heat. In addition, Wall et al. (1983) stated that the cost of purchase and operation of evaporators is 0.03 $/L (Wall et al. 1983). Therefore, rather than evaporating and using thin stillage for feed, it might prove economically viable to isolate or enrich potentially valuable compounds or fractions from thin stillage. According to Thomas and Ingledew (1992), the mass of stillage is more than four times that of the associated ethanol. If global ethanol production does indeed reach 155 billion liters by the end of 2020 as stated previously,

there would be approximately 620 billion liters of thin stillage produced from the bioethanol industry and thus a very large quantity of liquid waste that could potentially be used for other purposes.

11.4.2 Production of Ethanol from Sugarcane

Sugarcane is a crop that has a high yield of sugar per area, and the sucrose obtained does not need to be broken down like cornstarch. Therefore, the production of sugarcane ethanol requires fewer processing steps than corn-derived ethanol, which reduces investment and operational costs (Vohra et al. 2014). On the other hand, sugarcane is a seasonal crop requiring warm climates, which results in a lack of supply during certain periods of the year and could increase the price of ethanol.

The process for the production of ethanol from sugarcane consists of harvesting stalks, washing to remove impurities, and crushing to extract the juice, which contains the sucrose. The juice undergoes a treatment, whereby high temperatures are applied, and calcium oxide is added to aid in the precipitation of fibers and other impurities (Palacios-Bereche et al. 2014; Vohra et al. 2014). The mixture is then filtered and sent for the production of ethanol and/or sugar depending on the configuration of the plant. Juice sent for ethanol production must be concentrated in evaporators to achieve a high efficiency of fermentation.

Brazil is one of the largest producers of ethanol from sugarcane. In the typical Brazilian fermentation process, also known as the Melle-Boinot process, yeast cells are intensively recycled from one fermentation and then reused in the next one (Zanin et al. 2000). The process, however, leads to very high yeast cell density in the fermenter, which in turn contributes to a very short fermentation time (4–12 h) resulting in a relatively low final ethanol concentration compared to corn-processing facilities. The shorter fermentation duration may maximize the amount of sugarcane processed, which is important because sugarcane is a seasonal feedstock (Vohra et al. 2014; Lopes et al. 2016). This fermentation process is extensively used in Brazil and other countries due to its low operational and production costs, high efficiency, and simple operation (Zanin et al. 2000). The resulting liquor, which contains the ethanol, is then passed through distillation columns to produce hydrated ethanol, followed by a molecular sieve or distillation step with cyclohexane to produce anhydrous ethanol (Dias et al. 2015).

11.4.3 Production of Ethanol from Plant Cell Walls

The general process of producing bioethanol from lignocellulosic materials, which varies in its specifics depending on the type of biomass or technology chosen, begins with the reduction of biomass size to increase the surface area for subsequent chemical and enzymatic reactions (Vohra et al. 2014). The main steps of the process are

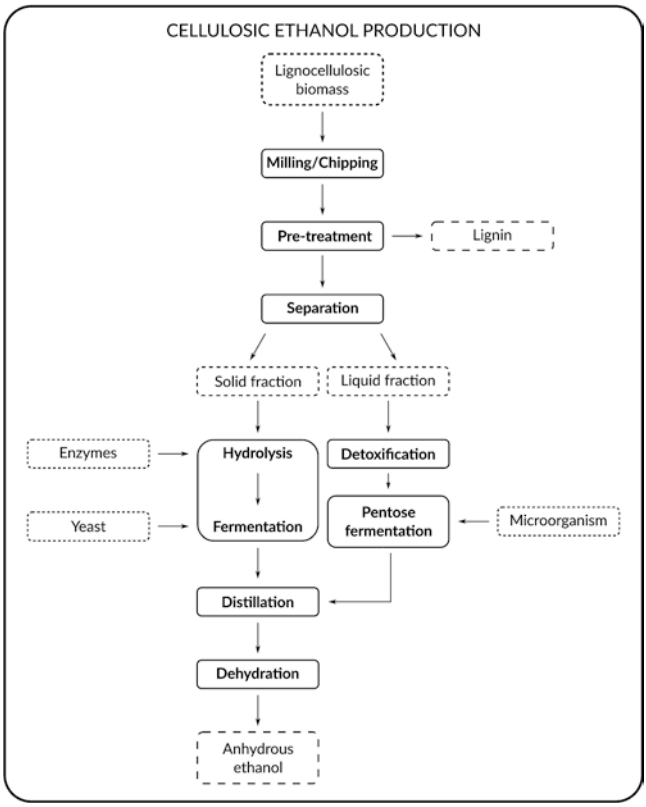

Fig. 11.5 Generic schematic for the production of lignocellulosic ethanol

summarized in Fig. 11.5. The pretreatment of biomass is necessary to expose the cellulose fibers for subsequent enzymatic treatment (Silveira et al. 2015; Kim et al. 2016). Typically, the pretreatment step subjects the biomass to harsh conditions, such as high temperature and pressure, which may cause loss of biomass. In addition, these steps are costly because of the amount of energy required. Due to these reasons, more efficient means of pretreatment still need to be developed to make the production of lignocellulosic ethanol economically feasible. The ideal process would allow efficient hydrolysis without loss of sugars, not require a lot of energy, minimize the number of steps, maximize the recovery of lignin, and not produce inhibitory compounds (Jorgensen et al. 2007; Kim 2013; Silveira et al. 2015; Kim et al. 2016).

Pretreatment methods can be divided into biological, chemical, physical, and chemical/physical methods (Zheng et al. 2009; Bensah and Mensah 2013; Silveira et al. 2015; Kim et al. 2016). Biological methods rely on microorganisms and enzymes to degrade biomass fibers, especially lignin and hemicellulose. They are environmentally friendly processes that do not cause high losses of sugars, but they

require long reaction times for high yields, which increase costs (Silveira et al. 2015). Physical methods are energy-intensive approaches that aim to reduce the size of the biomass and include milling, chipping, and grinding. Chemical methods rely on the use of acids, bases, or organic solvents to break and dissolve fibers. These processes may produce undesirable compounds that affect hydrolysis or fermentation yield and require a further step to neutralize or remove them. In addition, they often require high temperatures, which increase processing costs. A combination of chemical and physical methods produces better results and reduces the cost of pretreatment. For example, milling can be combined with acids, bases, or other catalysts that help break chemical bonds, which saves energy (Silveira et al. 2015). Other chemical/physical methods include the application of steam and ultrasound in combination with use of acids or bases.

Regardless of the pretreatment method, lignin is removed, and the remaining mixture is fractionated to yield a solid fraction (cellulose) and a liquid fraction (a solution of hydrolyzed hemicellulose; Kim 2013; Vohra et al. 2014). Purified lignin can be recovered and sold as a high-value compound or burned to generate power. The liquid fraction contained pentoses and fermentation inhibitors. Once the fermentation inhibitors are removed, the liquid fraction can be used as carbon source for certain microorganisms which can consume pentoses to produce ethanol. The solid fraction can also be used for ethanol production, by first hydrolyzing using an acid or via the catalytic action of enzymes. Both hydroxylation methods, however, have some shortcomings to be overcome. While acid treatment is faster, the process will degrade Glc and thus result in a lower ethanol yield during fermentation. In addition, acid treatment will produce wastewater that has to be further treated (Vohra et al. 2014). The enzymatic method requires the use of cellulases, which are produced by certain microorganisms and are expensive, to break down cellulose. The duration of the enzymatic process is also limiting and requires improvement, since it takes much longer than acid hydrolysis (Vohra et al. 2014). Following fermentation, the broth is distilled to obtain hydrated ethanol, which is then directed to a dehydration unit to obtain anhydrous ethanol.

In order to be as competitive as corn- or sugarcane-derived ethanol, the production of lignocellulosic ethanol must overcome the limitations described above, especially a reduction in the costs of pretreatment and cellulases, as well as the development of processes resulting in high yields of ethanol. In order to achieve this, relevant plant species could potentially be genetically engineered to have altered lignin configuration and/or content, which would reduce the cost of pretreatment (Cheng and Timilsina 2011). Similarly, an alternative to the use of microbial cellulases is the heterologous expression genes encoding these enzymes in plants during their growth (Lambertz et al. 2014). In addition, a proposed configuration called **consolidated bioprocessing** focuses on the simultaneous production of enzymes, saccharification, and fermentation, which would reduce costs by eliminating steps. Finally, improving the productivity of the crops using conventional breeding, biotechnology, and/or superior crop management techniques would increase the amount of cellulose available for ethanol production, which would have a direct impact on the processing prices (Balan 2014).

11.5 Enhanced Biorefinery: Utilization and Refinery of Fermentation By-Products

The treatment and utilization of the large amount of fermentation by-products and liquid waste are challenging; however, with efficient processes and refinery, they have the potential to provide valuable products. Here, thin stillage generated in the ethanol fermentation process is used as an example.

Thin stillage may be used as a medium for dissolving macromolecules present in biomass (Reaney and Ratanapariyanuch 2013) that have mass in excess of a 1,000 molecular weight cut off. These compounds may include proteins, peptides, gums, mucilaginous compounds, polyphenolic compounds, and complex polymers of carbohydrates and gums. Thin stillage also contains numerous ions and organic compounds that are smaller than the molecular weight cut off; these compounds are generally nontoxic and may be recovered following the concentration of the extracted biomass solution.

For example, the manufacture of enriched protein fractions from oilseed meal requires large volumes of treated water, and since thin stillage from ethanol production is available in large volumes, it has been found to be suitable for extracting protein-rich materials (Ratanapariyanuch et al. 2012). The use of thin stillage, in lieu of water, for protein extraction would decrease the energy requirements and waste disposal costs of both the protein isolation and biofuel production processes. Besides the use of thin stillage for protein extraction, it can also be utilized as media for the extraction of other compounds, such as soluble polysaccharide mucilage (Table 11.1).

11.6 Platform Chemicals Arising from the Biorefinery

After using thin stillage to extract macromolecules, organic compounds remain in thin stillage. Ratanapariyanuch et al. (2011) studied the composition and properties of wheat-based thin stillage and found that the osmotic potential of thin stillage was lower than that of water, whereas both the density and viscosity of thin stillage were higher than that of water. The pH of thin stillage was typically 3.7–3.8, and the total

Table 11.1 Primary yield and viscosity of mucilage extracted by 0.5 M $NaHCO_3$ versus thin stillage

Solvent	Time (min)	Grams of mucilage (dry weight)	Viscosity of the mucilage (centipoise)
0.5 M $NaHCO_3$	15	0.61 ± 0.03	3.15 ± 0.07
	30	0.66 ± 0.01	3.20 ± 0.00
	45	0.67 ± 0.01	3.30 ± 0.00
	60	0.68 ± 0.01	3.35 ± 0.07
Thin stillage	30	0.58 ± 0.01	3.25 ± 0.07

Table 11.2 Organic components of thin stillage

Constituent	Concentration (g/L)[a]	Amount of constituent (kilotonnes) from 620 billion liters of thin stillage
Dextrin	10.04	6224.8
Maltotriose	0.62	384.4
Maltose monohydrate	0.59	365.8
Glycerol	5.85	3627
Isopropanol	0.32	198.4
Ethanol	0.30	186
Lactic acid	5.55	3441
1,3-Propanediol	1.22	756.4
Acetic acid	1.27	787.4
Succinic acid	0.79	489.8
Glycerophosphorylcholine	1.00	620
Betaine	0.88	545.6
Phenethyl alcohol	0.29	179.8

[a]Mean values of organic compounds from four batches of thin stillage (Ratanapariyanuch et al. 2011)

Kjeldahl nitrogen was approximately 0.08–0.10% (w/w). The constituents of thin stillage can be categorized into three groups: (1) yeast metabolites including glycerol (Russell 2003), ethanol (Wilkie et al. 2000), succinic acid (Russell 2003), glycerophosphorylcholine (Almaguer et al. 2006), and phenethyl alcohol (Schrader et al. 2004); (2) bacterial metabolites including isopropanol (Lovitt et al. 1988), acetic acid, lactic acid (Chin and Ingledew 1993), and 1,3-propanediol (Cheng et al. 2006); and (3) wheat metabolites such as betaine (Kampen 1993). In addition, yeasts, bacteria, and fungi were also found. Thin stillage was also shown to contain $CaCl_2$, NaCl, K_2SO_4, $NaNO_3$, $Mg(OH)_2$, Na_2SO_4, and KOH. As previously stated, there could be approximately 620 billion liters of thin stillage produced from the bioethanol industry by 2020. Therefore, the amount of organic compounds that could potentially be recovered is very large (Table 11.2), if isolation procedures are developed to recover them.

11.7 Closing Comments

Biorefining is the sustainable processing of biomass into valuable products and energy. Based on the biorefinery concept, it is clear that biomass can be used as material to create new production lines for food, feed, biofuel, power, and chemicals. Value-added compounds could also arise from the biorefinery process. As shown in the description of the processing of seed materials, biorefinery is an efficient process for maximizing the use of plant biomass to produce valuable bioproducts. Depending on the type of starting biomass, it may be possible to produce food and feed in addition to industrial bioproducts. For example, the biorefining of the

seed of a *Brassica* oilseed species can potentially lead to production of oil for biodiesel production and meal to serve as feed for livestock. In addition, along with the development of agricultural biotechnology and the requirement of bioproducts with different properties, biorefinery processes are accordingly under continuous development and optimization.

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Chapter 12
The Food Versus Feed/Fuel Debate

Stuart J. Smyth and Simona Lubieniechi

Chapter Highlights

- Over a short period of time beginning in 2007 and stretching into 2008, there was a dramatic rise in commodity prices.
- As a result of the increased commodity price of corn, rice, and wheat, food products based on these commodities also began to rise in price.
- Many nongovernmental organizations were quick to accuse the increased production of biofuels as the reason for the increased food prices – since more corn was being used to produce ethanol, there was less corn going into food production, hence the increase in prices.
- The debate is ongoing as to how policies that encouraged increased biofuel production and the events of the food price crisis are related and whether the cause was as simple as critics have claimed.

12.1 Introduction

Scientific principles such as "nothing happens in a vacuum" or "that for every action, there is an equal and opposite reaction" can also be extended into the realm of government policy, meaning that the implementation of government policy can and will impact other sectors of the economy and not necessarily in ways that were intended or even envisioned. In the decade of the 2000s, numerous industrial country governments began to implement domestic policies that supported development and production of biofuels, particularly ethanol. The rationale for this was twofold: first, it was part of many domestic governments' commitment to addressing climate

S. J. Smyth (✉) · S. Lubieniechi
Department of Agricultural and Resource Economics, University of Saskatchewan, Saskatoon, SK, Canada
e-mail: stuart.smyth@usask.ca

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change and, second, to reduce domestic dependence on fossil fuels. One event that occurred in a period that stretched from early 2007 to the fall of 2008 was a sharp increase in food prices. Many, especially within the media, were quick to accuse the expansion of corn-based ethanol production as being the cause for the increase in food products. Others, such as the biofuel industry and government departments that supported increased biofuel production, were quick to respond with opposing viewpoints. The objective of this chapter is to provide a detailed analysis of the literature from both sides of the spectrum.

Biofuels are frequently championed as an alternative to petroleum-based fuels and a potential climate change mitigation strategy. The expansion of biofuel production, however, has raised considerable debate among the media and in academic literature about whether biofuels actually lower greenhouse gas (GHG) emissions, their impact on food security, and the extent of environmental impacts (Pimentel and Patzek 2005; Farrell et al. 2006; Runge and Senauer 2007; Bailey 2008; Nuffield 2011). In spite of these criticisms, governments in many countries have implemented both biofuel mandates and policies, supporting the development of biofuels. Industry statistics show that the United States (USA), Brazil, and the European Union (EU) are the leading biofuel producers (Renewable Fuels Association [RFA] 2017a). In the USA, biofuel production consists mainly of ethanol from corn, in Brazil of ethanol from sugar cane, and in the EU of biodiesel from rapeseed oil.

Canada currently has 17 operating ethanol plants with a total capacity of 1.85 billion liters per year (Ethanol Producer Magazine 2017) and 9 operating biodiesel plants with a total capacity of 586 million liters per year (Biodiesel Magazine 2017). As of 2017, three ethanol plants and two biodiesel plants were planned to be built or were under construction. The main feedstocks used for domestic ethanol production are corn and wheat. There are operational facilities that can produce commercial-scale levels of ethanol from wood waste and municipal waste but because of economic reasons produce methanol instead (Dessureault 2016). Based on previous production data, Dessureault (2016) estimated that in 2016, 75% of Canadian ethanol was derived from corn, 23% from wheat, and 2% from other feedstocks. Most biodiesel is produced from canola, animal fats, and cooking oils (Canadian Renewable Fuels Association[1] [CRFA] 2010). The main ethanol feedstocks are corn in Ontario and wheat in Western Canada.

The 2007–2008 spike in food prices had impacts around the world. Protests, and even riots, against the higher price of staple commodities were commonplace in numerous nations, predominantly developing nations. In many instances the rise in the price of basic staples, such as rice and corn-based food products, adversely affected low-income segments of society. Many of the vocal opponents to this rise in food prices were quick to blame biofuel policies of industrial countries, particularly the USA, as being the reason for the price rise. In-depth analysis and assessment of this issue required considerable amounts of data to begin to make any kind

[1] Established in 1984 as a nonprofit Canadian organization that promotes the role of renewable fuels and value-added products made from renewable resources, the Canadian Renewable Fuels Association was relaunched in 2016 under the name Renewable Industries Canada – RICanada.

of observation, and in reality identifying a single driver responsible for the price increases was extremely difficult.

This chapter provides the background to biofuel policies in the leading biofuel-producing nations, of the USA, the EU, Brazil, as well as Canada. This is followed by a contextual overview of what occurred regarding commodity prices in the 2007–2008 period. Subsequently, a discourse of the price spike assessments is offered, providing insights into just how complicated an issue this turned out to be.

12.2 Background

The global production of biofuel has known periods of both fast and slow growth, depending on the applied policy support and feedstock availability. Total world biofuel production has increased almost 5 times between 2002 and 2012, from 23 billion liters to 110 billion liters (United Nations Conference on Trade and Development [UNCTAD] 2016). Among the developed countries, the major producers are, and are expected to remain, the USA, Brazil, and the EU. In 2011, the USA and Brazil produced approximately 87% of biofuel production, while Canada contributed 2% (Campbell et al. 2016). The most recent data from 2015 shows the USA and Brazil producing 70% of the world biofuel production (Araújo et al. 2017). According to the Organisation for Economic Co-operation and Development [OECD]-Food and Agricultural Organization [FAO] (2017), global ethanol production was 120 billion liters in 2016, and it is projected to increase to 137 billion liters by 2026. This increase is mainly due to Brazil, with a projected ethanol contribution of 60%, followed by the USA, China, and Thailand. World biodiesel production is projected to increase from 37 billion liters in 2016 to 40.5 billion liters by 2026. At a global level, the main feedstocks used in ethanol production are corn and sugar crops, while vegetable oil and municipal waste are mainly used in biodiesel production. OECD-FAO (2017) estimates that ethanol production will use 15% and 20% of world corn and sugar cane production, respectively, in 2026; ethanol obtained from biomass is expected to account for 0.5% of world ethanol production.

In the USA and EU, production and consumption are driven by the policies in place, the renewed Renewable Fuel Standard (RFS2) in the USA and the Renewable Energy Directive (RED II) in the EU. In Brazil, at present, production and use are driven by the development of flex-fuel vehicles and the differential taxation system which favors hydrous ethanol (OECD-FAO 2017).

12.2.1 Canada

The Canadian biofuel industry expanded following the turn of the millennium, largely due to federal policies targeting solutions for climate change, energy supply diversification, and rural development (CRFA 2010). Federal renewable fuels

initiatives such as policy redesign, excise tax exemptions, and research grants were implemented to facilitate this growth. The Canadian government's Renewable Fuels Strategy has four components (Government of Canada 2017a). The first, "Increasing the retail availability of renewable fuels through regulations," is led by Environment and Climate Change Canada and refers to the 5% ethanol and 2% biodiesel **blending mandates**, implemented in December 2010 and June 2011, respectively. The second, "Supporting the expansion of Canadian production of renewable fuels" (named ecoENERGY for Biofuels), is comprised of investment initiatives (C$1.5 billion) to boost biofuel production, as well as eliminating excise tax exemptions for biofuels. In 2013, the Canadian government ceased subsidies for building new ethanol and biodiesel plants as biodiesel producers failed to meet production targets (McCarthy 2013). Only existing commitments were met until the program's expiration in 2017. Currently, the program is geared toward operating incentives for biofuel producers, based on production and sales volumes. Also, since 2008, the program implemented incentive rates for renewable alternatives to gasoline (starting at C$0.10/L) and for renewable alternatives for diesel (starting at C$0.26/L) (Natural Resources Canada 2016a). This program is being gradually phased out as the rates have declined to C$0.03 for ethanol and C$0.04 for biodiesel (OECD-FAO 2017).

Third, "Assisting farmers to seize new opportunities" comprised of two parts: ecoAgriculture Biofuels Capital Initiatives – ecoABC – and Agri-Opportunities Program. The ecoABC program involved making capital available (C$200 million in repayable capital) to farmers for building or expanding production facilities and for developing business proposals and feasibility studies. This program ended in March 2013 at which point it had invested C$159 million. The Agri-Opportunity Program was a C$134 million program that provided funds to accelerate the commercialization of innovative value-added agricultural, agri-food and agri-based products, services, and processes not commercially produced or available in Canada. This program ended in March 2011.

The final part of the strategy, "Accelerating the commercialization of new technologies," the Next-Generation Biofuels Fund (NextGen Biofuels Fund), is available for producing **second-generation or advanced biofuels**. Sustainable Development Technology Canada (SDTC) is able to invest with the private sector in establishing large-scale demonstration facilities for the production of next-generation renewable biofuels and coproducts from nonfeed stocks (Natural Resources Canada 2016b). At a federal level, Canada's Renewable Fuels Strategy requires a blend of 5% renewable content in the Canadian gasoline pool and a blend of 2% renewable content in the distillate pool, excluding heating oil (CRFA 2014). Provincial government mandates and initiatives, however, preceded the federal ones which helped eliminate interprovincial trade distortions (Dessureault 2016).

For instance, provincial governments have supported biofuel development through reductions in provincial fuel taxes for gasoline containing ethanol (Manitoba), exemptions from excise taxes (Ontario), grants for ethanol blend distributors (Saskatchewan), refundable tax credits for ethanol production (Quebec), capital assistance for ethanol production facilities, operating grants, and numerous other forms of support. Federal and provincial blend mandates for ethanol and

Table 12.1 Provincial and federal biofuel blend mandates in Canada

Province	Ethanol	Date of adoption	Biodiesel	Date of adoption
Ontario	5%	January 1, 2007	2–4%[a]	April 1, 2014
Saskatchewan	7.5%	January 15, 2007	2%	July 1, 2012
Manitoba	8.5%	January 1, 2008	2%	November 1, 2009
British Columbia	5%	January 1, 2010	4%	January 1, 2012
Alberta	5%	April 2011	2%	April, 2011
Quebec	5%	2012[b]	n/a	n/a
Federal	5%	December 15, 2010	2%	July 1, 2011

[a]Depending on the amount of greenhouse gas emissions reductions
[b]This is not a mandate, it is a planned target to be implemented with second generation ethanol
Sources: Adapted from CRFA (2010), Biofleet (2011), Dessureault (2016), Sapp (2014)

biodiesel are shown in Table 12.1. Canadian subsidization for biodiesel has lagged behind ethanol, so the biodiesel industry has taken longer to develop and is smaller than the ethanol industry (Laan et al. 2009). At a provincial level, the ethanol mandates range between 5% and 8.5%, and the biodiesel mandates range between 2% and 4% in British Columbia, Alberta, Saskatchewan, Manitoba, and Ontario. Quebec has a 5% aspirational mandate to be implemented with advanced ethanol. In addition to the mandates, British Columbia adopted a low-carbon fuel standard in 2008 based on a requirement to reduce the carbon intensity of fuels by 10% by 2020 from a 2010 baseline (Government of Canada 2017b). For both ethanol and biodiesel, Atlantic Canada and Quebec are exempt from the federal gasoline policy (CRFA 2014).

The Canadian ethanol blending mandates require over 2 billion liters of fuel-grade ethanol. As of 2016, Canadian biofuel production capacity was 1.775 billion liters, which means that Canada has to import the difference (Dessureault 2016).

The CRFA annual report (CRFA 2010) illustrated the challenges of building a biofuels industry from scratch, in the absence of, or certainly very limited, domestic market demand. Canadian biofuel subsidies have been provided through a mix of policy regulations and measures such as direct payments, tax exemptions, interest-free loans, grants, and consumption mandates (Table 12.2). Because information was scarce on subsidized production, sales, and payments which the Canadian biofuel industry had received, Laan et al. (2009) performed a comprehensive analysis of the subsidization process and range of payments.

Compared to investment and support in the USA and Brazil, biofuel subsidies in Canada are more recent and modest. Relatively low subsidies were targeted at ethanol research in the mid-1980s, followed by excise tax exemptions and investment incentives in the 1990s (Laan et al. 2009). A major federal policy strategy, the Ethanol Expansion Program was launched in 2003, offering loans for the construction of new ethanol plants. From 2006 to 2008, biofuel support was directed to virtually all stages of the supply chain, averaging C$300 million per year (Laan et al. 2009). Thus, the mandates implemented at a federal and provincial level, along with the subsidies and support for the domestic Canadian industry, have created

Table 12.2 Examples of government biofuel support in Canada

Stage of production	Subsidy types
Research, development, and demonstration	Grants and low-interest loans
Business planning	Grants for feasibility studies and market development
Plant construction	Grants and low-interest loans, accelerated depreciation
Production	Fuel tax exemption, producer payments
Price support	Mandated biofuel blending requirements and tariffs
Distribution	Grants for storage and distribution infrastructure
Operating incentives	Incentive payments paid on a per liter of biofuel basis
Consumption	Tax breaks for the purchase of biofuel-consuming vehicles, government procurement and dissemination of information to consumers

Source: Adapted from Laan et al. (2009) and Natural Resources Canada (2016a)

a policy-driven demand for ethanol in Canada (Campbell et al. 2016). Regarding advanced biofuels, the federal and provincial governments have initiated various research and development investments over time (Table 12.3). Advanced biofuels are produced from lignocellulosic plant materials such as agricultural residues, forestry by-products, and energy crops grown on marginal lands. Also municipal waste and algae are used for producing advanced biofuels. Canada has the advantage of a large stock of lignocellulosic biomass from agricultural waste and tree biomass, but competing with the Brazilian or American industry on this market raises serious challenges.

In Canada, the federal NextGen Biofuels Fund promotes the development of first-of-a-kind large demonstration scale facilities for the production of advanced biofuels (Sustainable Development Technology Canada [SDTC] 2018). The initial endowed budget was approximately $500 million. As of 2018, this fund stopped accepting applications, but will continue supporting existing projects until 2017 (SDTC 2018).

By comparison, in the USA, between 2007 and 2014, departments of Defense, Agriculture, and Energy provided more than US $1.7 billion in grants and loans for advanced biofuels along with tax incentives, government policies, and price incentives (UNCTAD 2016). In 2014, a public-private partnership between the European Community and the Bio-Based Industries Consortium, called the Bio-Based Industries Joint Undertaking, provided €3.7 billion to support converting biomass into common consumer products through innovative technologies by biorefineries (Flach et al. 2017).

However, despite the ongoing support for advanced biofuels, in the most recent OECD-FAO biofuels report (2017), one of the main world issues listed is that, due to the lack of policy signals and the low energy prices, investment in research and development for advanced biofuels produced from lignocellulosic biomass, waste, or nonfood feedstock is not supported. The main barriers for investment are the high research and development costs, production costs, and the regulatory uncertainty (Flach et al. 2017).

Table 12.3 Various Canadian federal government early support for second-generation biofuel research and development

Year	Total amount invested (million)	Program/initiative
Mid-1980s to Mid-1990s	$18	National Research Council Natural Resources Canada and Agriculture and Agri-Food Canada supported research by Iogen corporation to develop cellulosic ethanol production technologies
1999	$18	The federal government provided partial funding for Iogen's $40 million commercial-scale demonstration plant through loans repayable from future profits
2002	$2.7	Federal government awarded to Iogen a cost-shared research contract
2004	$550	SD tech fund managed by Sustainable Development Technology Canada (SDTC) provided $19 million between 2004 and 2008 for second-generation ethanol
2006	$145	Agricultural Bioproducts innovation program (ABIP) was a multiyear grant program to support new and existing research networks in the areas of bioproducts and bioprocesses, in addition to biofuels and other forms of bioenergy. ABIP funded the cellulosic biofuels network with $19.9 M in 2009 to develop a network to provide expertise, technology, and processes associated with cellulosic ethanol production
2007	$500	NextGen biofuels fund provided interest-free loans for large-scale demonstration facilities producing second-generation biofuels
2007	$134	Agri-opportunities Program provided funds for commercialization of new agricultural products, processes, or services

Source: Adapted from Laan et al. (2009)

For the most part, Canadian policies to support the development of the biofuels industry have been the implementation of biofuel blend mandates and the use of fiscal stimulus packages to encourage the advancement of biofuel processing technology as well as infrastructure investment regarding the construction of biofuel plants. As noted above, Canada is not considered a large player in the global biofuel market and the blend mandates and policy decisions would have had, at best, a minimal impact on the increase in food prices.

12.2.2 USA

American biofuel mandates require renewable fuels that satisfy environmental sustainability criteria such as GHG emissions savings relative to fossil fuels and **indirect land use change** (ILUC) restrictions. In 2005, the USA created the Energy Policy Act which was driven by the need to increase US energy security. This Act

implemented renewable fuel blend mandates as a means of reducing the existing dependence on foreign oil imports. Other main policy drivers were promoting rural development through job creation, mitigating climate change through GHG emissions reduction, and enhancing competitiveness through innovative technologies (Mondou and Skogstad 2012). As of 2016, the gasoline consumed in the USA contained more than 10% ethanol on average (Renewable Fuels Association [RFA] 2017b). This increase in gasoline average ethanol content is attributed to the growing consumption of E15 (gasoline blends containing 15% ethanol), mid-level blends (20–50% ethanol), and flex fuels (51–83% ethanol) (RFA 2017a). In the USA, the two major low-carbon fuel policies in place are the Renewable Fuels Standards (RFS)1 created under the EPA in 2005 and the revised RFS or RFS2 created in 2010. The renewable fuel categories under RFS1 and RFS2 are biomass-based diesel, cellulosic biofuel, advanced biofuel, and total renewable fuel. RFS1 was created under the Energy Policy Act, which amended the Clean Air Act. The Energy and Security Act (EISA) of 2007 further amended the Clean Air Act by expanding the RFS to what is known as the revised RFS (US-EPA 2017).

The original RFS1 required 28.3 billion liters of renewable fuel to be blended in gasoline by 2012. When RFS1 was expanded under the EISA, the volume of renewable fuel to be blended into transportation fuel increased from 34 billion liters in 2009 to 36 billion liters by 2022, with 57 billion liters of corn ethanol and 21 billion liters of advanced biofuel by 2015 and 79.5 billion liters of advanced biofuels by 2022. Another important regulation regarding biofuel sustainability was the introduction of life cycle GHG performance threshold standards, ensuring that each category of renewable fuels emits fewer GHG than the fuel it replaces.

In 2010, under the RFS2 program, many regulations were included, clarified, and expanded, as the RFS2 program covers all transportation fuels used in road, rail, and marine transportation. The RFS2 requires a combined use of 140.4 billion liters of biofuels by 2022, of which 58.5 billion liters are conventional first-generation (corn ethanol) biofuels and 81.9 billion liters from advanced (cellulosic, biomass-derived diesel, and others) biofuels. The RFS2 requires specific GHG emission reductions depending on the fuel category, 20% for **first-generation biofuels**, 50% for advanced (second-generation) biofuels, and 60% for cellulosic (third-generation) biofuels. The methodology used for calculating GHG emissions should include all life cycle GHG emissions of fuel, including ILUC emissions (Scarlat and Dalemand 2011). The US Renewable Fuel Standard requires the creation of credits, representing volumes of renewable fuels, and has a credit trading system (Government of Canada 2017b).

The Environmental Protection Agency (EPA) annually provides minimum quantities for each of the four classes of biofuels required. In 2013 and 2017, the EPA decided to reduce total, advanced, and cellulosic mandates as the production capacity for cellulosic ethanol has been lagging (OECD-FAO 2015, Prentice et al. 2017).

American policy was targeted at setting aggressive blend mandates for the inclusion of biofuels. Biofuel production was subsidized through these policies, many new biofuel plants were constructed, and considerably greater levels of corn production were witnessed. The reality of the RFS1 policy was that the blend

requirements for second-generation biofuels were simply too aggressive and the industry was not able to meet these mandates due to the technology gap that exists in converting cellulosic materials and waste into biofuels in an economical and efficient manner.

12.2.3 EU

The EU strategy for biofuels industry development is driven by motivating policy incentives, with the three main policy drivers being energy security, the continuous effort to reduce GHG emissions, as well as rural development interests (Swinbank 2009).

The EU RED requires that 20% of overall EU energy consumption be sourced from renewables, and a mandatory 10% minimum target for all member states for the consumption share of renewable energy in transportation Paragraph 18 of RED defines the 10% target as that share of final energy consumed in transport, which is to be achieved from renewable sources as a whole, and not solely from biofuels. In addition, second-generation biofuels' contribution to the target is twice that made by other biofuels (Article 21(2)).

The EU sustainability criteria came into effect in December 2010 in Articles 17, 18, and 19 of the RED (European Commission 2009). The criteria are concentrated on GHG savings, high-biodiversity value land, high-carbon stock land, and agro-environmental practices. The European renewables framework focuses on promoting renewable energy, setting mandatory national renewable energy targets, such as achieving a 20% share of renewable energy in final energy consumption and a 10% share of energy from renewable sources in transport by 2020. These goals contribute to the European 2020 growth strategy as they "contribute to Europe's industrial innovation and technological leadership as well as reducing emissions, improving the security of our energy supply and reducing our energy import dependence" (European Commission 2013). The 2013 European Commission report on the progress of renewable energy, however, observes that, after a good start to the project, there has been a slower than expected removal of barriers to renewable energy development, while some member states need to take additional efforts to achieve the proposed goals. The progress analysis reveals that the European economic crisis along with the administrative and infrastructure barriers, coupled with policy and support schemes disruption, are responsible for the target achievement delays.

The European energy market is not open and competitive. The current policies strive to compensate for market failures through the use of support schemes, standards, and administrative rules designed to promote renewable energy development. The European Commission (EC) report (2013) observes that the planned trajectory for biofuels production for 2020 will also result in a deficit. Thus, considering that one of the amendments that the Commission proposed for biofuels was a greater use of nonfood feedstock, additional measures will be required to achieve the 2020 targets.

The 2009 RED initially required a 10% share of renewable energy use in the transportation sector by 2020. Related to this is the Fuel Quality Directive 2009/30/EC which set a 6% reduction in the GHG intensity of fuel used in transportation by 2020. Shortly following the implementation of this directive, numerous stakeholders expressed concerns that such a high mandatory usage of first-generation biofuels would lead to a massive cultivation of biofuel crops, either to the detriment of existing agricultural production or the expansion of cropland (Ernst and Young 2011). This is known as ILUC and relates to the unintended consequences of releasing a substantial amount of carbon emissions into the atmosphere as a result of changing the land use to the dedicated production of ethanol feedstocks or biodiesel crops. In October 2012, the EC amended the existing legislation on both the RED and the Fuel Quality Directive, capping the share of first-generation biofuels that can be used at 5%, down from the 10% renewable energy target by 2020 (European Commission 2012). One of the motivations of this amendment is to stimulate the development of advanced (second- and third-generation) biofuels that will further reduce GHG emissions. Additionally, ILUC factors will be considered when assessing the GHG performance of biofuels. Market incentives for biofuels with no, or low, ILUC emissions in particular for second- and **third-generation biofuels** were provided. The aim of the biofuel sustainability criteria is to prevent the direct conversion of forests, wetlands, and areas with a high biodiversity value to biofuel production.

RED was amended in 2015 by an EU Directive to a 7% cap on the share of conventional biofuels and a not-binding national target for advanced biofuels of 0.5%. In November 2016, the EC released the RED II for the period 2021–2030 to ensure that the EU will adhere to the target of producing at least 27% of its energy from renewable sources by 2030 (International Council on Clean Transportation [ICCT] 2017). To minimize the ILUC impacts, the RED II introduces a cap on conventional biofuels, toward the EU renewable energy target, starting at 7% in 2021 and going down gradually to 3.8% in 2030. In addition, the RED II encourages the use of advanced biofuels with a minimum share of 1.5% in 2021 to 6.8% by 2030. Furthermore, advanced biofuels should emit at least 70% fewer GHG emissions than fossil fuel (ICCT 2017).

The EU ethanol production rose from 5.1 billion liters in 2011 to an estimated 5.3 billion liters in 2015 (Flach et al. 2017). Cellulosic ethanol production began in 2014 with 50 million liters and is estimated to increase to 60 million liters in 2018. The most used feedstocks for producing ethanol are sugar beets, wheat, corn, and rye.

Biodiesel is the most important biofuel in the EU, and it has been commercially produced since 1992. The EU is the world's largest biodiesel producer followed by the USA, Argentina, Brazil, Indonesia, and Thailand (OECD-FAO 2017). Vegetable oil and municipal waste are the main feedstock sources for biodiesel production.

With the majority of the technology development in Europe focused on biodiesel as the preferred biofuel energy options, the EU imports considerable amounts of ethanol. The increased ethanol demands did put pressure on other markets to increase their ethanol production to serve the EU demands. As part of the EU's

policy approach to the production and importing of ethanol, the EU has implemented some of the most rigorous land use regulations in existence. Not only can land use not change to increase ethanol production, that is, land that was not previously in cultivation cannot begin to be cultivated to produce biofuel crops, but the EU imposes many of its land use restriction on the nations that it imports ethanol from. The European policy approach to biofuels could be said to be largely driven by the environmental improvement criteria and climate change mitigation strategies (European Commission 2012).

12.2.4 Brazil

Until a few years ago, Brazil was the global leader in ethanol production and exports. Brazil is considered to be the worlds' most efficient ethanol producer (Sorda et al. 2010; Solomon 2010), even though it took a long time and supportive policies to achieve this. Brazil's advantages consist of its history of ethanol use, the well-established infrastructure, and its low-cost feedstock, sugarcane. Although the Brazilian biofuel industry was initially subsidized, particularly for building the infrastructure, the industry is viable without large government subsidies (Nass et al. 2007, Solomon 2010).

The expansion of the biofuel industry has also been driven by the development of the flex-fuel vehicle industry (OECD-FAO 2012), which began in 1970 and was launched through the Proalcohol Programme. The program implemented tax incentives and final consumer prices for both cars and ethanol (Kamimura and Sauer 2008). The Brazilian car manufacturing industry developed flexible-fuel vehicles over time that can run on increasingly higher percentages of ethanol. The growing Brazilian flex-fuel fleet can be powered by hydrous ethanol (E100), a mixture of ethanol and a small quantity of water, with no gasoline added. Another option for ethanol use in these vehicles is gasohol, a mixture of gasoline and 20% or 35% hydrous ethanol (Wisner 2012). The Brazilian ethanol mandates were initially increased from 18% to 25%, decreased in 2011 to 20% due to a low sugarcane production and a low ethanol supply, and then increased again at 27% in 2015 (OECD-FAO 2015). The 27% mandate and a differential taxation system in some Brazilian states, which favors hydrous gasoline in relation to gasohol, drive the ethanol production in Brazil (OECD-FAO 2015). Brazilian ethanol production is thus projected to increase from 29.2 billion liters in 2016 to 36.3 billion liters in 2026 (OECD-FAO 2017).

As of 2017, Brazil is the third biodiesel producer in the world, contributing 36% to global production, and it is projected that it will maintain its position (OECD-FAO 2017). The Brazilian biodiesel mandate gradually increased from 2% in 2006 to 8% in 2017 and a proposed 9% and 10% in 2018 and 2019, respectively. If technically feasible, it is intended to increase the mandate to 15% (Barros 2016). Brazilian biodiesel production was 4 billion liters in 2015 (Barros 2016), and it is projected to increase to 5.4 billion liters by 2026 (OECD-FAO 2017).

With the intent of reaching the GHG emissions targets set at the United Nations Framework Convention on Climate Change in Paris 2016, Brazil has announced the intention to implement the RenovaBio program which, as of November 2017, has not been approved yet by the Brazilian congress. Through RenovaBio, Brazil is supposed to reduce its GHG emissions by 43% of 2005 levels by 2030. Within this program, producers and importers will be issued decarbonization credits which will be traded on an exchange market. Fuel distributors will have to achieve their own individual decarbonization goals (Phillips 2017).

Brazil's domination of biofuel production and export was surpassed by that of the USA. Brazil has a long history of biofuel use and has developed and promoted the growth of the industry through the use of government policy designed to support a domestic vehicle development program. The use of fiscal and economic incentives has created a strong domestic industry that is now witnessing a removal of the original subsidies to industry. Brazil's ethanol production advantage lies in its abundance of sugarcane, the most economical feedstock available for ethanol production.

12.3 Food Price Crisis of 2007–2008

Over the 24-month period from the start of 2007 to the end of 2008, global food prices witnessed a drastic increase, especially cereals. The rise in food prices is best reflected from the increase in the Food and Agriculture Organization's Food Price Index.[2] The **Food Price Index** (FPI) is a combined measurement of commodity prices for meat, dairy, cereals, oils and fats, and sugar. By collecting commodity prices over time, it is possible to compile a price index that reflects the average trading prices for commodities at any specific moment in time. The overall FPI rose from an average of 158.9 in 2007 to an average of 199.8 in 2008 (FAO 2013) – a rise of 26%. It was the **Cereals Price Index** (CPI) that rose the most sharply and accounted for most of the increase. The CPI, which is comprised of cereal and rice trading prices, rose from a 2007 average of 166.9 to a 2008 average of 237.8, an increase of 43%. In 2009, the FPI dropped by 21% with the CPI dropping by 27%.

A more detailed examination of what has been dubbed "the price crisis" reveals the rise in prices from the start of 2007 to the peak of prices in 2008, to be even sharper. The FPI rose from 134 at the start of 2007 to a peak at 224.4 in June 2008, an increase of 67%. The rise in the CPI was considerably more dramatic, rising from 144 at the start of 2007 and peaking in April 2008 at 274.1, a rise of 90%. Appeals for food aid were issued by 36 countries (US Department of State 2011). The rapid rise and decline in both indexes over the period of 2007–2008 are illustrated in Fig. 12.1. Ultimately, these events triggered the FAO's November 2009 World Summit on Food Security.

Delving down a step further by more closely examining the price increase of the three staple food commodities of corn, rice, and wheat, it is possible to gain further

[2] Online at: http://www.fao.org/worldfoodsituation/foodpricesindex/en/

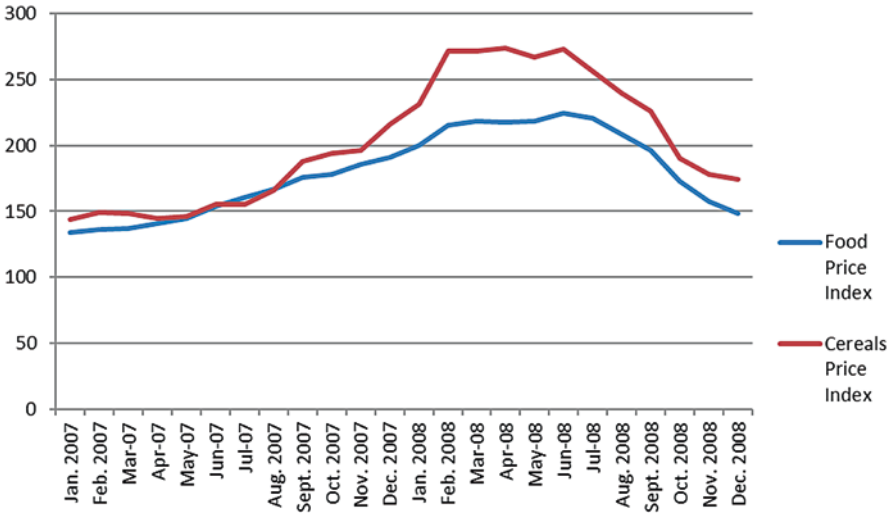

Fig. 12.1 Food and cereal price indexes, 2007–2008 (Source: FAO 2013)

insight into the hardships that resulted from the price increases. These three commodities account for the dominant intake for household nutrition on a daily basis. An in-depth assessment of the food crisis by the International Food Policy Research Institute found that in some nations, monthly year-over-year price increases were in excess of 100% in some instances (Headey and Fan 2010). A selection of examples of commodity price increases is provided in Table 12.4.

Rice prices rose rapidly throughout the fall of 2007 and the early months of 2008. A report by the Economic Research Services (ERS) of the US Department of Agriculture (USDA) indicates that the rice benchmark for global trading prices (Thailand's 100 Percent Grade B long-grain milled rice) was trading in excess of US$1,000 per ton in April of 2008 (USDA-ERS 2009). This price was double the trading price in February 2008 and triple that of November 2007. The percentage of rice price increases between the second quarter of 2007 and the second quarter of 2008 is documented in Fig. 12.2.

Increasing corn prices impacted most Latin American countries, particularly Mexico. Many of Mexico's poor rely on tortillas as their main dietary staple. While specific data is not easily discernible, one study estimates that between 2005 and 2011, the price of tortillas rose by 69% (Wise 2012). Wise goes on to estimate that the **Tortilla Price Index** rose from a level of 110 in the fall of 2006 to a level of 130 by the summer of 2008, an increase of 18% in less than 2 years.

The third staple commodity, wheat, also witnessed record price increases during this period. Using the commodity trade benchmark of US No. 1 hard red winter wheat, the price of wheat rose from US$195 per tonne in May 2007 to a high of US$440 per tonne in March 2008 (Index Mundi 2013). This represents an increase of 125% in less than 12 months.

Table 12.4 Staple commodity price changes, 2008

Country	Commodity	Average price increase, M-07 to M-08
Thailand	Rice	75%
Haiti	Rice	27%
Nicaragua	Corn	38%
Bolivia	Wheat	41%
Chile	Rice	46%
Bangladesh	Rice	31%
Ethiopia	Corn	115%
Uganda	Corn	109%
Malawi	Corn	116%
Tanzania	Corn	73%
Afghanistan	Wheat	71%
India	Rice	16%

Source: Adapted from Headey and Fan (2010) with permission from the International Food Policy Research Institute (www.ifpri.org)

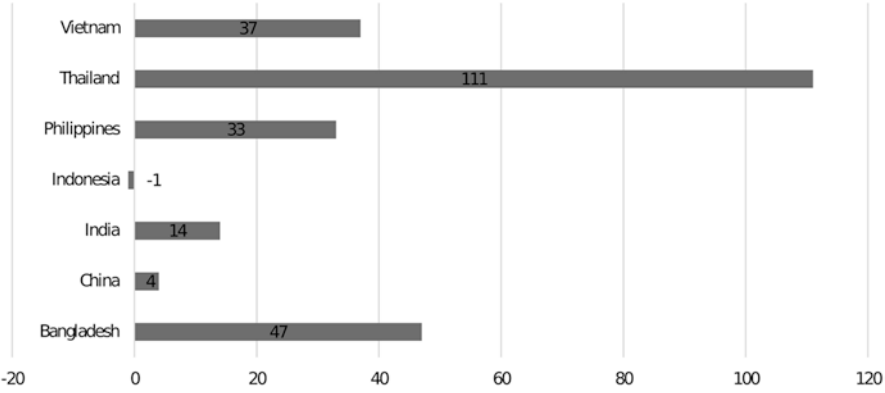

Fig. 12.2 Rice price increases, 2007–2008 (quarter two, year-over-year, % age) (Source: Dawe 2009)

Since commodity prices around the world rose drastically in such a short period of time, substantial price increases resulted for many staple food products based on rice, corn, and wheat. Civil unrest occurred in many nations due to the inability of the poorest in society to be able to afford to buy these food products at inflated prices. These rapid price increases were quite unexpected and triggered great consternation within many governments and international organizations and agencies.

The chapter now turns to discussing what reason(s) or causes were responsible for the food price crisis. The gist of the broad debate on the topic can be condensed into the following question: Did the increase in US biofuel production trigger the

food price crisis? While many commentators were quick to come to this conclusion, time and factual-based research has shown that the correlation is not as evident as critics have assumed.

12.4 Analysis of the Food Price Crisis

Without a doubt, biofuel production around the globe dramatically increased during the first decade of this century. While the EU focuses much of its domestic technology development on biodiesel, it still imports vast quantities of biofuel. Canada is not a significant player in the international biofuel market, so increased biofuel demands in Canada had minimal impact at an international level. Brazil's production increased, as did production in the USA. The increase in the amount of corn production being utilized by the American biofuel industry is highlighted in Fig. 12.3. The percentage of US corn production increased from 5% at the turn of the millennium to almost 40% by the end of the decade. Correspondingly, the production of corn has also increased as is shown in Fig. 12.4.

Critics of industrial agriculture were quick to conclude that the food price crisis was directly triggered by the US increase in biofuel production, as increasing amounts of corn were being diverted away from food use to biofuel use. While there are numerous examples to draw upon of the arguments put forth by the critics of the biofuel industry, a report released by ActionAid International provides the essence of these arguments. ActionAid is an antipoverty nongovernmental organization (NGO) that is based in the UK. In a report that is typical of NGO criticism of many North American agricultural practices, ActionAid (2012: 3) states, "There is widespread agreement among experts that the recent surge in global biofuels production has been an important contributor to the rise in global food prices over the last six years.… The increase in corn ethanol production in the US has contributed to rising corn prices in several ways." Unfortunately, ActionAid provides no details as to who the experts are, or for that matter, what qualifies them to be experts. The fact that

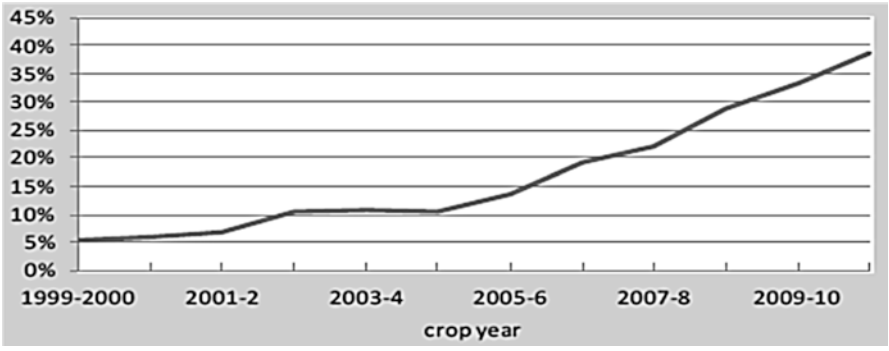

Fig. 12.3 Ethanol share of US corn production (Source: Wise 2012)

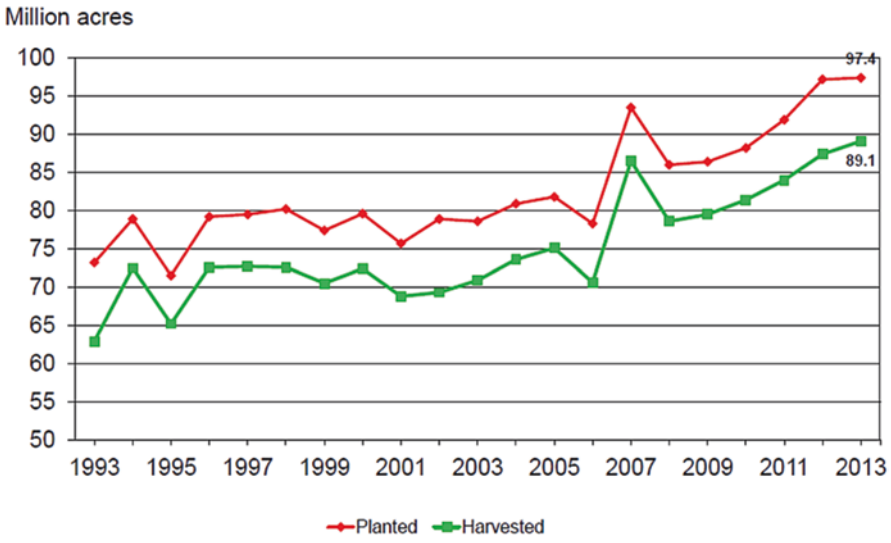

Fig. 12.4 US corn production (Source: USDA-NASS 2013)

this report came out 4 years after the food crisis and ignores other, more balanced, research is indicative of the biased research that is far too common within the NGO community.

A more balanced assessment of the food price crisis is offered by Hays (2008) who indicates that food prices increased due to a number of reasons: (1) the sale of foodstuffs, namely, corn and soybeans, to biofuel producers; (2) increased demand in places like China and India, where people were becoming richer and demanding more and better food like crop-consuming meat; (3) weather problems and droughts in bread baskets like Ukraine and Australia as well in sub-Saharan Africa; (4) the high cost of fertilizer due to high oil prices; (5) hoarding of grains by nations wanting to ensure their domestic supplies stayed put; and (6) panic buying, hoarding, and speculation.

While Hays includes increased biofuel production as the leading driver of the food price crisis, he does acknowledge other issues also drove food price increases. Non-peer-reviewed online studies, reports, and commentaries, however, offer minimal value in trying to understand what triggered the food price crisis of 2007–2008. Auld (2012) estimates that there are in excess of 1,000 reports, studies, book chapters, and journal articles pertaining to the topic of biofuel production and the price of commodities. Obviously a discussion on this volume of literature is beyond the scope of any publication. To gain insight into this issue, an examination of fact-based data analysis research is required. It is to this literature that we now turn our attention.

As was discussed previously in this chapter, governments have utilized a variety of policy measures to support and encourage the growth of the biofuel industry. One common policy measure that has been used is subsidies. The problem with using

subsidies, whether they are targeted at farmers or ethanol plants, is that they distort the market and create inefficiencies. For example, if a production subsidy is available for corn, many farmers will plant corn to take advantage of the subsidy but would not have planted corn had the subsidy not been available. Another policy option used by most governments has been the implementation of fuel/biofuel blend mandates that require a certain percentage of fuel to contain biofuel. de Gorter and Just (2010) observe that the benefits resulting from blend mandates can easily be lost with the inclusion of biofuel subsidies into the policy fix.

One economic relationship that is crucial to understanding this issue is the relationship between gasoline prices, ethanol prices, and the price of corn. Economies the world over are growing; hence there are greater energy demands in these economies. Simply put, as economic growth occurs within a country, the demands for fuel increase in parallel. These increased energy demands can be to power a new manufacturing plant and transport products from one location to another or for planting more cropland. When a government has implemented blend mandates into its energy policy, as the demand for fuel increases, so too will the demand for ethanol and biofuels to be blended. What then, is the impact on corn prices? de Gorter and Just (2010) indicate that prior to 2004, there does not appear to be any correlation between ethanol prices and corn prices, but following 2004, the authors indicate that the price of corn is directly linked to the price of ethanol. Thus, when the demand for ethanol to be blended into gasoline increases, so does the price of corn used to produce that ethanol.

Increases in the price of oil also contribute to inflating food prices. Farmers face higher input costs through higher fertilizer prices and higher fuel costs. Rail companies and grain handlers face higher fuel costs to move commodities from rural collection points to either commodity processing plants or export facilities. Additionally, food products are widely transported, both nationally and internationally, and higher oil prices result in higher fuel costs, contributing to increased food costs. Fuel costs account for a large portion of transportation costs pertaining to food prices, whether it is for bulk commodities or process foods.

As presented, energy policies and economic growth have an impact on commodity prices and hence food prices. In an effort to distinguish the impact of these two factors on food price inflation, Hochman et al. (2011) model the effects of economic growth, increased energy demands, biofuel expansion, fluctuations in the rates of currency exchange, and levels of crop inventories. Hochman et al. (2011) posit that when these factors are taken into consideration, they "explain 70 percent of the price increase for corn, 55 percent for soybean, 54 percent for wheat, and 47 percent for rice during the 2001–2007 period." The authors speculate that factors such as commodity trading, trade policy, and adverse weather may account for the balance of the price increase.

Beginning in 2002, the US dollar began to experience a significant decline relative to other major currencies. Charlebois and Hamann (2010) analyzed the consequences of US dollar depreciation and highlight that this was a contributing factor to the 2008 agricultural price increases. The world price for major crops, and the international trading of these crops, is typically denominated in US dollars; thus a declining

value of the American dollar will eventually lead to increased prices (Flammini 2008). This rationale is reinforced in a World Bank Report (Mitchell 2008), which shows that another factor that contributed to the rapid increase in the world market prices for major food commodities, along with biofuels, rising energy prices (and implicit price increase in fertilizers and chemicals), increased costs of production, or protectionist exporting policies, was the declining US dollar. Mitchell (2008) calculates that the dollar depreciation against the Euro in the 2002–2008 interval was of approximately 35%. Hence, the depreciation of the American dollar during this period led to increasing commodity prices (Gilbert 1989; Baffes 1997).

A policy choice that further inflated food prices was the decision by some governments to ban the export of certain staple commodities, which resulted in a contraction of the global supply available for purchase by other countries, hence further inflating the price of that commodity. Hochman et al. (2011) identify that this policy option was implemented by both China and India in the 2007–2008 period. As a means of ensuring consistent domestic supplies, governments of both countries banned the export of raw commodities. In the case of China, their government banned the export of rice and corn, while the Indian government also banned the export of rice. An additional 14 countries followed suit and banned the export of basic commodities to ensure that domestic supplies could be maintained, but the cost of this policy was substantial as the panic that resulted from this drove the price of those commodities even higher, resulting in higher costs to these governments when faced with importing food products.

Swinnen et al. (2011) make an interesting observation in the discussion of the food price crisis. They observe that numerous NGOs and international agencies have long suggested that poverty and food insecurity in developing countries were due in part to low commodity prices. Following the food price crisis of 2007–2008, these same organizations completely reversed their position and argued, however, that rising commodity and food prices threatened food security. The authors posit that many of the NGOs operating in the agriculture, food security, and sustainable development field are guilty of biased communication policies and simply champion whatever message gets them the greatest degree of media attention. Many large, international NGOs are predominantly fixated on raising money and will frequently bias their communications with whatever message will generate the greatest level of donations; hence they lack any real commitment to the actual issue they are communicating about.

In their International Food Policy Research Institute report, Headey and Fan (2010) examined evidence that would link certain food price increase factors to the rise in food prices. They determined that, based on the evidence they reviewed, three factors drove the increase in food prices. First, rising oil prices due to economic growth; second, increased demand for biofuels due to high blend mandates from numerous governments; and third, trade shocks on commodity prices. Agriculture is an energy-intensive industry, and as discussed above, rising oil and fuel prices contributed to increased food prices. Trade shocks include export bans on staple commodities by some governments and the panic buying behavior that resulted with many government purchasing agencies. The report observes that the rice export

bans enacted by both the Indian and Vietnamese governments strongly contributed to the price volatility that was witnessed in the international rice market. The hoarding of staple commodities created considerable consternation within importing nations, resulting in the bidding up of commodity prices in an attempt to secure enough supply to satisfy domestic demands. One contributing factor to the increase in wheat prices was the severe drought that was experienced by Australia during the 2005 and 2006 crop years, which reduced international wheat stocks. Headey and Fan determine that speculation by commodity brokers, which was suggested as one of the major reasons for increased food prices, did not have sufficient evidence to be a determining factor in the food price crisis.

Piesse and Thirtle (2009) observe that the when taken in context, the price increases from 2007 to 2008 were considerably below those of the 1970s. When inflation is factored into food prices, the increase in food prices during the 2007–2008 crises was actually 50% lower than the food shortage of 1973. Piesse and Thirtle believe that yet a further factor was responsible for the rapid increase in food prices, which is that of low international commodity stocks heading into this period. They show that in periods of low stocks, commodity markets face increasing volatility. As is shown in Fig. 12.5, the stock-to-utilization ratio was at a lower point than it had been during the 1973 food shortage and, coupled with the Australian droughts of 2005 and 2006, created the right environment for commodity price volatility to expand, hence the rapid increase in food prices. Timmer (2010) observed that the Philippines' government policy to purchase import supplies at any price was one of the main triggers for the panic that ensued in the commodity markets, with prices rising rapidly.

One aspect of the discussion of the various factors and aspects affecting and relating to the food price crises that remains to be discussed is: what was the impact in terms of increased poverty and reduced food security in developing countries? Headey (2011) identified that the World Bank estimated that in the 2007–2008 period, over 130 million people worldwide were driven into poverty. In addition to that, a further 75 million people became malnourished, meaning that due to their

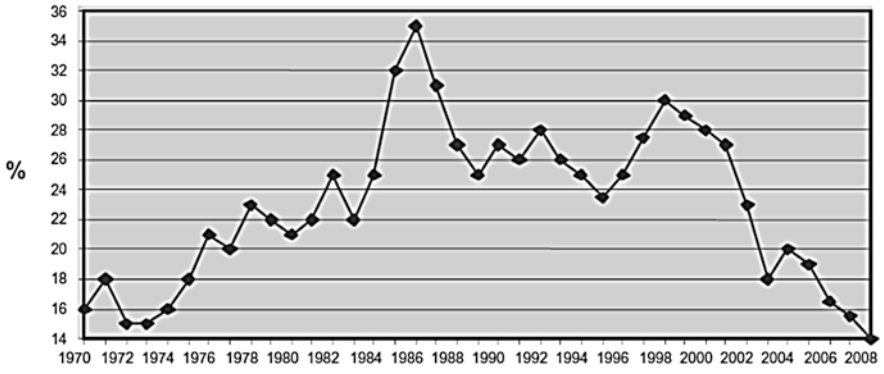

Fig. 12.5 Stock-to-utilization ratio, 1970–2008 (Source: Piesse and Thirtle 2009)

limited revenue, the higher food prices meant that these people could no longer purchase enough food on a daily basis to sustain their nutritional requirements. The costs of this in terms of pain and suffering can never be calculated.

Clearly a multitude of factors came into effect and all contributed to some degree to the increase in food prices. Some of these factors had a substantially higher effect than did others. As is demonstrated above, even those that can be called experts in the area cannot agree as to whether or not the same factors carried the same degree of impact. Some of the literature presented above argues that some factors did not have an impact on increasing food prices, while other experts suggest that the same factor had a considerable impact. This illustrates just how complicated an issue this is and how, depending on the data used to derive the results, the impacts of one factor may vary considerably.

12.5 Closing Comments

The focus of this chapter has been to determine whether the increased production of ethanol from corn in the USA triggered a dramatic rise in food prices in 2007–2008. The answer according to many nongovernmental organizations was obviously a positive one and that increased US ethanol production was the leading reason for increased food prices. Less passionate and more reasoned analysis, however, suggests that, yes, the increased production of corn-based ethanol did play a role in increasing food prices, but it was one of several factors that occurred simultaneously that ultimately was responsible for the increase in food prices.

On its own, increased ethanol production from corn may have resulted in a modest increase in food prices at this timeframe, possibly a few percentage points, but the increased use of corn to produce ethanol was not responsible for price increases in the magnitude that has been described above. The rise in food prices were due to the combined effects of the following market circumstances and events: rising oil prices; declining value of the American dollar; government policies that implemented aggressive biofuel blend mandates; increased demand for and production of biofuels; low commodity stocks; trade barriers, such as preventing the sale and export of staple commodities; and panic buying of staple commodities by some governments.

More often than not, any single event or crisis is never triggered due to the results of a single action or policy. As is evident from this discussion, the increase in food prices was due to a combination of at a minimum, eight different major factors. Initially, the entire process was the result of the American and EU implementation of blend mandates that exceeded existing domestic capacities, and as a result of the mandates, industry responded by increasing biofuel production, which, when taken in combination with other market factors, resulted in the food price crisis. Of course, no individual or theoretical model could have predicted the impacts that ultimately resulted from the implementation of government policies that required blend mandates.

As part of their recognition that the implemented blend mandates were overly aggressive given the existing technology and the inability of industry to overcome the transition to second-generation biofuels as quickly as government policy makers had hoped, both the EU and USA have extended the deadlines for blend mandates as well as reduced the level of blending that is mandated. Both of these policy revisions have assisted in reducing the pressure on the biofuels industry to meet the blend mandates, especially the mandates for the inclusion of second-generation biofuels, which has resulted in food prices experiencing reduced volatility.

What lessons have been learned from this experience? There would appear to be three key take-away messages that are crucial for the biofuels and bioproducts industries and their relationship with agriculture and food production. First, food price spikes are not one-time events; they occur periodically, and unfortunately, there will be another one. As was commented on above, there was a food price crisis in the early 1970s, when oil prices spiked. While economists and statisticians are getting better at modeling trends in the price of oil, there will always be events that are unpredictable and that will ultimately have tragic consequences. Therefore, future food price spikes are not preventable, any more than an earthquake is preventable, but what is possible is to plan and prepare for the next one, such that the effects of the price spike are not as drastic or as widespread as was experienced in 2007–2008. International development agencies like the FAO and the World Bank have held several planning sessions and events to share experiences and knowledge about what transpired as a means of ensuring that this knowledge is widely disseminated such that when the next price spike is triggered, governments the world over will have a better understanding of what policies should, and what policies should not, be implemented as a means of managing the crisis.

Second, domestic energy policy is an extremely complicated field, requiring reams of data and considerable thought and reflection prior to implementing a new policy or revising existing policies. As is evident, a socially desirable policy such as implementing a biofuel blend mandate as a means of reducing GHG emissions can have unintended effects. No policy analyst has a crystal ball in which to gaze and determine the future course of events and is therefore able to plan perfectly. Hence, those responsible for developing government energy policies have to be extremely diligent to ensure that they are utilizing the most complete datasets possible to assess the impacts of future policies and consult with leading experts in industry and academia to ensure that gaps in the development of the policy are recognized early in the policy development process and can be accordingly corrected or mitigated and the monitoring of the circumstances pertaining to existing policies not be ignored. To a large degree, it is this last pertinent part of the policy process that created the market circumstances for the food price increases as governments in the EU and USA were unable to respond quickly enough to the market results from the aggressive blend mandates that had been enacted.

Third, trade barriers against the exporting of commodities in times of low commodity stocks only exacerbate the problems. While many governments and political parties struggle to trust the free market, it is better able to respond to critical events than when governments attempt to intervene in the economy in their attempts to

offset or minimize the crisis. The hoarding of commodities by some governments disrupted the international trading of those commodities, resulting in dramatic price distortions. While many remain unconvinced that markets are efficient at allocating resources in times of scarcity, it is evident from the government policies that banned the export of staple commodities that government intervention in the market during times of crisis does not produce results that improve the situation. Obviously, no one is capable of forcing governments not to intervene in the market during critical events; all that can be done is to ensure that information about the social cost of such intervention is widely available.

The food price crisis of 2007–2008 had tragic consequences in many developing nations, the cost of which in terms of human suffering will never be accurately, or adequately, measured. The best that can be hoped for is that government policy makers have gained new insight into the unintentional impacts of changing energy policies and that they will be more cognizant in the future about monitoring newly implemented policies to ensure that unintended consequences are not brewing in the background. As has been said many times, those who do not remember history are doomed to repeat it.

Where could policy-efforts be best directed to ensure that when food prices begin to rapidly increase the next time, that buffers have been developed and implemented that are designed to mitigate the worst of the impacts? One important policy area would be the further investment in lignocellulosic biofuel technology development. An increase in research and development funding would allow for the use of nonfood crops to offset the demand for corn as the main feedstock for biofuel production. Technology improvements, so that wood waste, post-harvest waste, and urban waste are capable of becoming efficient feedstocks, would alleviate some of the pressure by shifting land from the production of corn for biofuel to the production of other food crop alternatives. Research into increasing the yield ability of biomass crops (e.g., *Panicum virgatum*, *Mithcanthus* spp.) that would be better suited for production on marginal lands could contribute to shifting biofuel feedstock production away from food-producing land. Regardless of what policy option is pursued, one key policy is the investment in innovation. In the absence of innovation investment, the next food price crisis could be expected to be of equivalent scale and scope.

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Glossary

Acetoacetyl-CoA reductase an enzyme that catalyzes the production of 3-hydroxybutyryl-CoA (Sect. 5.2.3.1).

Acetyl-CoA carboxylase (ACCase) an enzyme that catalyzes the formation of malonyl-CoA from acetyl-CoA (Sect. 4.7.1).

Acetyl-coenzyme A (Acetyl-CoA) a molecule that participates in various intracellular biochemical reactions. One of its main functions is to deliver acetyl groups to the Krebs cycle for the production of energy (Sect. 2.7).

Acidic sugars derivatives of monosaccharides that have a carboxyl (-COOH) group in place of the $-CH_2OH$ in the sixth carbon position (Sect. 6.2.1).

Acidulant a compound that adds flavor to foods; flavoring agent (Sect. 10.5.1).

Active site a specialized region of an enzyme that can interact with the substrate and facilitates the reaction (Sect. 2.6).

Acyl carrier protein (ACP) a protein that attaches the fatty acid chain to the fatty acid synthase complex (Sect. 4.7.1).

Acyl-CoA a group of coenzymes involved in the metabolism of fatty acids: after moving across the plastidial envelope, the exported fatty acids are reactivated to acyl-CoAs on the outside of the plastid (Sect. 4.7.1).

Adherents adhesive materials and the surfaces of materials for adhesives (or glues) that hold objects together (Sect. 9.2.2).

Adhesives nonmetallic liquids or gels that bind the surfaces of materials together and resist separation (Sect. 3.4).

Adsorption a process that occurs when adhesive molecules are attracted to a specific site on a solid surface through weak van der Waals forces or chemisorption via covalent bonding (Sect. 9.2.2).

Advanced biofuels see second-generation biofuels.

Agronomy a branch of agriculture dealing with field crop production and soil management (Sect. 3.1).

Albedo the amount of solar radiation reflected back into the atmosphere (Sect. 1.1).

Albumins storage proteins found in most land plants (Sect. 8.2.6).

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Aldose a monosaccharide that contains a carbonyl group at the end of the carbon chain (Sect. 6.2.1).

Alginate a natural polymer that exists widely in many species of brown seaweed (Sect. 9.2.3).

Alkyl glycosides (or alkyl polyglycosides) a class of surfactants derived from fatty alcohols that are used in the production of biodegradable detergents (Sect. 10.5.7).

Alternative splicing a process leading to the formation of more than one protein based on the information from one gene (Sect. 3.3).

Amino acid organic compounds containing amine and carboxyl functional groups. The central carbon is known as the α-carbon and to it is bonded a carboxyl group, a hydrogen atom, an amino group, and a variable side chain or R group (Sect. 2.5).

Amphipathic a molecule possessing both hydrophilic (water-loving) and hydrophobic (water-fearing) properties (Sect. 2.3).

Amylopectin a type of highly branched polysaccharide with chains of glucosyl moieties linked with α-1, 4 glycosidic bonds and α-1, 6 glycosidic bonds; one of the two components (the other is amylose) of starch (Sect. 6.2.2).

Amyloplasts specialized organelles that synthesize and accumulate starch (Sect. 6.3.2).

Amylose a type of linear polysaccharide with chains of glucosyl moieties linked with α-1, 4 glycosidic bonds; one of the two components (the other is amylopectin) of starch (Sect. 6.2.2).

Anabolism metabolic processes that construct molecules from smaller units (Sect. 2.7).

Anomeric carbon the carbon atom that forms the carbonyl group when a molecule is in a linear form (Sect. 2.4).

Anthropogenic emissions pollutants originating from human activity (Sect. 1.1).

Apomixis crops that reproduce through asexual reproduction (Sect. 3.3).

Autotrophs organisms that can convert energy from light into primary metabolites (Sect. 2.7).

Availability a reliable supply that is available year-round (Sect. 6.4).

Azlon the common generic name for all fibers regenerated from plant proteins (Sect. 9.2.3.2).

Bagasse the biomass residue that is usually burned to generate power in processing plants (Sect. 6.4.2).

Bio-oil the liquid phase of pyrolysis, which is made up of a complex mixture that contains water and a high content of oxygen (Sect. 4.3).

Bioactive oils oils that occur naturally in certain foods and have the potential ability to promote human health (Sect. 5.1).

Biocatalysis the use of enzymes or cells to speed up chemical reactions (Sect. 3.1).

Biochar charcoal made from biomass via pyrolysis (Sect. 1.2).

Biochemical coupling coupled reactions in biochemical processes/pathways (Sect. 5.4.2).

Bioconversion the conversion of organic materials, such as plant or animal waste, into usable products or energy sources through biological processes or agents, such as certain microorganisms (Sect. 3.1).

Biodegradability capacity to decay through the action of living organisms. The concept was introduced in Chap. 9 to describe the degradation of films by microorganisms in composting environments at the end of their life cycle by naturally occurring microorganisms into water, carbon dioxide, methane, biomass, and mineral residues (Sect. 7.7).

Biodiesel diesel fuel produced from biologically derived oils or fats using a specific procedure, called transesterification. It can be used as a vehicle fuel in its pure form or as a diesel additive (Sect. 1.4).

Biofuel fuel produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than the geological processes involved in the formation of fossil fuels, such as coal and petroleum, from prehistoric biological matter (Sect. 1.2).

Biomass biological material from living or recently living organisms, most often referring to plants or plant-based materials that are not used for food or feed. It can also include animal- or microorganism-derived materials (Sect. 1.2).

Bioproducts industrial and consumer goods manufactured wholly or in part from renewable biomass, which may be derived from crops, trees, marine plants, microorganisms, and animals (Sect. 1.3).

Biorefinery the sustainable processing of biomass into a spectrum of marketable products and energy (Sect. 3.1).

Biorefining sustainable processing of biomass into marketable products and energy.

Biotechnology the use of living organisms or biological processes for the purpose of developing useful agricultural, industrial, or medical products, especially through the use of methods such as genetic engineering (Sect. 3.1).

Blending mandate a governmental requirement for a minimum percentage content of biofuels in transportation fuels (Sect. 12.2.1).

Bois Durci or hardened wood an extruded plastic composite material manufactured from ebony or rosewood sawdust and diluted egg albumin (Sect. 9.1).

Break point the maximum stress a particular plastic can withstand while being pulled or stretched without breaking or the point at which the film breaks (section "Mechanical Properties of the Protein Films").

Building block chemicals a limited number of low-value chemicals with simple structures that are the precursor of many products (Sect. 10.1).

C-terminus the end of polypeptide chains that contains the free carboxylic group (Sect. 8.1).

Carbohydrates compounds which can be thought of as sugars and sugar polymers. They consist of carbon, oxygen, and hydrogen and have the general formula $(CH_2O)_n$ (Sect. 2.4).

Carbon capture and storage a process in which carbon dioxide is captured, converted into another carbon molecule, and stored underground (Sect. 1.2).

Catabolism metabolic processes that break down biomolecules (Sect. 2.7).

Cellulose an unbranched β-1,4 glucan that is the most abundant terrestrial organic polysaccharide (Sect. 2.4).

Cell wall the outer boundary of the cell (Sect. 2.3).

Cereal Price Index (CPI) an index compiled using the International Grains Council wheat price index, as well as maize and rice trading prices. For each rice variety considered, a simple average of the relative prices of appropriate quotations is calculated, and then the average relative prices of each variety are combined by weighting them with their assumed (fixed) trade shares (Sect. 12.3).

Cetane number (CN) an indicator of the combustion speed of diesel fuel and compression needed for ignition. It can be used to relatively measure the delay between fuel injection and ignition, which decreases as CN increases (Sect. 4.5).

Chloroplast specialized plastids containing chlorophyll and involved in photosynthesis in plants and algae (Sect. 2.3).

Climate change a change in the statistical distribution of weather patterns when that change lasts for an extended period of time (i.e., decades to millions of years). Climate change may refer to a change in average weather conditions, or in the time variation of weather within the context of longer-term average conditions (Sect. 1.1).

Cloud point the temperature below which crystallization occurs in fuels and causes a cloudy appearance (Sect. 4.5).

Coatings a term used to describe the films formed from proteins directly on the surfaces of objects, providing some separation from the environment (Sect. 9.2.1).

Conformation the spatial arrangements of a molecule that can be obtained by rotation of the atoms about a single bond (Sect. 2.4).

β-conglycinin 7S fraction of soy proteins (Sect. 8.4.3).

Consolidated bioprocessing a proposed configuration for ethanol production via fermentation whereby there is a simultaneous production of enzymes, saccharification, and fermentation (Sect. 11.4.3).

Corn stover refers to the cobs, leaves, and stalks that remain in the field after harvest (Sect. 6.5.3).

Cytochrome P450 a superfamily of protein. Some of them contribute to the biosynthesis of epoxy fatty acids in certain species (Sect. 5.2.1.3).

Covalent bond the bond formed by two atoms sharing electrons (Sect. 2.2).

Cytoplasm the content of the cell minus the nucleus in a eukaryote (Sect. 2.3).

Cytosol the soluble component of the cytoplasm (without subcellular organelles and internal membranes) in a eukaryotic cell (Sect. 2.3).

D- and L- forms enantiomers (mirror images) of monosaccharides (Sect. 6.2.1).

Densification compaction of the feedstock prior to transportation (Sect. 6.4).

Deoxy sugars derivatives of monosaccharides that contain a hydrogen atom instead of the terminal hydroxyl group (Sect. 6.2.1).

Dextrins low molecular mass products of starch digestion (Sect. 7.5).

Diacylglycerol acyltransferase (DGAT) an enzyme that catalyzes the acyl-CoA-dependent acylation of diacylglycerol (DAG) to produce TAG (Sect. 4.7.1).

Dipole a distribution of charge whereby one end of a molecule is positive and the other is negative (Sect. 2.3).

Directed evolution a process whereby enzyme variants are generated based on the introduction of random mutations into DNA encoding the enzyme, with the hope that some of the mutated genes result in the production of a more active enzyme when produced in a host such as yeast (Sect. 4.7.3).

Disaccharide a dimer of two monosaccharides (Sect. 2.4).

Dried grains with solubles (DDGS) made by drying wet distillers grains with solubles, the co-product of ethanol production. DDGS has increased shelf-life and decreased transportation costs (Sect. 11.4.1).

Drop-in strategy an approach whereby intermediates produced from biomass are the same as those produced using petrochemicals; thus, they already have a well-established market and chemical route for their use as feedstocks (Sect. 10.4).

Dry milling a process in grain (e.g., corn) biorefinery that yields germ and endosperm fractions. The process consists of dehulling, conditioning, removal of the germ, grinding, sifting, purifying, and aspirating grits (Sect. 11.3.2).

Elastic modulus (EM) a parameter used to measure the stiffness of a film (section "Mechanical Properties of the Protein Films").

Electrostatic attractions occurs when electrons are transferred across an interface, thus creating positive and negative charges that attract one another (Sect. 9.2.2).

Elongase system an extraplastidial enzyme complex that catalyzes the sequential elongation of oleoyl-CoA in lipid biosynthesis (Sect. 5.2.1.2).

Energy crops (or biomass crops) crops that have only biomass as their final product (Sect. 6.5.3).

Enogen a transgenic corn plant developed by Syngenta USA to express alpha-amylase (Sect. 9.3).

Enzymes proteins that can act as biological catalysts to speed up chemical reactions such as condensation and hydrolysis (Sect. 2.6).

Eukaryotic cells cells that have a nucleus and other organelles enclosed within membranes (Sect. 2.3).

Extractability the ability to separate desirable and undesirable components of a feedstock (Sect. 6.4).

FAD2-related hydroxylase an enzyme that generates the hydroxy fatty acid ricinoleic acid (12-hydroxy-18:1Δ^{9cis}) from oleic acid (Sect. 5.2.1.3).

FAD2-related conjugases enzymes that catalyze the formation of conjugated fatty acids, a group of fatty acids containing non-methylene-interrupted double bonds within their structure, from either linoleic acid or α-linolenic acid (Sect. 5.2.1.3).

FAD2-related epoxygenase an enzyme that catalyzes the formation of epoxy fatty acids in certain species (Sect. 5.2.1.3).

Fatty acid reductase (FAR) a reductase that converts fatty acids to fatty alcohols (Sect. 5.2.2).

Fatty acid synthase (FAS) complex a group of enzymes that catalyze fatty acid synthesis (Sect. 4.7.1).

Feedstock a starting material for the production of industrial products (Sect. 2.3).

Fermentation an intracellular process whereby some microorganisms use pyruvate or other organic compounds as the final electron acceptor in a process to produce energy (in the form of adenosine triphosphate [ATP]) in the absence of oxygen (Sect. 6.5.2).

Fiber a continuous filament or discrete, elongated, piece of material (Sect. 3.2).
First-generation biofuels also known as conventional biofuels; are made directly from food crops explicitly grown on arable land for fuel production (Sect. 12.2.2).
Food Price Index (FPI) a measure of the monthly change in international prices of a basket of food commodities. It consists of the average of five commodity group price indices (cereal, vegetable, dairy, meat, and sugar), weighted with the average export shares of each of the groups (Sect. 12.3).
Foundry resins resins used in the manufacturing of furan fiber-reinforced plastics, which are ideal for piping materials that must be resistant to corrosives and solvents (Sect. 10.5.11).
Furanose monosaccharide made up of a five-membered ring (Sect. 6.2.1).
Gel an intermediate state between solid and liquid (Sect. 8.3.2).
Gene the basic physical unit of heredity; it represents a linear sequence of nucleotides along a segment of DNA that provides coded instructions for the synthesis of mRNA, which, when translated into protein, leads to the expression of a hereditary character (Sect. 3.3).
Genome editing a process/technique that allows the creation of site-specific changes in a genome (Sect. 3.3).
Genome an organism's complete set of DNA, including all of its genes (Sect. 3.3).
Genomics the study of the way genes and genetic information are organized within the genome, the methods for collecting and analyzing this information, and how this organization determines their biological functionality (Sect. 3.3).
Genotype the genetic makeup of an organism (Sect. 3.3).
Globulins storage proteins found in most land plants. They are the most abundant class of proteins in soy protein isolates (Sect. 8.2.6).
Glutelins cereal storage proteins that are soluble in dilute acid or base and not coagulated by heat (Sect. 8.4.1).
Glutens proteins comprising gliadins and glutenins found in wheat and related grains (Sect. 8.4.2).
Glycinin 11S fraction of soy proteins (Sect. 8.4.3).
Glycosidic bond bond formed between the anomeric carbon of a monosaccharide and the oxygen of a hydroxyl group of another molecule (Sect. 6.2.2).
Glycosylation the addition of oligosaccharide chains called glycans to proteins during or after their synthesis in the endoplasmic reticulum (ER) and/or Golgi apparatus (GA) of the cell (Sect. 8.2.5).
Green chemistry a chemical process that minimizes the use and production of hazardous substances (Sect. 3.1).
Greenhouse gases gases present in the earth's atmosphere that absorb and emit radiant energy from the sun. Examples of greenhouse gases include water vapor, carbon dioxide, methane, nitrous oxide, ozone, and fluorocarbons (Sect. 1.1).
Hardened wood see Bois Durci.
Hardwoods woody plants such as poplar that produce biomass that is usually denser than agricultural residues and softwoods (Sect. 6.5.3).
Haworth projection a common way of writing a structural formula that can show the cyclic structure of monosaccharides with a simple three-dimensional perspective, as shown in Fig. 2.7 (Sect. 2.4).

Heat of combustion the energy density of a fuel (Sect. 4.5).

α-helices right-handed spirals within a polypeptide chain stabilized by a hydrogen bond between a carbonyl oxygen (at position n) and the amide hydrogen of the fourth amino acid (n+4) toward the C-terminus (Sect. 8.2.2).

Hemicellulose a polymer of monosaccharides that usually makes up less than half of the dry mass of cell walls (Sect. 6.3.1).

Heterologous expression expression of a gene or part of a gene in a host organism that does not naturally contain this gene or gene fragment (Sect. 3.3).

Hexose a monosaccharide with six carbons (Sect. 6.2).

Hybridization a process involving the crossing of two highly inbred parental lines, each possessing at least one desired characteristic, to obtain progeny with both traits (Sect. 3.2).

Hydration properties (of proteins) a result of the amino acid composition, in particular the ratio of polar versus non-polar and ionic versus neutral amino acids (Sect. 8.3.1).

Hydrogen bonding a force forming a special type of weak chemical bond when the slightly positive hydrogen atom of a polar covalent bond in one molecule is attracted to the slightly negative atom of a polar covalent bond in another molecule or in another region of the same molecule (Sect. 2.3).

Hydrolysis reaction chemical reaction whereby chemical bonds are broken down by the addition of a molecule of water (Sect. 10.4).

Hydrophilic (polar) amino acids amino acids that contain polar side chains (Sect. 8.3.1).

Hydrophobic "water-fearing" (Sect. 2.3).

Hydrophobic (nonpolar) amino acids amino acids that contain hydrophobic side chains (Sect. 8.3.1).

Indirect land use change (ILUC) a concept relating to the unintended consequences of releasing more carbon emissions due to land-use changes around the world induced by the expansion of croplands for ethanol or biodiesel production in response to the increased global demand for biofuels (Sect. 12.2.2).

Industrial waste waste produced by industrial activity. Some of it can be used as a feedstock for the production of bioethanol (e.g., waste from cellulose plants) (Sect. 6.5.3).

Interdiffusion a process occurring when an adhesive dissolves and diffuses into the substrate material (Sect. 9.2.2).

Kennedy pathway a lipid biosynthetic pathway involving the sequential acylation of a glycerol backbone in the form of G3P (derived from glycolysis), to ultimately form TAG (Sect. 4.7.1).

Ketose a monosaccharide that does not contain a carbonyl group at the end of the carbon chain (Sect. 6.2.1).

β-ketothiolase an enzyme that catalyzes the synthesis of acetoacetyl-CoA (Sect. 5.2.3.1).

Legumin the fraction of globulin from bean protein that has a coefficient of sedimentation of 11S (Sect. 8.4.4).

Lignin a major component of lignocellulose that is responsible for the strength and rigidity of plant tissues. Aids in water transport and functions to protect against attack by insects and infection by microorganisms (Sect. 10.6).

Lignocellulose the most abundant form of biomass on earth, composed of cellulose, hemicellulose, and lignin (Sect. 10.6).

Lipids fatty acids and their derivatives and substances related biosynthetically or functionally to these compounds (Sect. 2.3).

Liquefaction dextrin formation from starch (Sect. 11.4.1).

Lubricity the ability of a diesel fuel to lubricate engine surfaces (Sect. 4.5).

Marine mussel adhesive proteins proteins that allow the adhesion of objects in seawater and contain a substantial quantity of 3,4-dihydroxyphenylalanine (DOPA) (Sect. 9.2.2).

Mechanical interlocking a process occurring when an adhesive flows into pores or solidifies around projections (Sect. 9.2.2).

Mechanical properties commonly measured properties for protein films, including their strength, elasticity, and plasticity (section "Mechanical Properties of the Protein Films").

Metabolic engineering the redirection or modulation of carbon flow through a metabolic pathway (Sect. 3.3).

Metabolic evolution the consecutive growth of a microorganism strain in different culture conditions to select desirable characteristics (Sect. 10.5.3.1).

Metabolomics the global/systematic analysis of the metabolite status of cell or tissue type (Sect. 3.3).

Mitochondria organelles in eukaryotic cells that are known as the "power houses" of the cell because of respiration and the formation of adenosine triphosphate (ATP), the universal energy currency within them (Sect. 2.3).

Monolignols phenylpropanoids that form lignin (Sect. 10.6).

Monomeric protein a single polypeptide chain (Sect. 2.5).

Monosaccharide a sugar that exists mainly in ring form in solution (Sect. 2.4).

Monounsaturated fatty acid a type of fatty acid that has one point of unsaturation in the fatty acid chain outside of the carboxyl group (Sect. 2.3).

Municipal solid waste everyday items that are discarded by the public. Some of them can be used as feedstocks for the production of bioethanol (e.g., paperboard products and woody materials) (Sect. 6.5.3).

N-terminus the end of polypeptide chains that contain the free amino group (Sect. 8.1).

Nanofiber-based wound dressings (NFDs) wound dressings containing an electrospun-nanofibrous layer applied to a basic support fabric material (Sect. 9.2.3.2).

Natural rubber a high molecular weight polymer of isoprene (most often *cis*-1,4-polyisoprene), with other ill-defined minor components that contribute to its functionality (Sect. 5.2.3.2).

Non-glutens comprise albumins and globulins (Sect. 8.4.2).

Nutraceuticals compounds that have the potential to offer health benefits beyond normal nutrition (Sect. 5.3).

Oligosaccharide a polymer of several (e.g., roughly 3 to 20) monosaccharides (Sect. 6.2).

Omega-3 very long-chain polyunsaturated fatty acids (VLC-PUFAs) fatty acids 20 carbons or more in length with at least 3 methylene-interrupted double bonds in the *cis*-position, the first of which is located three carbons from the methyl end of the chain; one of the most beneficial types of bioactive lipids (Sect. 5.3.1).

Organic molecule (or compound) compound containing carbon atoms that share electrons (or form covalent bonds) with hydrogen and possibly other elements (Sect. 2.2).

β-oxidation an intracellular catabolic process of degradation of fatty acids (Sect. 5.4.1).

Pectins a group of heteropolysaccharides that are rich in galacturonic acid (Sect. 6.3.1).

Pentose a monosaccharide with five carbons (Sect. 6.2).

Peptide (amide) bond bond established between the carboxylic group of a nascent peptide or protein and the amino group of the additional amino acid, resulting in the release of a water molecule (Sect. 2.5).

Peroxisome an organelle in eukaryotic cells containing enzymes that transfer hydrogen atoms from various substrates to oxygen, producing and then degrading hydrogen peroxide (Sect. 2.3).

PHA synthase enzyme that catalyzes the polymerization of hydroxyalkanoates (Sect. 5.2.3.1).

Phenotype the observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences (Sect. 3.3).

Phospholipids a class of lipids involved in the formation of cellular membranes, which usually consists of two layers of phospholipid molecules (Sect. 2.3).

Plant biomass see biomass.

Plant breeding the active selection of individuals with desirable traits (Sect. 3.1).

Plant genetic engineering a process/method for modifying the DNA of a plant or introducing DNA from another source to generate a crop with a specific beneficial trait (Sect. 3.3).

Plant phenomics the high-throughput analysis of the plant phenome (Sect. 3.3).

Plants with novel traits (PNTs) plants that contain a trait which is both new to the local environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health. These traits can be introduced using biotechnology, mutagenesis, or conventional breeding methods (Sect. 3.3).

Plasma membrane the membrane at the boundary of the cell which regulate the cell's chemical composition by serving as a selective barrier (Sect. 2.3).

Plasticizers low molecular weight and low volatility substances that work as spacers to reduce the strength of intermolecular attractive forces and lower the glass transition temperature of amorphous or partially crystalline protein films (Sect. 9.2.1).

Plastids organelles found in photosynthetic eukaryotic cells that include chloroplasts, chromoplasts, and amyloplasts (Sect. 2.3).

Platform chemicals building block chemicals.

Polybutylene succinate (PBS) a bioplastic that has properties comparable to those of polypropylene but can be degraded by microorganisms in compost or freshwater; can be manufactured using succinic acid as a feedstock (Sect. 10.5.3).

Polybutylene terephthalate (PBT) a polymer that is better than PET for injection molding due to its fast crystallization properties (Sect. 10.5.5).

Polyhydroxyalkanoates (PHAs) linear polymers of 3-hydroxy fatty acids with various side chains that give them different properties (Sect. 5.2.3.1).

Polylactic acid (PLA) a biodegradable plastic that can be synthesized through the polycondensation of lactic acid or via the polymerization of lactide produced from lactic acid (Sect. 7.3).

Polymers large molecules that are made up of a number of repeated monomer subunits covalently linked through the process of polymerization (Sect. 5.2.3).

Polyploidy cells and organisms that contain more than two paired sets of chromosomes (diploid) and often outperform their diploid relatives (Sect. 3.2).

Polysaccharide various monosaccharides linked in a chain that can vary in molecular weight (Sect. 2.4).

Polytrimethylene terephthalate (PTT) a polymer with better stain and ultraviolet resistance than other polymers such as PET (Sect. 10.5.4).

Polyunsaturated fatty acid fatty acids that have more than one double bond, or point of unsaturation, in the interior of the fatty acid chain (Sect. 2.3).

Pour point the temperature at which gelation occurs in fuels (Sect. 4.5).

Primary metabolism fundamental biochemical processes that are essential to the survival of a cell (Sect. 2.7).

Primary structure the sequence of amino acids in a polypeptide chain (Sect. 2.5).

Products (of an enzymatic reaction) the molecules/products derived from enzyme-catalyzed reactions (Sect. 2.6).

Prokaryotic cells cells that lack a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle (Sect. 2.3).

Prolamin a seed storage, globular, water-ethanol soluble protein containing large amounts of proline and glutamine amino acids and small amounts of arginine, lysine, and histidine amino acids (Sect. 8.2.6).

Prolons fibers from soybean protein and corn zein (Sect. 9.2.3).

Protein film an independently produced sheet or membrane formed from a protein isolate and a plasticizer by solvent casting or extrusion methods, which have known physicochemical properties that are suitable for a particular use (Sect. 9.2.1).

Protein fusion technology a technique being tested to overcome two significant challenges of plant-based systems: low accumulation of the protein and difficulty in terms of purification (Sect. 9.3).

Protein structure the structure of a protein, which is determined by the basic structure of the peptide bond, the sequence of its amino acids, folding of the primary chain and interactions, as well as intra-protein and inter-protein cross-links among its constituent amino acid side chains (Sect. 8.2).

Proteins polymers of amino acids that have a great variety of functional, structural, and regulatory roles in organisms (Sect. 2.5).

Proteomics the global analysis of proteins (Sect. 3.3).

Proximity distance between the harvest location and the end user (Sect. 6.4).

Pyranose monosaccharide made up of a six-member ring consisting of five carbon atoms and one oxygen atom (Sect. 6.2.1).

Pyrolysis a high temperature reaction in the absence of oxygen, converting free fatty acids (which can be obtained from crude TAG feedstocks through an initial hydrolysis step) to mainly alkanes and alkenes (Sect. 1.2).

Quaternary structure identical or different subunits, which have their own tertiary structure associated through various interactions (Sect. 2.5).

Racemic mixture a mixture of two enantiomers usually produced as products of chemical reactions (Sect. 10.5.2.1).

Renewable sources substances and materials that can be naturally replaced or reproduced (Sect. 10.1).

Rubber transferase an enzyme complex that is embedded within a membrane surrounding the rubber particle core (Sect. 5.2.3.2).

Saccharification production of Glc monomers from dextrins (Sect. 11.4.1).

Saturated fatty acid all of the carbons, other than the carbon in the carboxyl group, are saturated with hydrogens (Sect. 2.3).

Second-generation biofuels also known as advanced biofuels; made from various types of biomass (Sect. 12.2.1).

Secondary metabolism reactions that aid in the growth and development of plants but are not absolutely required for their survival (Sect. 2.7).

Secondary metabolites compounds that are not directly involved in the normal growth, development, and reproduction of an organism; the absence of these compounds does not result in immediate death of the organism (Sect. 2.7).

Secondary structure shorter segments within a polypeptide chain exhibit different types of folding, including the α-helix and β-strand (Sect. 2.5).

Selectivity the preference of an enzyme to use a particular substrate when presented with a mix of possible substrates (Sect. 5.2.1.1).

β-sheets the primary amino acid chain is folded back on itself so that interactions can occur at the sides of the chain for some length to create a planar surface (Sect. 8.2.2).

Softwoods woody plants that produce such as spruce and pine that produce biomass that is usually denser than agricultural residues but less dense than hardwoods (Sect. 6.5.3).

Specificity the ability of an enzyme to use a particular substrate when presented in isolation from others (Sect. 5.2.1.1).

Starch a storage polysaccharide in plants with the major components amylose and amylopectin (Sect. 2.4).

Stearoyl-ACP desaturase (SAD) a class of enzymes that convert stearoyl-ACP to oleoyl-ACP (Sect. 4.7.1).

Stillage residue from the manufacture of ethanol/alcohol from grains; the unevaporated residue of distillation (Sect. 11.4.1).

Strain the geometrical measure of deformation, which is expressed as percent elongation (E) and represents the relative displacement between particles in the material body (section "Mechanical Properties of the Protein Films").

Styrene-butadiene rubber (SBR) rubber produced from the polymerization of 1,3-butadiene and styrene; one of the main components of tires (Sect. 10.2).

Substrates the molecules first interacting with an enzyme in an enzyme-catalyzed reaction (Sect. 2.6).

Svedberg (S) numbers a measurement of sedimentation during ultracentrifugation. Larger S numbers indicate larger molecular weight (Sect. 8.4.3).

Synthetic biology the use of synthetic DNA and genetic circuits to produce value-added products (Sect. 3.4).

Synthons synthetic fibers manufactured from petroleum (Sect. 9.2.3).

Tensile strength (TS) the amount of force per unit of the original cross-sectional area to pull a film to the point where it breaks (section "Mechanical Properties of the Protein Films").

Tensometer a piece of equipment used to generate stress-strain curves of a film sample (section "Mechanical Properties of the Protein Films").

Terephthalic acid a derivative of para-xylene that can be used as a feedstock in the production of diverse polyesters, such as PTT, PBT, and PET (Sect. 10.5.9).

Tertiary structure the overall three-dimensional arrangement of a polypeptide chain; stabilized by various interactions between the side chains of the amino acid residues (Sect. 2.5).

Thioesterase (TE) an enzyme that releases fatty acid chains from the FAS complex (Sect. 4.7.1).

Third-generation biofuels biofuels obtained from algae (Sect. 12.2.3).

Tortilla Price Index a consumer price index, which includes the weighted average of the price of tortilla in the interval of 2005–2011 relative to their prices in 2005 (Sect. 12.3).

Transcription factors proteins that interact with regulatory sequences in genes to influence the extent of gene expression (Sect. 4.7.3).

Transcriptomics the global analysis of mRNA sequences (Sect. 3.3).

Transesterification a process whereby plant oils are reacted with an alcohol in the presence of a strong alkali, such as potassium hydroxide, to produce alkyl esters with viscosity and ignition properties comparable to conventional diesel (Sect. 4.2).

Triacylglycerol a compound in which fatty acids are "esterified" to a three-carbon glycerol backbone. It is the major component found in plant seed oils and is the main lipid feedstock for producing biodiesel and other bioproducts (Sect. 2.3).

TrypZean trade name of a bovine trypsin expressed in corn grains (Sect. 9.3).

Uniformity a measure of variation in the composition of feedstocks (Sect. 6.4).

Van der Waals forces weak, short-range electrostatic attractive forces between molecules (section "Mechanical Properties of the Protein Films").

Vacuoles multifunctional organelles in cells. In seeds, vacuoles serve as storage sites for reserve proteins and soluble carbohydrates (Sect. 2.3).

Vicilin/phaseolin fraction of globulin from bean protein that has a coefficient of sedimentation of 7S (Sect. 8.4.4).

Viscosity the resistance to flow of a liquid due to internal friction (Sect. 8.3.2).

Wax esters compounds made up of a long-chain fatty acid esterified to a long-chain fatty alcohol (Sect. 5.2.2).

Wax synthase (WS) enzyme that catalyzes the esterification of a fatty alcohol to a fatty acid (Sect. 5.2.2).

Wet distillers grains with solubles (WDGS) the co-product of ethanol production, including the residues of thin stillage after evaporation mixed with wet cake (Sect. 11.4.1).

Wet milling a process of grain (e.g., corn) biorefinery which involves a previous steeping of the seeds to facilitate separation of hull, germ, and endosperm (Sect. 11.3.2).

Wheat gluten meal the fine, protein-rich supernatant produced from wheat after centrifugal removal of most of the starch from wet-milled endosperm; gluten is extracted from it (Sect. 8.5.2).

Yield point prior to this point, films deform elastically and will return to their original shape and size when stress is released (Sect. 9.2.1.2).

Zein a class of protein that makes up 50% or more of corn kernel proteins (Sect. 8.4.1).

α-zein zein rich in nonpolar amino acids, including glutamine (21–26%), leucine (20%), proline (10%), alanine (10%), and phenylalanine (8%), but deficient in basic and acidic amino acids (Sect. 8.4.1).

β-zein zein rich in methionine (10%) and tyrosine (8%) (Sect. 8.4.1).

γ-zein zein rich in proline (25%) and histidine (8%) amino acids (Sect. 8.4.1).

δ-zein zein rich in sulfur-containing amino acids (methionine and cysteine) (Sect. 8.4.1).

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