Chapter 5
The Obtainment of Bioplastics

5.1 The Controversial Definition of Bioplastics

Under the pressure of the social requirement of a more natural style of life, many commercial products were rendered more attractive with a more or less valid superficial coverage of bio-related aspect; these products are often mixed without any scientific rule with environmentally friendly products with misleading information for the users.

The aim of this chapter is then to provide a survey of information about what we can find under the name of bioplastics and helping the reader in identifying the really biologically originated properties and how they can be modulated in the direction of more technological features typically present in fossil-originated plastics.

Indeed, it must be considered that any commercial product to be available to a large number of people needs the availability of an adequate amount of raw material and the existence of a suitable production process involving, in general, an appropriate formulation and combination with other compounds. Up till now, no natural product is ready for use in a competitive high-technology world.

The involvement of several industrial steps for the production of a polymeric material that can be classified in some way as bioplastics affects the level of natural character (Kirk et al. 1991). As already reported in the previous chapter, monomers are the starting material for polymer production; the same monomer can have both fossil and natural origin. In the latter case, the bio-monomer is converted into the corresponding polymer through a man-made polymerization process. The obtained polymer is considered here as bioplastics even if the same polymer can be obtained from the monomer with the same chemical composition but fossil origin. Therefore, the same polymer can have different origins but the same environmental impact. A typical example of this type is offered by ethylene monomer that can be obtained from

F. Ciardelli et al., *Polymers from Fossil and Renewable Resources*,
https://doi.org/10.1007/978-3-319-94434-0_5

fossil or from renewable sources. In the last case, it is called as 'bioethylene'. Ethylene and bioethylene are identical from the molecule point of view and the corresponding polyethylenes obtained by the same man-made polymerization process cannot be distinguished in structure and properties. Nature can, however, supply directly bio-generated polymers, the biopolymers, that have to be considered real bioplastics both for their origin (completely natural) and their environmental compatibility. Then, the name bioplastics is attributed to polymer materials derived from natural monomers or polymers independently of their properties but this cannot be applied to monomers and polymers derived from fossil even if the materials are highly accepted by the environment.

These aspects deserve to be discussed in the following sections, where the various possibilities are presented separately to clarify more evidently the role of the nature and the bio-features of the produced materials for the production of the commercially classified bioplastics.

5.2 Biopolymers from Natural Resources

In this section, examples of the more relevant biopolymers are reported, polymers spontaneously produced in nature, which can assume plastic behaviour together with their availability in the world, isolation possibilities and application characteristics.

Many are the macromolecules produced in nature (Bailey 1991). Most of them belong to polysaccharide or protein classes. DNA and RNA are natural macromolecules, the importance of them in the life is well-known. Their commercial counterpart is usually extracted from microorganism, such as for instance yeast, and at present, they are investigated for possible application in sensors. However, to the best of our knowledge, they have not found yet any actual application and for this reason, they will not be considered in details in this context.

Nature produces few polyhydroxyalkanoates and few aromatic polymers also, such as lignin and the low-molecular weight shellac resin. Polyhydroxyalkanoates are very important emerging materials since they exhibit thermoplastic behaviour similar to that of the widely used man-made polyolefins. However, they will be discussed specifically in Sect. 5.3 since engineered bacteria are used at industrial level for their production. Shellac, instead, is a rare and expensive material. It is a resin secreted by the female lac bug on trees, and it is collected from thousands of years by scrapping the bark of the trees. After the coming up of synthetic polymers, its use has been limited quite only to restoring ancient furniture and thus will not be considered longer here.

Lignin represents the second polymer on the Earth and the only large-volume renewable feedstock of aromatics. It is found in all vascular plants where it has a support function. It can be described as a cross-linked polymer of substituted phenols. Its aromatic nature and the presence of many cross-linkings make it a rigid polymer providing the structural stability to plants (Heinze 2008). Because of its chemical structure, lignin is insoluble, it has a very high-glass transition temperature and it

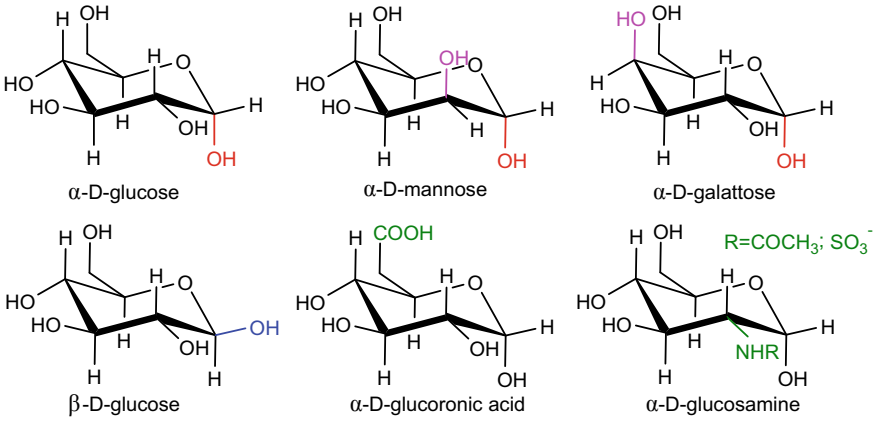


Fig. 5.1 Principal repetitive units of polysaccharides

does not melt, being amorphous. For all these reasons, it cannot be extracted and used in its native status and up till now, it has been exploited in the past only as an energy source by burning. At present, it is widely investigated as a possible renewable source of low-molecular weight aromatic chemicals since it is a very abundant by-product of the paper industry and biorefinery. In any case, it is not used or studied as polymer and thus it is not relevant for the topic of this paragraph.

Natural polymers of practical interest, in fact, are restricted to few polysaccharides and proteins, with the former being much more important than the latter. In fact, polysaccharides represent 75% of the annual biomass production (around 170 billion tons). They play key roles in nature basically for their structural function (cellulose, pectin in plants and chitin in animals), elasticity regulation in the connective tissue of animals (anionic polysaccharides such as glycosaminoglycans), energy storage function (starch and glycogen), control of the migration of water and cations from and to the cells. Other anionic polysaccharides (alginates, carragenans and pectates) act as an extracellular matrix in plants.

From a chemical point of view, polysaccharides are chains composed of monosaccharide units (Fig. 5.1) bound together by glycosidic linkages (Fig. 5.2). These last are formed between the hemiacetal or hemiketal group of a monosaccharide and any hydroxyl group of another monomer (Fig. 5.2). In addition to the hydroxyl groups, the repetitive units can carry other functionalities such as carboxylic groups (i.e. glucuronic, mannuronic and galacturonic acids), sulphate groups (i.e. chondroitin 4-sulphate, chondroitin 6-sulphate and dermatan sulphate) and amine or acetylamide groups (Fig. 5.1). Moreover, polysaccharides may be composed of a single type of glycosyl unit (homoglycans), as in the case of glucans (i.e. cellulose, starch, glycogen, dextran, …), or from two–six different glycosyl units (heteroglycans) in the form of alternating copolymers (carragenans, glycosaminoglycans) or block copolymers (alginates).

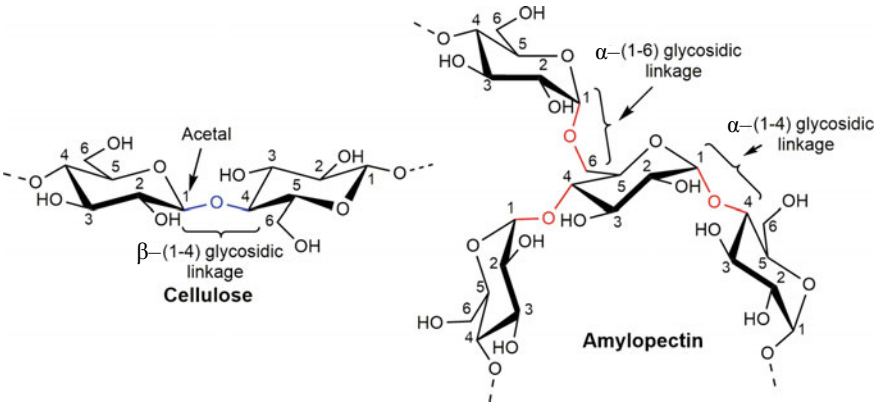


Fig. 5.2 Glycoside linkages in cellulose and amylopectin

The number of bonds for each repeating unit influences the structure of the polysaccharide, since it can bring to linear or branched chains and the position of glycoside bonds influences the polysaccharide structures in terms of the possible chain conformations. Since the cyclic aliphatic units are relatively rigid, the flexibility of the polymer is essential due to the possible rotation of the monomers around the glycoside bonds. From a structural point of view, polymer chains can be linear (such as cellulose, dextran, amylose) or branched (such as amylopectin, glycogen) and branches can be made of single monomeric units, as for scleroglucans and some galactomannans, or they can be extended, as for amylopectin and glycogen (Fig. 5.2). The conformation of polysaccharide chains and the spatial organization are influenced by the configuration of the carbon atoms, particularly the C-1 (α or β). For example, β(1-4) glucan chains correspond to extended linear structures (stripes), while a large helicoidal structure is originated by α(1-4) connected chains. Hydrogen bonds play a key role in the stabilization of polysaccharide chain conformations and supramolecular structures creating stable intra- and intermolecular interactions. The conformation of polysaccharides suggests their biochemical function, since stripe-like polysaccharides have structural and protective functions (i.e. cellulose), while polysaccharides with storage function (i.e. starch) have a large helicoidal structure that facilitates the enzymes accessibility.

In any case, in spite of the wide variety of possible monomers and structures, the polysaccharides of practical interest are only few with cellulose and starch being the most important.

Among the natural polymers, cellulose is the most abundant on the Earth (Klemm et al. 2001). It has support function in all plants including the ones where there is not lignin. Dry softwood and hardwood are made up to 40–45% cellulose, and are the most important source of cellulose for paper fabrication. The second economical source of industrial cellulose is cotton that is made up to 95% cellulose. Moreover, cellulose can be found in all vegetables and fruits (Table 5.1) and it is produced

Table 5.1 Chemical composition of some typical cellulose-containing natural materials[a]

Natural source	Main components (%)			
	Cellulose	Hemicelluloses	Lignin	Others
Cotton	95	2	1	0.4
Hemp	70–80	10–22	6	2–3
Agave	73–78	4–14	11–17	2–4
Ramie	76	17	1	6
Flax and Jute	63–71	12–21	2–13	2–13
Hardwood	43–47	25–35	16–24	2–8
Softwood	40–44	25–29	25–31	1–5
Kenaf	36	21	18	2
Bagasse and grain stubbes	35–45	25–50	15–35	5–10
Coconut fibre	32–43	10–20	43–49	4

[a]Data from Klemm, D.; Schmauder, H.-P.; Heinze, T., Cellulose. In Biopolymers, Steinbüchel, A.; Hofrichter, M. Volume 6 of the series Biopolymers, Viley-VCH 2001

by unicellular plankton or algae in the oceans by the same carbon dioxide fixation process that is found in photosynthesis of land plants.

The formation of β(1-4)-glycosidic bonds between D-anhydroglucopyranose units gives the linear high-molecular weight homopolymer of cellulose (Fig. 5.2). The polymerization degree (DP) depends on the origin and ranges from 1000 to 14,000, with corresponding average molecular weights (M_n) from 162 to 2268 kDa. Whatever the source, in nature, cellulose is found in hierarchical organized fibre structures composing the primary cell wall (Fig. 5.3). Indeed, living structures are usually made of several or often many components. Accordingly, cellulose-containing materials include other chemical components such as hemicellulose, lignin, pectin, salts in different percentage ratios as summarized in Table 5.1. The best situation is found in cotton fibre sources that are made up to 95% of cellulose (Table 5.1).

As mentioned above, cellulose is mostly included in fibres constituting the plant cell wall. These fibres are composed of twisted microfibril boundless that are aligned in the fibre direction (Fig. 5.3). Whereas, microfibers are 15–18 nm thickness hierarchic structures composed of elementary fibrils, which are made by the macromolecular chains of cellulose in highly ordered regions (i.e. crystalline), alternate with disordered domains (i.e. amorphous). Crystalline cellulose occurs in several polymorphs or allomorphs. Moon et al. gives in their review, a detailed description of cellulose and of the cellulose crystalline structure.

The hierarchical structure of cellulose is stabilized by intra- and intermolecular hydrogen bonds between hydroxyl groups. The resulting network provides stiffness to the straight chains and promotes the aggregation into the crystalline structures of cellulose.

As mentioned above, in these ordered structures, cellulose is usually accompanied by other chemical components and separation processes are usually required to get

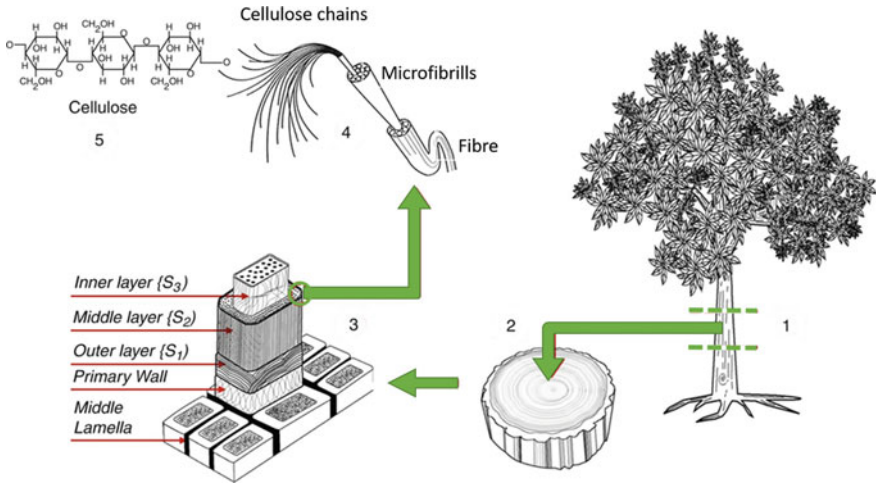


Fig. 5.3 Structure of cellulose fibre as it occurs in a plant cell wall. Adapted by permission from Springer: Springer Berlin Heidelberg, Electrospun Cellulose Composite Nanofibers. In Handbook of Polymer Nanocomposites. Processing, Performance and Application: Volume C: Polymer Nanocomposites of Cellulose Nanoparticles by Abdul Khalil, H. P. S.; Davoudpour, Y.; Bhat, A. H.; Rosamah, E.; Tahir, P. M.; Pandey, J. K.; Takagi, H.; Nakagaito, A. N.; Kim, H.-J., COPYRIGHT Springer Nature (2015)

pure cellulose. In the case of wood, two processes are currently used: the sulphite and the prehydrolysis Kraft pulping. Cellulose with purity larger than 97% is obtained by processing under high pressure in the presence of chemicals to separate lignin and hemicelluloses. Figure 5.4 shows the scheme of a typical Kraft sulphate process.

The pulp produced in the Kraft process is typically used to fabricate high-quality paper. In general, the term pulp is referred to wood or other lignocellulosic materials that have been physically and/or chemically broken down such that (more or less) discrete fibres are liberated and can be dispersed in water.

The purity, molecular weight and structure of cellulose affect its properties and the corresponding actual applications. In general, the high-molecular regularity and order result in high crystallinity degree providing stiffness and rigidity. On the other hand, the presence of amorphous domains provides flexibility. Crystalline cellulose has a negligible accessibility to water and chemicals: chemical attack can occur only on amorphous domains and on crystal surface. This results in a high inertness to chemicals and in a scarce solubility: cellulose can be solubilized only in solvents with strong ability to interrupt hydrogen bonds such as ionic liquid or binary mixtures (Biermann 1996) like electrolytes in strongly dipolar aprotic solvents (LiCl in N,N-dimethyl acetamide, in N-methyl-2-pyrrolidone, or in 1,3-dimethyl-2-imidazolidinone). This highly stable structure prevents cellulose from melting and it degrades first. In other words, cellulose does not exhibit thermoplastic behaviour. Traditional methods to process cellulose are based on chemical derivatization such as in the Celophane® process that is based on the transformation of cellulose in cel-

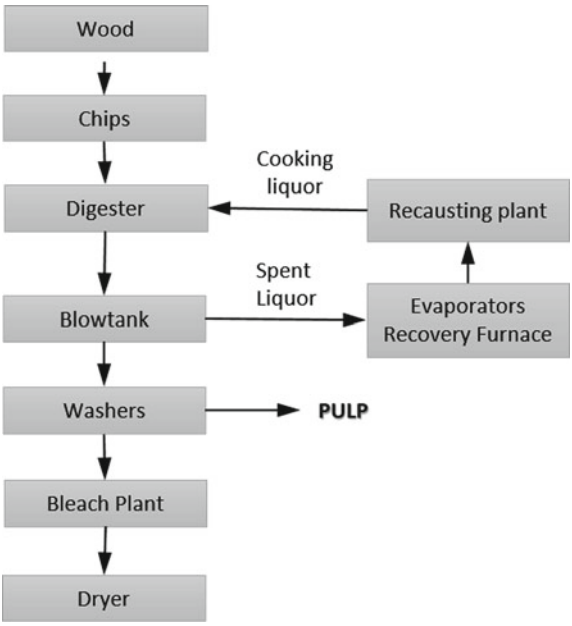


Fig. 5.4 Scheme of Kraft pulping process

Table 5.2 Properties of native starch granules[a]

Granule properties	Potato[a]	Maize[a]	Wheat[a]	Tapioca[a]	Peas[b]	Rice[c]
Diameter (μm)	5–100	3–26	1–40	4–35	2–40	3–9
Lipid (%w/w)	0.05	0.60	0.15	0.10	n.a.	–
Protein (%w/w)	0.06	0.35	0.40	0.10	0.6	n.a.
Phosphorus (%w/w)	0.08	0.02	0.06	0.01	n.a.	0.06
Amylose (%w/w)	–	–	–	–	33–49	5–28
Amylopectin (%)	21	28	28	17	–	–
Degree of polymerization (DP_n)						
Amylose	3000	800	800	3000	1300	1000
Amylopectin × 10^6	2	2	2	2	80	9
Peak Viscosity (mPa s)	3000	600	300	1000	Not present	
Swelling ability at 95 °C (%)	1153	24	21	71	20	23–30

[a]Data from (a) Ellis et al. (1998). (b) Ratnayake, W. S.; Hoover, R.; Warkentin, T., Pea Starch: Composition, Structure and Properties—A Review. Starch - Stärke 2002, 54, 217–234. (c) Singh, N.; Singh, J.; Kaur, L.; Singh Sodhi, N.; Singh Gill, B., Morphological, thermal and rheological properties of starches from different botanical sources. Food Chem 2003, 81, 219–231. And references cited therein

lulose xanthate by reaction with carbon disulphide, and on the regeneration back of cellulose as semicrystalline material by extruding xanthate in NaOH solution.

On the other hand, many derivatives of cellulose such as cellulose acetate, nitrocellulose, cellulose ethers exhibit thermoplastic behaviour: the conversion of the –OH groups into other functionality reduces the chains regularity, the crystallinity degree and the chain stiffness.

After cellulose, starch is the most available polysaccharide (BeMiller et al. 2009). It is relevant to human and animal diet. It has many uses in non-food applications also, in particular as a sizing agent in papermaking, textile, as chemical (e.g. adhesives, starch-based plastics) and as a pharmaceutical excipient. Its industrial, non-food use is growing in production volume particularly for the preparation of starch-based plastics.

Actually, starch is not a single polymer. It occurs in plants as a multi-scale granular structure composed mainly of amylose and amylopectin. Granules size is in the micrometre range even if the exact value, as well as the granule shape, depending on the botanical origin (Table 5.2).

Amylose makes up about 20–30% of the starch granule (Fig. 5.5); the remainder consists of the branched amylopectin. The linear and branched polymers are arranged to give alternate lamellae of crystalline and amorphous regions, with branches included in the amorphous one. Lamellae are 9 nm thick with a spherical morphology and form concentric multilayers within the granule with a total thickness of a few hundreds of nanometres. These well-arranged multilayers are periodically alternate with full amorphous zones of comparable thickness composed of amylopectin and amylose in poorly regular arrangements. One repeat on this scale is known as a ‘growth ring’.

As in the case of cellulose, starch granules are not made only of pure amylose and amylopectin but proteins, enzymes, amino acids, nucleic acids and lipids are included. Their proportion depends on the plant of origin and impacts on the extractability of starch (Table 5.1). Accordingly, the botanical origin of starch affects starch production processes including costs (Purves et al. 2015). In general, starches from maize, wheat, barley, sorghum, potato, sweet potatoes, cassava and pea can be extracted via two methods (dry and wet). Isolation of starch from tubers (potato, cassava and tapioca) is relatively easy and cheap due to the simple structure of tuber tissue and the low protein and fat contents. Isolation from cereals is more difficult and expensive because starch is included only in the endosperm of kernel. Germ and pericarp that are rich in fats, proteins and fibres, respectively, need to be removed. In the case of tubers, after sieving, washing and eventually peeling, roots are crushed in the presence of water to give a slurry containing both the starch granules and the other minor components (proteins, non-starch polysaccharides, amino acids, salts). Starch granules and high-molecular weight compounds are separated by sedimentation or filtration from the smaller components. Purification from non-starch polysaccharides and soluble proteins can be eventually performed by centrifugation and countercurrent washing, respectively. After these stages, a highly pure starch powder can be obtained simply by drying.

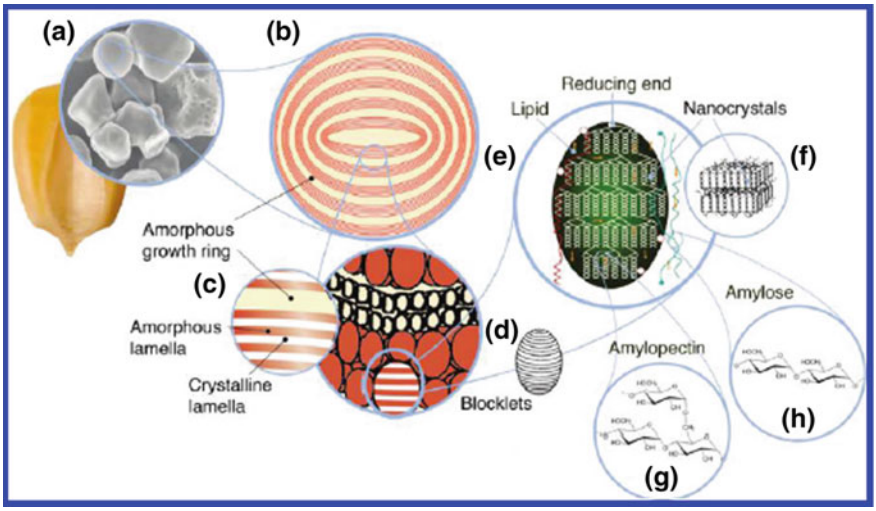


Fig. 5.5 Starch multi-scale structure: **a** starch granules from normal maize (30 μm), **b** amorphous and semicrystalline growth rings (120–500 nm), **c** amorphous and crystalline lamellae (9 nm), magnified details of the semicrystalline growth ring, **d** blocklets (20–50 nm) constituting a unit of the growth rings, **e** amylopectin double helixes forming the crystalline lamellae of the blocklets, **f** nanocrystals: other representation of the crystalline lamellae called starch nanocrystals when separated by acid hydrolysis, **g** amylopectin's molecular structure and **h** amylose's molecular structure (0.1–1 nm). Reprinted with permission from Le Corre et al. (2010) Copyright (2019) American Chemical Society. The figure was made by combining Fig. 1 from Tang, H.; Mitsunaga, T.; Kawamura, Y., 2006. Molecular arrangement in blocklets and starch granule architecture. Carbohydrate Polymers 63:555–560 and Fig. 11 from Gallant, D. J.; Bouchet, B.; Baldwin, P. M., 1997. Microscopy of starch: evidence of a new level of granule organization. Carbohydrate Polymers, 32:177–191. Copyright (2019), with permission from Elsevier

Potatoes are the major source of starch only in cold countries, such as, for instance, the Netherlands. The major source of starch in the world is maize (also named corn) (Table 5.3) and starch is extracted, mainly by wet milling process. This process was optimized over 150 years to extract not only starch but also the other vaporizable components of the corn kernel. The process begins by steepening the kernels in a dilute sulphur dioxide solution into large tanks (steep tanks). The resulting softened kernels are then processed to obtain the germ and a starch–gluten slurry. The former is thus processed to obtain oil and the fibre. The latter fraction is sent to centrifugal separators where the two main components are separated based on their different density, lower for gluten and higher for starch. The starch is then washed and dried or modified and dried.

The transformation of starch including its extraction and possible modification as well as the physical properties relevant for application and use are largely dependent on the structural organization of the granule in amorphous and crystalline domains, even if strongly affected by the presence of water. When native starch granules are

Table 5.3 Main starch crops in the world

Crops	World production in 2013 (P tons)[a]
Maize	1020
Rice (Paddy)	74.1
Wheat	71.6
Potatoes	37.6
Cassava	27.7
Bananas	10.7
Yams	0.631
Sorghum	0.623

[a]P = peta = 10^{15}

left to stand in water, they undergo limited reversible swelling at room temperature. On heating at a temperature in the 58–78 °C range, depending on the starch origin, the granules begin to swell irreversibly. This phenomenon is called gelatinization and the temperature at which it starts to occur is named as gelatinization temperature. The gelatinization proceeds with the disorganization of granular structure by breaking of H-bonds inside the granule. Some properties like as swelling degree, melting temperature, birefringence, soluble fraction and viscosity in water are irreversibly changed. Solubilization occurs by leaching of amylose and amylopectin from the granules. The relative amount of the two polysaccharides that can be solubilized depends on the origin of the starch and on the heat–shear conditions used.

Generally, on cooling a polymer solution, chain rigidity gradually increases and phase separation between solvent and polymer occurs. The exclusion of water from starch is accompanied by a decrease in the granule swelling and in the recovery of molecular order. The ordering called 'retrogradation' is a recrystallization process occurring after the crystalline order lose during gelatinization. As a whole, gelatinization and retrodegradation can be seen as an annealing process promoted by the solvent in which the crystalline order is initially destroyed thanks to the ability of water to interact by hydrogen bonds with the hydroxyl groups of starch.

Interactions of water with starch occur strongly during the early stages of sorption when molecules diffuse as isolate species. Because of the interaction with water, intermolecular bonds between starch units becomes weaker resulting in an increase in the distance between starch chains and then in a higher mobility of the macromolecular chains. Both these effects indicate the role as plasticizer of water on starch at a molecular level and are accompanied by a decrease in the T_g values. At high water content, clusters of water molecules form with strong and well-oriented intramolec-

ular hydrogen bonds among them. At this stage, water exhibits typical liquid-like properties and forms weaker bonds with starch. T_g value of dry starch is in the 225–235 °C range; it decreases with moisture sorption and level off below 0 °C at water content above 30%. Under this condition water forms a separate phase outside the granules. The plasticization effect of water is due to its ability to enter into the very rigid hydrogen bonds network of the dry starch and to interrupt the networks by replacing some hydrogen bonds either intra-chains and interchains, the former between neighbouring repeating units and the latter between closed chains.

Polyols, such as sugars and alditols, including glycerol and ethylene glycol have also the capacity to plasticize starch as well. Several thermoplastic materials based on starch and formulate with polyols have been patented. They are produced by using conventional techniques employed for commodity plastics, such as extrusion, compression moulding, injection and blow moulding (see Sect. 4.3.5). The key step is the destructurization of starch under extrusion condition in the presence of plasticizers. In fact, with water as plasticizer, starch destructures under extrusion at water content lower than 20%, while for gelatinization water must be in excess (>30%). The final properties of processed starch depend on the starch type, as well as on the processing parameters such as temperature, shear, cooling regime and amount of plasticizers. On the other hand, mechanical properties of extruded starch depend on the plasticization degree: the fracture mechanism changes from a rapid, brittle fracture at plasticizer content below 8% to slow, plastic fracture (tearing) with content close to 20% (Bastioli 1998).

Commercial thermoplastic starch usually includes destructurized starch in mixture with plasticizers and polymers. For instance, thermoplastic starches commercialized by Novon are blends with various hydrophilic polymers, such as ethylene–vinyl alcohol copolymers (EVOH). Hydrophobic polymers and additives, such as plasticizers and lubricants, could also be included. Mater-Bi® is the trade name of the formulations produced by Novamont that are typically comprised of at least 60% starch. Other natural additives and hydrophilic, biodegradable synthetic polymers are included in the blends forming interpenetrated or semi-interpenetrated structures at the molecular level. The resulting ultimate properties are comparable to the ones of low- and high-density PE.

Others nature-made polymers of practical interest are fibre proteins such as collagen, silk and keratin. None of this exhibit thermoplastic behaviour, or in their native status or after extraction. In order to become thermoplastic, they need to be processed like cellulose (in the case of fibre proteins) or starch. They must be destructurized and plasticized to assume thermoplastic behaviour.

5.3 Bioplastics in Engineered Systems

The concept of Metabolic Engineering was introduced over 20 years ago from Bailey. The metabolism of an organism is modified by genetic engineering. More recently, the concept has undergone a transformation and actually, metabolic engineering is

not only used for improving the production of native metabolites but also for producing any desired molecule. It usually involves the optimization or modification of biochemical pathways already existing by the introduction of new pathway components, most commonly in bacteria, yeast or plants. The goal is the exploitation of these organisms in a cost-effective production at the industrial level of valuable substances. In this field, the recent increase in the variety of molecules produced from engineered cellular and the growing of commercial interest in bio-based polymers and plastics with a particular interest in products with improved functionalities and obtained with sustainable and environmental-friendly synthetic approaches can be combined.

From a general point of view, biopolymers can be synthesized from monomers produced in vivo and then polymerized in vitro. Alternative and very appealing approaches produce the polymer directly by the microbe. Both methods allow a diversification of the final product by using a simplified production scheme with respect to traditional industrial methods with the control in molecular weight. During the last two decades, the knowledge on genes, proteins and metabolites are improved, the costs of oligonucleotides synthesis are decreased and much more precise techniques for investigating the metabolism inside the cells have been developed. For this reason, the target of metabolic engineering is actually the possibility of manipulating the entire cell instead the perturbation of single pathways to. New approaches are now considered including the possibility of controlling the gene expression and modulating the regulatory networks throughout the cell. Traditional methods instead were simply based on deleting and/or over-expressing endogenous genes with eventual introduction also of heterologous genes.

In addition to monomers (see Sect. 5.2), the use of the microbial cell factories for the production of full-length polymers is attracting the interest of the scientific community. The most significant example of bioplastic produced in engineered system is poly(hydroxyalkanoates) as showed is below in Fig. 5.6.

It is already known that some bacteria are able to accumulate polyhydroxyalkanoates (PHAs), in the presence of an excess of carbon source and under nutrient-limited conditions. PHAs are intrinsically biocompatible and biodegradable. Others example of polymers produced microbially are, cellulose and spider silk others. Cellulose can be bio-synthesized by cyanobacteria *Synechococcus leopoliensis* constitutes, e.g. a feedstock of CO_2, because it can be easily digested by cellulose due to its non-crystalline structure. Silk proteins with an optimized molecular weight were expressed in *E. coli* by inducing the glycine production in the cell.

Even if some monomeric constituents can be produced by microbial fermentation (such as lactic acid) and used in chemical synthesis of the corresponding bio-based polyesters, PHAs and their polythioester analogues (see the general chemical structures in Fig. 5.7) are the only relevant examples of polymers produced and accumulated in vivo and whose structure can be manipulated by metabolic engineering. The host enzyme, which is present in the cell, affects the monomeric composition and as a consequence, the polymer properties by supplying the PHA-synthase and hydroxyacil-CoA thioester precursors involved in the metabolic pathway.

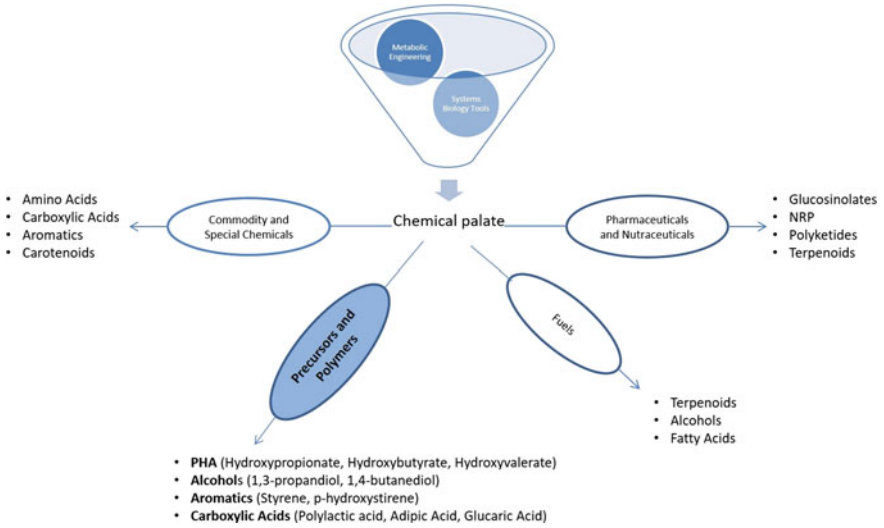
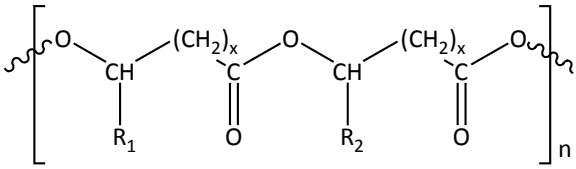


Fig. 5.6 The main classes of polymers produced in engineered systems

Fig. 5.7 General structure of polyhydroxyalkanoates



R_1, R_2 = alkyl groups C1-C13

X = 1 - 4

n = 100 - 30.000

From the pioneeristic studies in the 1920s of Maurice Lemoigne with the discovery of poly(3-hydroxybutyrate) (PHB), as a product of accumulation in *Bacillus megaterium*, many others polyhydroxyalkanoates with different chemical structure (PHA, Fig. 5.7) have been reported to form in a wide range of organisms (e.g. *Psedomonas* sp., *Bacillus* sp. and *Methylobacterium* sp.). Some of them are homopolymers of a *R*-hydroxyalkanoic acid (HA) while others are copolymers based on two or more HAs.

PHB plays the role of intracellular reserve for carbon and energy in bacteria similarly to starch and glycogen in plants and animals. At the beginning of the scientific investigation, 3-hydroxybytyrate unit was believed to be the only hydroxyalkanoate (HA) constituent of the microbial reserve polymer. Instead, up to now approximately, 125 different HAs are known to be present as building blocks in a more general class of polymers named as polyhydroxyalkanoates (PHA).

The growing interest in these polymers derives from their rapid degradation (3–9 months) under both aerobic, with the development of carbon dioxide and water, and anaerobic conditions, with the development of methane. They are degraded without the need of any special environmental set-up by different kinds of microorganisms. In particular, they degrade in the see and ocean also.

Gram-positive and Gram-negative bacteria from *Necator*, *Cupriavidus*, *Ralstonia*, *Pseudomonas*, *Aeromonas*, *Bacillus*, *Alcaligenes*, *Enterobacter*, *Rhodobacte*r and some cyanobacteria and halophiles can naturally synthesize polyhydroxyalkanoic acids as insoluble inclusions accumulated in the cytoplasm and playing the role of storage compounds, if carbon is in excess, especially if other essential nutrients, such as nitrogen or phosphorus, are in limited amount. However, *Azobacter vinelandii UWD*, *Azobacter eutrophus*, *Azobacter latus* and a *mutant Azobacter vinelandii* are able to biosynthesize PHAs under non-limiting conditions. The type of microorganisms, the availability of low cost of carbon source, culture conditions, growth rate, polymer yield from the substrate affect the final composition of PHA and its properties. Renewable resources such as starch, cellulose, hemicellulose, sucrose, triglycerides, wheat, sub-products such as glycerol, rice bran, molasses, whey, organic acids, fossil resources such as methane, mineral oil, lignin and wastes such as wastewaters and palm oil mill represent suitable substrates for bacteria in PHAs production at lab scale. The choice of the substrates is one of the weaknesses of the production because it largely affects the PHA production costs. Only a few kinds of bacteria are able to be used for industrial low-volume scale production of low-cost materials, but with high-value biomedical application for their versatility and biocompatibility, such as *Azotobacter vinelandii*, *C. Necator*, *Methylobacterium organophillum*, *A. latus*, *Protomonas extorquens*, *Pseudomonas olovorans*, *recombinant E. Coli and Paracoccus denitrificans*.

The classification of PHAs is based on the number of the carbon atoms in the monomer units and split them into two main groups. PHAs with a short-chain length of C2–C5 atoms (scl-PHAs) are brittle, stiff and have high crystallinity content. The increase in the chain length of the monomer from C4 to C14 (mcl-PHAs) produces an increase in flexibility and melting point and a decrease in crystallinity degree and tensile strength. There are also some examples of PHA-copolymers synthesized from a combination of short-chain and medium-chain monomers (e.g. P(3HB-*co*-4HB), P(3HB-*co*-3HV), P(3HB-*co*-3HHx)).

The chemical structure of the monomers (i.e. molecular weight, presence and structure of branches, presence or heteroatoms) and the distance between the ester linkages largely affect all the properties of PHAs. The nature of the microorganism, the culture conditions and carbon source determines the type of monomer incorporated during the polymerization. The type of microorganisms with their activity, fermentation conditions, state of inoculum, the nature of the medium composition and the type of processing are used to affect the molecular weight of PHAs. The $\overline{M_w}$ values of *mcl*-PHAs moved in the range 60,000–412,000 independently by the presence of both saturated and unsaturated pendant groups and $\overline{M_n}$ between 40,000 and 231,000. They are generally lower than the values of scl-PHAs. The polydispersity ranges between 1.6 and 4.4 and it is higher with unsaturated monomers.

All PHAs are semicrystalline polymers. In the case of *mcl*-PHAs, the values of T_g are in the range from −25 to 65 °C and T_m from 42 to 65 °C (Table 3.6). The T_g value decreases with the increase in the average length of the pendant group. A higher degree of crystallization is observed only in the case of higher T_m values. A different crystalline packaging can be due to the presence of odd or even numbered monomers. A layer-like order arrangement involves both the backbone and the side chains and the structural regularity of the repeating units affects the crystallinity degree and promotes the formation of lamellar crystals. For this reason, a low crystallinity degree is the consequence of large and irregular pendant side groups. PHAs are enantiomerically pure (all in *D*(-) configuration) and their isotactic and syndiotactic sequences help the crystallization process (see Sect. 3.4).

PHAs with medium-chain length show elastomeric behaviour thanks to the presence of crystalline domains acting as physical cross-links. The properties are completely different for the ones of short-chain length PHAs. On the other hand, an increase in the toughness and in the flexibility, measured as elongation at break and a decrease of the stiffness, measured as Young's modulus (Table 3.6) can be obtained with the presence of a comonomer into the polymer backbone.

The most important issue towards the industrial use of PHAs on a large scale is the high production costs because of the microorganism cost. In alternative, the use of a microorganism's population selected from the variety already present in wastewater was proposed. This methodology resulted in an enhanced PHA-producing capacity and in the reduction of expensive feedstock use, with respect to the traditional pure culture bacterial fermentation. The consequence is a reduction not only of the production costs but also of the environmental impact of the whole process. Examples of wastewaters used at lab scale are from the paper mill and food industries. The effectiveness of mixed microbial cultures (MMCs) production processes is based on the selection and enrichment of the PHA-accumulating microorganisms and a maximum PHA content of 89% of dry cell weight is obtained. MMCs can be exposed to biomass for repeated periods of feasts under aerobic, anaerobic and/or anoxic (a total depletion in the level of oxygen) dynamic feed conditions to enrich them with PHA-storing bacteria.

In-silico genomic studies have also allowed the investigation of bacterial strains able to produce at the same time H_2 and PHB polymers under dark fermentative conditions. It was found that a single isolated bacterial suffers from the risk to be contaminated because a rapid increase in the amount of contaminants can take place with the elimination of the original culture. Instead, the use of a mixture of well-defined bacterial cultures with different and high metabolic activities can help to solve this aspect and to enlarge the operative physiological conditions. The use of biowastes as feed represents a promising possibility to produce H_2 and PHB in a sustainable manner. At the same time, the immobilization of the bacterial cultures on natural, high biocompatible and biocompatible substrates such as coconut coir, groundnut shells or banana sleeves allows solving the difficulties to retain a large population of free-floating bacteria in a continuous culture mode.

Recently, the production of novel biopolymers in plants represented a challenge to provide renewable sources of materials of industrial interests from agriculture. Plants cells play the role of miniature factories because they produce biochemicals and materials necessary for their growth, reproduction, defence and for monitoring and interacting with their environment. Cellulose, lignin, starch and hemicelluloses are materials traditionally produced from plants and used by humans for their lives. It is known from ancient times that the production of natural rubber is from *Hevea brasiliensis* or Para rubber tree. Anyway, the addition of genes encoding enzyme activities can convert the endogenous plant metabolite to a polymeric structure. PHAs, collagen, silk, elastin and cyanophycin are produced *in planta* with this strategy.

As previously reported, the industrial PHA production by microorganisms must overcome the disadvantages of the high production costs. Therefore, the use of pure cheaper carbon sources instead of glucose or sucrose is of real interest. In this direction, glycerol, which is a coproduct, produced in large scale, of many industrial processes such as in the production of biodiesel, represents an interesting solution. In particular, some studies showed that some bacteria and archaea strains are able to produce PHB oligomers in the presence of glycerol. In particular, it was demonstrated that glycerol by-product from biodiesel production can be used as a liquid phase in the cultivation of osmophilic organism in the presence of hydrolysates from meat and bone meal (free from prions of bovine spongiform encephalopathy) that acts as sources of nitrogen and phosphorus.

5.4 Monomers from Nature and Their Polymerization Derivatives

Several molecules with the suitable structure to be converted into macromolecules by man-made polymerization processes can be obtained either during natural processes (lactic acid) or by synthetic routes using bio raw materials (ethylene from bioethanol).

Renewable resources constitute an extremely rich and varied array of molecules. Most of them have more than one functional group and therefore, under the definition of polymerizable monomers, they are suitable building blocks to prepare long polymeric chains. However, in spite of the huge variety, only a limited number of them have found practical applications. Among these, we can mention monoterpenes from Pinewood that found applications mainly as adhesive components and spray adjuvant in the form of low-molecular-weight hydrocarbon-like resins. Rosin, also known as colophony, is the term commonly used for the non-volatile residue obtained after the distillation of conifer tree resins. It is mainly composed of aromatic acids. It looks like a semitransparent and brittle solid. Pine trees (*Pinus genus*) is the most important source of rosin. In spite of the broad variety, often-natural molecules are available in low amount or are difficult to recover in pure form, since they are

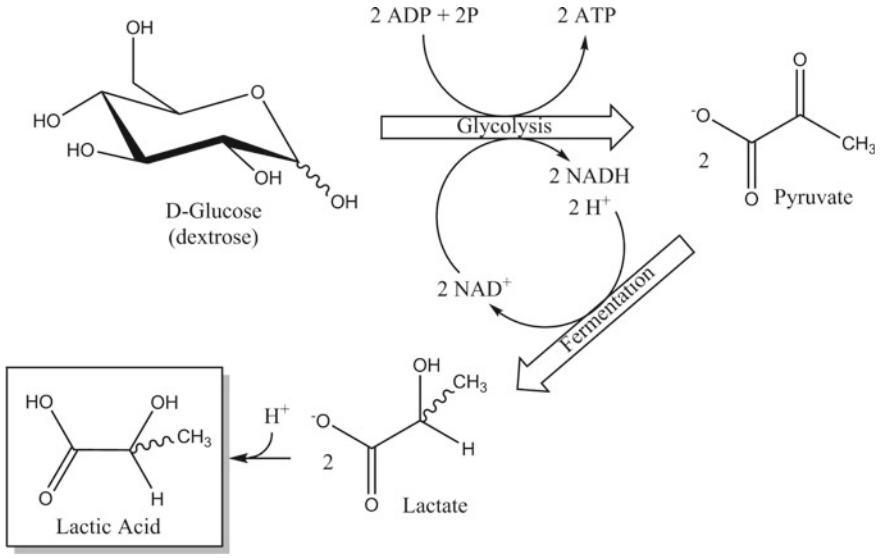


Fig. 5.8 Biosynthesis of Lactic Acid from dextrose

included in complex biological systems. A representative example is provided by lactic acid, the monomer of polylactic acid (PLA). It is the well-known by-product of muscles when they work under anaerobic conditions producing energy from glucose. It is responsible for the fatigue disease since it accumulates in muscles after intensive work. Like other chemicals, it is a naturally occurring molecule, but to be available in large amounts, it must be produced through industrial process. In this case, its natural origin is preserved by using renewable resources as raw material for its production. Actually, lactic acid has been produced on large scale from maize by NatureWorks since 2001 in Nebraska (USA). At present, it is produced by many other companies around the world. The process adopted by NatureWorks starts with the extraction of starch from maize and the separation from the other components of the kernel (proteins, fats, fibres, ash and water). A wet milling process is adopted (see Sect. 5.2) and the extracted starch is then hydrolysed to D-glucose (dextrose) in the presence of an enzyme, in the second process stage. The resulting sugar solution is transferred to a fermentation process where lactate is produced by fermentation with a microbial inoculum under anoxic condition (Fig. 5.8). Calcium hydroxide is used to control the pH during the fermentation and to achieve high conversion. Finally, a pure lactic acid monomer is obtained by acidification with sulphuric acid, filtration-off of the precipitated by-products (mostly $CaSO_4$) and evaporation of water from the solution.

The industrial process of lactic acid production is a multistep process based on the transformation of an agriculture crop into a chemical by using biological and chemical processes (Fig. 5.9). This is what typical occurs in a modern biorefinery,

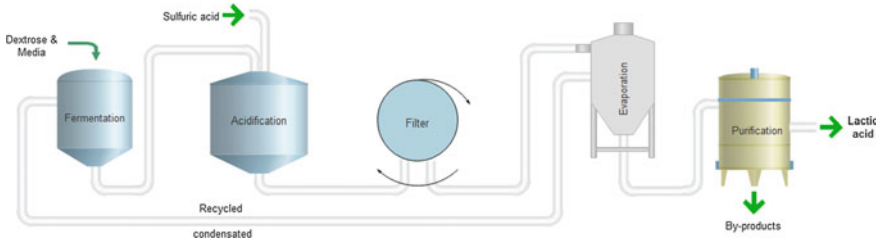


Fig. 5.9 Lactic acid production process

even if this last is usually more complex integrating several processes of biomass conversion to produce not only fuels and chemicals but also power energy and heat.

In other words, the biorefinery must be considered quite similar to today's petroleum refinery, which produces multiple fuels and products from petroleum; the main difference being the starting raw materials. By obtaining multiple products, a biorefinery gives values to and takes advantage of the different components that are present in biomass and their intermediates. The inherent value of biomass feedstock can thus be fully exploited and promoted. A biorefinery typically produces chemical or nutraceutical products in low-volume but high-value, fuel and commodity chemicals in high-volume and low-value such as biodiesel and bioethanol, respectively (see also alcohol fuel). The latter being used as raw materials for the production of many others derived chemicals such as for instance monomers for polymer and solvents. In biorefinery electricity, the heat needed for the process is usually obtained by combustion of the produced fuel through combined heat and power (CHP) technology. Exceeding energy is usually sold as electricity to the local utility. A typical example of this sort is the production of ethylene as high-value derivative of ethanol, the primary product of the biorefinery (bioethanol). Ethylene is the monomer, which produces any polymer belonging to the well-known commodity thermoplastic polyethylene family.

Ethanol is currently produced from different kinds of renewable raw materials, including starch, cellulose, hemicellulose and lignin-containing crops. In particular, first-generation processes used food-competing raw materials such as maize, while the most recent second-generation process is based on non-food crops and agriculture by-products, which are composed mainly of cellulose and hemicellulose. Third-generation processes use agriculture or urban organic wastes. The most part of plants operating while this book is in preparation produce ethanol mainly from sugar, cellulose or starch, with the last two being both sugar polymers (see Sect. 5.2). The first step of all processes have the aim to get sugars from the raw crops and the operative conditions adopted in each of these steps depend on raw material and on the used technology. In Brazil, for instance, sugarcane is mostly used as a substrate to

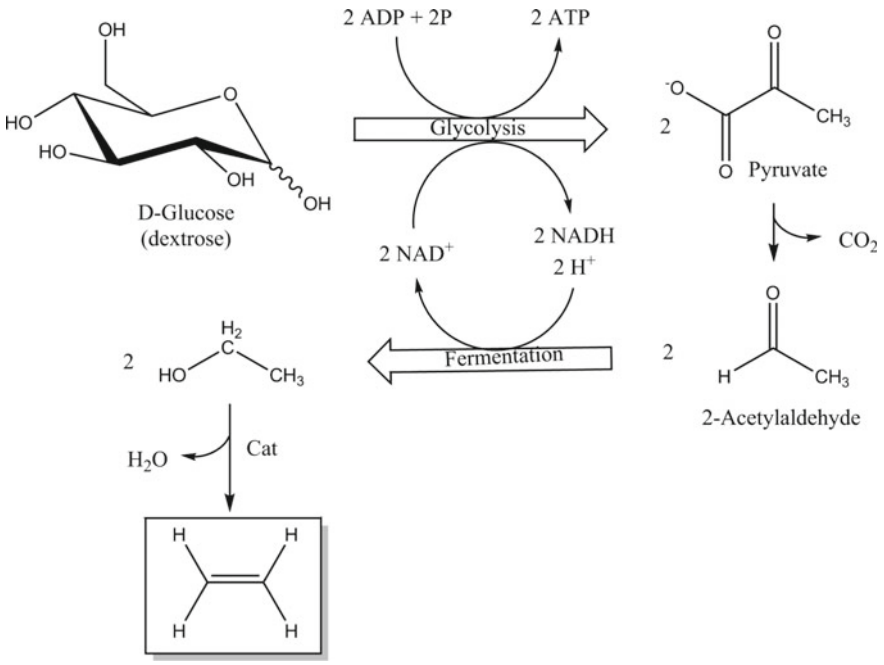


Fig. 5.10 Synthesis of ethylene from ethanol, which is obtained by biosynthesys from dextrose

get ethanol. Sugarcane is pressed to obtain the sugarcane juice. This is separated by filtration from the solid fibrous residuum named as cane bagasse. The juice is clarified and concentrated at first by water evaporation to promote sucrose crystallization and then centrifugated. The obtained sucrose-saturated viscous phase is called as cane molasses. It is made of 45–60% sucrose, 5–20% glucose plus a low amount of fructose. Sugarcane juice, molasses or a combination of them are used as fermentation substrates for ethanol production. In fermentation, the first reaction is usually the glycolysis to pyruvate (Fig. 5.10), then depending on the used bacteria and on the process conditions different products can be obtained by the true fermentation step. In most of the actual operating plans, ethanol is the target product since it can be used as fuel and chemical raw material, as well.

To be used as fuel, crude ethanol must be purified, in particular, it must be extracted from the aqueous fermentation broth. This is typically the most energy demanding and thus cost-effective part of the plant, since ethanol distill as azeotrope with water and further purification steps are need to get dry ethanol. This cost can be compensated if it can be used as a reagent to obtain other chemicals with high value. The Brazilian petrochemical company, Braskem is producing ethylene from bioethanol and then polyethylene, the first bio-PE certified in the world. Ethylene is obtained by dehydration in the presence of a suitable catalyst (Fig. 5.10).

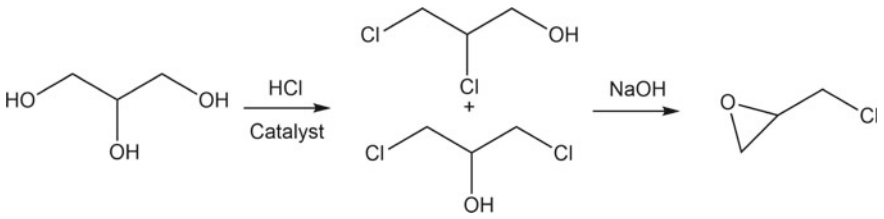


Fig. 5.11 Epichlorihydrin synthesis from glycerol

The two examples so far mentioned (lactic acid and ethylene production) are both based on sugar fermentation platforms. An alternative platform for monomer production is based on the use of fatty acids as raw materials. In fact, glycerol is becoming available at a low price on the market since it is a by-product in biodiesel production. Glycerol is an interesting raw material for the production of monomers with even carbons, which are difficult to obtain from petrol. Among these 1,3-propandiol, which can be used to prepare polyesters and polyurethanes, as well as epichlorohydrin. This last is mostly converted into epoxide monomers, and acrolein, which is an intermediate for the preparation of acrylic monomers. The first two examples are already operating on pilot plant by DuPont and Solvay, respectively. A process for the production of acrolein from glycerol on gas phase has been patented by Arkema and developed at pilot scale. However, the industrial scale-up has not been carried out up to now because of economic reason. In fact, the price for the production of acrolein from petrol is still lower than the production from glycerol. Other companies are also developing others alternative processes for the production of acrolein or acrylic acid from biomass.

Epichlorohydrin produced by Solvay and commercialized as Epicerol® is obtained in a two-step process. In the first step, glycerine is reacted with hydrochloric acid to give dichloropropanol, an intermediate product. The second step is the dehydrochlorination of the product, which produces epichlorohydrin (Fig. 5.11). Different from the others mentioned examples, this process does not differ from traditional petrol-based process except for the origin of the starting reagent.

5.5 Bioplastic Formulation and Processing

The above classes of polymers are then going to end products for commercial use through a procedure similar to one of the fossil-derived polymers, which implies the use of additives to modulate their performances according to the market request. Biodegradable polymers, particularly those from renewable resources, allow solving the low sustainability of long-lasting polymers on short-lived applications and the difficulties to find accurate and economically viable outlets of traditional materials by taking also into account the negative environmental balance of the material recycling.

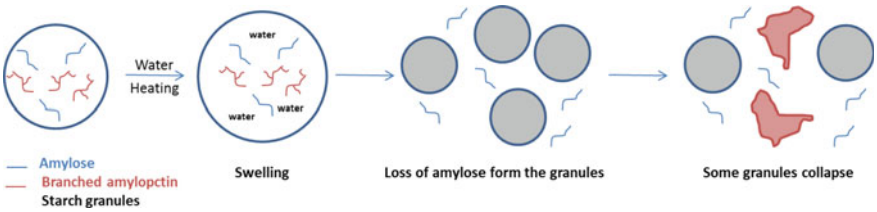


Fig. 5.12 Gelatinization of starch

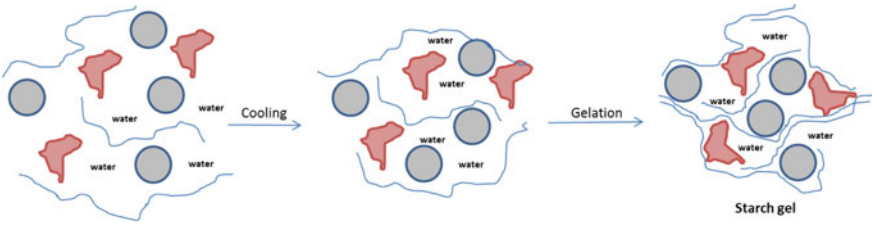


Fig. 5.13 Gelation of starch

Polysaccharides represent the most abundant family of agro-polymers, with cellulose, starch and chitin as the main actors. Their use can contribute to preserve petrol resources replacing fossil resources-based polymers for some applications, e.g. packaging, agricultural, biomedical uses.

In this class of polymers, starch is an important material, because it represents the main storage supply in botanical resources. It is available in large amounts at the raw state and can be easily processed and transformed for many industrial applications. For this reason, its processability is described in detail in this paragraph.

With the only exception of the use as a filler in reinforced plastics, native starch is typically modified by chemical or physical approaches and converted into thermoplastic polymer and then processed in the melt. The treatment of starch at high temperature in the presence of large amount of water causes the denaturation of the granular organization through the gelatinization process (Fig. 5.12).

Gelation (Fig. 5.13) step follows the gelatinization and the process concludes with the retrogradation of the starch gel.

During this process, starch forms a sort of paste and most of the intermacromolecular hydrogen bonds are destroyed. The melting and glass transition temperature of starch decrease as a consequence of the transformation. Different products for different applications can be obtained by playing with the process condition such as water content and thermomechanical inputs. In order to split the value of the melting temperature and the degradation temperature of starch, chemicals with boiling temperature higher than the processing one are used as plasticizer, i.e. glycerol, sorbitol, polyethylene glycol urea and high molecular weight amines. Water can play both the role of destructuring agent and plasticizer.

The disruption of native starch granules by the combination of thermal, mechanical, destructuring agents and plasticizers is generally carried out by extrusion processes. They can be carried out in one or two steps. In one-step process, a twin extruder is fed with starch granules and then water and plasticizer are added along the barrel. Exceeding water is eliminated. At the end of the process, starch is completely destructured and melted. In two-step processes, first, starch granules are mixed at dry state with the plasticizer, and then the mixture is heated up to allow the diffusion of the plasticizer into the granules. After that, water is added and the blend is introduced in the extruder. Along the barrel, starch is destructured, plasticized, melted and partially depolymerized. In any case, a homogeneous molten state is obtained. Plasticized starch shows strong modifications of the chemical–physical properties with respect to the native one. In particular, during plasticization, the crystallin phase decreases and modifies. Two kinds of crystallinity can be obtained: residual crystallinity from native starch and processing-induced crystallinity. The latter is influenced by the process parameters such as temperature, screw geometry and speed, plasticizer kind and content. Crystals form by fast recrystallization of amylose into single-helical structures even if co-crystallization of amylose and amylopectin is also proposed. The process is indicated to occurs mainly during extrusion even if recrystallization during ageing has been documented with some plasticizers. The presence of water affects some properties of plasticized starch, in particular, the mechanical ones that result to depend on the sorption–desorption of moisture with the environment. This equilibrium is affected by the presence of other plasticizers. Glycerol, e.g. can be differently linked to the polysaccharide chains as a consequence of the humidity content and total amount of the plasticizers. In the presence of moisture excess, a phase separation occurs with the formation of a multiphase system with rich and poor plasticizer domain. The ratio between water and plasticizer influence also the mechanical properties and permeability of plasticized starch. Anyway, the water permeability results higher because of the polar character of the material even if the oxygen permeability is lower than in other polyesters.

Similarly, water plays an important role in the denaturation of biological macromolecules such as proteins. It was shown that water does not play just the role of solvent, but it also takes part in the structure of biological polymers affecting their properties. Indeed, some water molecules are strongly bonded to proteins by hydrogen linkages, the exact amount depending on the chemical composition of the protein as well as on its spatial organization. Fibrillar macromolecules, such as collagen, with their larger exposed surface, have large amount of bonded water. On the contrary, globular proteins having highly ordered and compact structure with molecular mass less than about 30 kDa have lower amount of bonded water. The polypeptide chain is packed into well-defined conformations, which is determined by the sequence of the polar and apolar groups along the chain that is defined by its primary structure (i.e. the sequence of amino acid residues). The original conformation and the corresponding biological function is lost if proteins are submitted to severe conditions (e.g. high temperature, high pressure, high acidity, or high concentration of denaturants) promoting unfolding and denaturation. The denaturation process occurs in a short temperature range with extensive heat absorption. It is a highly cooperative

process involving a high number of contacts between nonpolar groups in the protein. The overall surface area of the nonpolar groups has to be rearranged and exposed to water upon globular protein unfolding. Significantly different is the unfolding of fibrillar proteins. Collagen represents an extreme example of this group with a very large exposed surface area per unit volume. The macromolecular chain of collagen is highly flexible and assumes the form of a random coil in aqueous solution. However, three chains of collagen can associate forming a stable rope-like poly-l-prolyl superhelix able to provide biological tissues with mechanical stress resistance over long distances. A feature of collagen is the loss of its regular rigid structure with the increase in the temperature above a critical value (above 40 °C) at which the chains are separated into three independent random coils. It is important to note that collagens obtained from different sources do not differ essentially in their structural organization but differ significantly in their thermal stability; this last depending on the imino acid content. Indeed, the number of possible conformations depends inversely on the amount of pyrrolidine ring in the chain. Thermal studies have demonstrated that, in contrast to globular proteins, there are no apolar groups in the collagen triple helix that might be exposed to water upon its unfolding, because hydroxyproline is in the third (Y) position of the triplet repeat. As a consequence, hydroxyl groups of prolines are able to interact with the water molecules that surround the collagen superhelix and form an extensive cooperative network of water hydrogen bonding. This extended network is suggested to be responsible for the very large enthalpy of collagen 'melting', which is much larger than the enthalpy of globular protein denaturation.

Similarly, to the process of synthetic macromolecules, extrusion of proteins must be performed after denaturation (often named melting for analogy with crystalline polymers) and far above the glass transition temperature so that the macromolecule chain mobility is high enough to allow chain flowing and eventually mixing with other components. However, temperature during extrusion must be kept below a limit value to avoid thermal-activated reactions, such as degradation with the formation of volatile compounds. For this reason, proteins can be processed only if their denaturation temperature is significantly below their degradation temperature. As previously reported, water is the most effective plasticizer agents for proteins: by increasing the moisture content from 0 to 20% w/w the denaturation temperature of proteins decreases from 200 to 80 °C. Extrusion of protein-based thermoplastics requires the following steps: protein chains denaturation, disassociation, unravel and alignment that are possible only if the chains are enough mobile and can form new molecular interactions on cooling. Even if proteins are considered as amorphous structures plasticized by water, some aspects related to the native protein structure (ordered regions, such as α-helices and β-sheets) and thermal behaviour (endothermic events) correlate with the behaviour of semicrystalline materials. A series of transitions must be taken into account: at the glass transition, the amorphous regions become mobile, at the melting temperature, the crystalline regions melt, at the degradation temperature the polymer chains thermally decompose.

As in polysaccharides, plasticizers with two or more hydroxyl groups such as glycerol, sorbitol, and di-, tri- or mono ethylene glycol are added to proteins in

combination with or instead of water for thermoplastic applications. The presence of a second plasticizer with water in protein mixture allows avoiding the increase in the T_g value and brittleness as the moisture content decreases. During extrusion, mechanical stress promotes protein de-aggregation, while heating promotes aggregation by hydrophobic interactions (physical cross-linking) and stabilization of the aggregated structures by disulphide bonds, reducing melt flow. Processing temperature is, therefore, an important parameter to control protein/protein association. Furthermore, degradation of the protein can occur because of the increase in the residence time, torque, and pressure. For protein processability, plasticizers as well as others additives such as thermal stabilizer and disulphide bonding inhibitors are need to reduce macromolecular associations during extrusion and to achieve a thermoplastic behaviour. On the contrary, an extensive cross-linking (>10%), can result in the formation of a thermoset material (an extended cross-linked material that can not be processed again). It is important to point out that the behaviour of natural polymers is difficult to generalize and differences can be observed because of the difference in composition that always exist even among polymers belonging to the same class but of different origin. Covalent cross-links are not the only inhibiting factor for the thermoplastic behaviour. Actually, the hydrophobic interactions have to be overcome and sometime this drawback can be negligible. For instance, in the case of water-insoluble and hydrophobic proteins, such as zein, amphiphilic molecules, such as sodium dodecyl sulphate, are need to enable thermoplastic processing. Ionic surfactants give electrostatic as well as hydrophobic interactions leading to the dissociation of protein chains. On the other hand, hydrophobic plasticizers usually give non-homogeneous and non-compatible blends with a reduction in water absorption. Indeed, the proteins ability to interact with water is not only influenced by covalent cross-linking and chain arrangement but also by the chemical additives.

Based on the possible ways to transform a natural polymer in a thermoplastic material, the most common bio-based and biodegradable polymers are classified into the following four categories depending on the synthetic route used to prepare them:

- Natural polymers: They are usually obtained by extraction from biomass, in particular from agro-resources and include mainly polysaccharides and proteins both from animals (whey, collagen) and plants (zein, soya and gluten) (see Sect. 5.3);
- polymers obtained by microbial production, e.g. poly(hydroxyalkanoates) (PHAs) (see Sect. 5.4);
- polymers made by man using monomers obtained from agro-resources, e.g. polylactic acid (PLA) (see Sect. 5.5);
- biodegradable polymers whose monomers are obtained by chemical synthesis from fossil resources, e.g. poly(ε-caprolactone) (PCL).

Actually, starch and PLA biopolymers are the most interesting since they are produced on an industrial scale and thus are commercially available in relatively large amounts. Furthermore, they have an interesting balance of properties. In particular, PLA shows excellent transparency and relatively good water resistance. The

high stiffness can be reduced to obtain flexible films by the addition of plasticizers. However, oxygen barrier decreases as a consequence.

Natural polymers have excellent oxygen barrier under dry conditions. However, they are usually too much stiff and difficult to process by using conventional equipment. These drawbacks can be overcome by taking advantage of their strong water sensitivity that allows biopolymer processing in the presence of moisture acting as a plasticizer. On the other hand, oxygen barrier properties can be recovered after processing by material drying. All biopolymers with commercial interest show excellent gas barrier properties in their optimum formulations, even if the large amount of plasticizers, necessary to get materials properties adequate for processing have a detrimental effect.

Polyhydroxyalkanoates show very high water barrier properties and they can be used in multilayer systems in combination with other natural polymers. However, all these materials usually suffer from relatively high production cost, and their commercial interest remains in competition with conventional thermoplastic materials. An innovative trend in polymer science with high practical interest is the addition of natural additives in bioplastic formulations. This approach is promising for food-packaging applications and allows the reduction or elimination of some of the main food spoilage causes, such as rancidity, colour loss/change, nutrient losses, dehydration, microbial proliferation, senescence, gas build-up and off-odour. Natural extracts from plants, essential oils or agricultural waste products can show antimicrobial activity against different pathogenic and spoilage microorganisms, including Gram-negative and Gram-positive bacteria and moulds. Examples are extracts of blueberry on the growth of *Listeria monocytogenes* and *Salmonella Enteritidis*, grape seed extracts against major food pathogens like *Salmonella Typhimurium*, *Escherichia coli* (*E. coli*), a green tea extract against *Staphylococcus* and some Gram-negative bacteria, such as *E. coli* or *Salmonella*. The use of natural extracts or their original compounds (including low-molecular-weight phenolic acids, tannins, proanthocyanidins, flavonoids, such as anthocyanins or flavonols) is growing. Disadvantages are their high volatile; to overcome this drawback, they are incorporated into the packaging materials as additives to be released to food by migration. With this purpose, several natural extracts have been incorporated into different types of biopolymers that were used in the development of antimicrobial active films. The addition of citrus extract to gelatine and methylcellulose gives films with no For instance, odour and negligible water solubility. polycaprolactone (PCL)/Alginate films containing three natural extracts from rosemary and Asian and Italian essential oils were developed and they showed good activity in controlling/inhibiting the growth of foodborne pathogens in fresh-cut vegetable. Active materials can be obtained also by incorporating lemon, propolis extracts and olive leaves as a source of polyphenols on PLA, and PCL films. The processing temperature must be selected below the temperature at which degradation of the polymer matrix starts as well as below the volatilization and degradation temperatures of the active compounds. Furthermore, the hydrophobic/hydrophilic nature of active compounds may affect in a positive or negative way, respectively, the water vapour permeability, the water solubility, and the biodegradation rates. The incorporation of natural extracts, such as rosmarinic acid, showed

their potential capability to control food pathogens with no significant modifications on tensile strength. The presence of additives in the polymer matrices allows their release during long periods of time from preparation, storage and distribution, resulting in the extended shelf life with spoilage decrease and maintenance of nutritional quality of foods. FAO (2013).

References

Bailey, J. E. (1991). Toward a science of metabolic engineering. *Science, 252,* 1668–1675.

Bastioli, C. (1998). Properties and application of mater-Bi starch-based materials. *Polymer Degradation and Stability, 59,* 263.

BeMiller, J. N., & Whistler, R. (2009). *Starch: Chemistry and technology* (S. L. Taylor Ed.). San Diego: Academic Press.

Biermann, C. J. (1996). *Handbook of pulping and papermaking*. Academic Press.

Ellis, R. P., Cochrane, M. P., Dale, M. F. B., Duffus, C. M., Lynn, A., Morrison, I. M., et al. (1998). Starch production and industrial use. *Journal of the Science of Food and Agriculture, 77*(3), 289.

FAO. (2013). FAOSTAT, *Food and Agriculture data. Rome*. Italy: FAO (Food and Agriculture Organization of the United Nations).

Heinze, T., & Petzold, K. (2008). Chapter 16—Cellulose chemistry: Novel products and synthesis paths. In M. N. B. Gandini (Ed.), *Monomers, polymers and composites from renewable resources* (pp. 343–368). Amsterdam: Elsevier.

Kirk, R. E., Othmer, D. F., Kroschwitz, J. I., & Howe-Grant, M. (1991). *Encyclopedia of chemical technology* (4th ed.). New York: Wiley.

Klemm, D., Schmauder, H.-P., & Heinze, T. (2001) Cellulose. In A. Steinbüchel & M. Hofrichter (Eds.), *Biopolymers*. Weinheim, Chichester: Wiley-VCH.

Le Corre, D., Bras, J., & Dufresne, A. (2010). Starch nanoparticles: A review. *Biomacromolecules, 11*(5), 1139–1153.

Moon, R. J., Martini, A., Nairn, J., Simonsen, J., & Youngblood, J. (2011). Cellulose nanomaterials review: Structure, properties and nanocomposites. *Chemical Society Reviews, 40*(7), 3941.

Purves, W. K., Orians, G. H., & Heller, H. C. (1995). *Life, the science of biology* (4th ed.). Mass: Sunderland.

Waterschoot, J., Gomand, S.V., Fierens, E., & Delcour, J. A. (2015). Production, structure, physicochemical and functional properties of maize, cassava, wheat, potato and rice starches. *Starch/Staerke, 67*(1–2), 14.