Chapter 2
Chemistry of Lactic Acid

Abstract The importance of lactic acid in the food industry is certainly correlated with its peculiar chemical and physical properties. According to the Joint FAO/WHO Food Standards Programme, lactic acid isomers and the racemic mixture can be used as acidity regulator in certain foods with the aim of contrasting certain acid-sensitive microorganisms. As a result, the description of food-related uses of lactic acid should involve also peculiar chemical and physical features. This chapter would give a brief and accurate overview of chemical and physical features of this additive. In addition, the chemical synthetic processes for the production of the so-called milk acid are described. Finally, fermentative pathways and related industrial strategies are discussed.

Keywords Distillation · Heterolactic fermentation · Homolactic fermentation · Hydrolysis · Lactic acid · Lactic acid bacteria · Racemic mixture

Abbreviations

IUPAC	International Union of Pure and Applied Chemistry
LAB	Lactic acid bacteria
H_2SO_4	Sulphuric acid

2.1 Lactic Acid and Chemical Features. An Introduction

The importance of lactic acid in the food industry is certainly correlated with its peculiar chemical and physical properties. According to the Joint FAO/WHO Food Standards Programme–Codex Committee on Food Additives (Codex Alimentarius Commission 2013), lactic acid (intended as the two *L* and *D* isomers with the additional racemic mixture) can be used as acidity regulator in certain foods (e.g. smoked fish and smoke-flavoured fish) with the aim of contrasting certain acid-sensitive microorganisms.

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As a result, the description of food-related uses of lactic acid should involve also peculiar chemical and physical features. This chapter would give a brief and accurate overview of chemical and physical features. In addition, the chemical synthesis of lactic acid is described; finally, these properties are given below.

2.1.1 Basic Properties

Lactic acid, also named ‘milk acid’, is an organic acid with the following chemical formula: $CH_3CH(OH)CO_2H$. The official name given by the International Union of Pure and Applied Chemistry (IUPAC) is 2-hydroxypropanoic acid (Table 2.1). This important acid can be naturally produced (Martinez et al. 2013), but its importance is correlated with synthetic productions. Pure lactic acid is a colourless and hydroscopic liquid; it can be defined a weak acid because of its partial dissociation in water (Eq. 2.1) and the correlated acid dissociation constant ($K_a = 1.38 \times 10^{-4}$).

$$H_3C-CH(OH)-COOH \rightleftharpoons H^+ + H_3C-CH(OH)-COO^- \qquad (2.1)$$

Table 2.1 shows the most important data related to lactic acid.

2.1.2 Isomers

Lactic acid is a chiral compound with a carbon chain composed of a central (chiral) atom and two terminal carbon atoms (Fig. 2.1). A hydroxyl group is attached to the

Table 2.1 Physical and chemical properties of lactic acid (Igoe 2011; Mohanty et al. 2015; Vaidya et al. 2005)

Identification parameters	Description
Compound name	Lactic acid
IUPAC name	2-Hydroxypropanoic acid
Chemical formula	$C_3H_6O_3$
Molecular mass	90.08 mol g^{-1}
Appearance	A colourless and syrupy liquid; alternatively, a white to yellow solid compound
Taste	Mild acid taste
Odour	Odourless
Boiling point	122 °C
Melting point	17 °C
Specific gravity/density	1.2
K_a	1.38×10^{-4}
pK_a	3.86

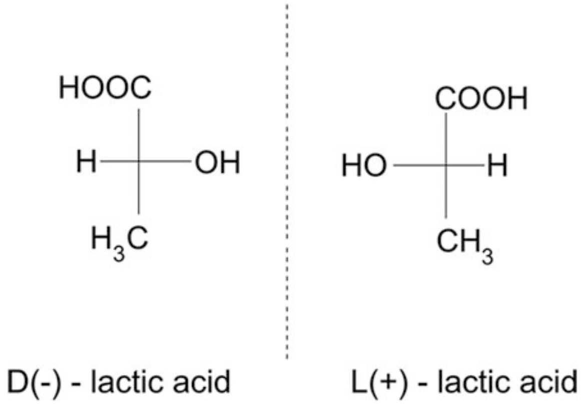

Fig. 2.1 Lactic acid is a chiral compound with a carbon chain composed of a central (chiral) atom and two terminal carbon atoms. A hydroxyl group is attached to the chiral carbon atom while one of the terminal carbon atoms is part of the carboxylic group and the other atom is part of the methyl group. Consequently, two optically active isomeric forms of lactic acid exist: L(+) form, also named (S)-lactic acid, and D(−) form, or (R)-lactic acid. L(+)-lactic acid is the biological isomer. BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html) has been used for drawing this structure

chiral carbon atom while one of the terminal carbon atoms is part of the carboxylic group and the other atom is part of the methyl group (Narayanan et al. 2004).

As a result, two optically active isomeric forms of lactic acid exist: L(+)-form, also named (S)-lactic acid, and D(−)-form, or (R)-lactic acid. Pure and anhydrous racemic mixture of lactic acid is a white crystalline solid with a low melting point. L(+)-lactic acid is the biological isomer as it is naturally present in the human body; consequently, the importance of this form of lactic acid depends of the known biochemical synthesis (Narayanan et al. 2004; Ou et al. 2011).

2.2 Synthesis of Lactic Acid

Basically, lactic acid can be produced by different chemical pathways and by microbiological synthesis. The first commercial production is ascribed to Charles E. Avery in 1881 (Carr et al. 2002; Kelkar and Mahanwar 2015).

2.2.1 Lactic Acid and Chemical Synthetic Strategies

2.2.1.1 Hydrolysis of Lactic Acid Derivatives

Lactic acid can be produced from the most part of its derivatives by means of suitable treatments (Ghaffar et al. 2014; Vaidya et al. 2005). Lactonitrile

(2-hydroxypropanenitrile, $CH_3CHOHCN$) is the most preferable of these compounds used in the chemical synthesis of lactic acid rather than other raw materials. Lactonitrile can be produced by the nucleophilic addition of hydrogen cyanide (HCN) to the liquid phase of acetaldehyde (CH_3CHO) in alkaline media under high pressure (Eq. 2.2).

$$HCN + H_3C-CHO \xrightarrow{\text{high/pressure}} H_3C-CHOH-CN \tag{2.2}$$

After recovery and distillation of the obtained impure lactonitrile (Narayanan et al. 2004), the purified compound can be treated (acid hydrolysis) by using concentrated hydrochloric acid (HCl) or concentrated sulphuric acid (H_2SO_4), with the resulting production of ammonium sulphate salt—$(NH_4)_2SO_4$—and crude lactic acid (Eq. 2.3).

$$\begin{aligned} &H_3C-CHOH-CN + H_2O + \frac{1}{2}H_2SO_4 \xrightarrow{\text{hydrolysis}} H_3C-CHOH-COOH \\ &\quad + \frac{1}{2}(NH_4)_2SO_4 \end{aligned} \tag{2.3}$$

The produced (crude) lactic acid needs to be concentrated and purified. Methanol (CH_3OH) can be used with the aim of producing methyl lactate ester, $CH_3CHOHCOOCH_3$ (Eq. 2.4).

$$H_3C-CHOH-COOH + H_3-C-OH \xrightarrow{\text{esterification}} H_3C-CHOH-COOCH_3 + H_2O \tag{2.4}$$

Methyl lactate ester is subsequently collected, purified by distillation, and hydrolysed in acidic aqueous solution to lactic acid, while methanol can be recycled in the same process (Eq. 2.5). The resulting product is a racemic mixture of lactic acid (Narayanan et al. 2004).

$$H_3C-CHOH-COOCH_3 + H_2O \xrightarrow{\text{hydrolysis}} H_3C-CHOH-COOH + H_3C-OH \tag{2.5}$$

2.2.1.2 Nitric Acid Oxidation of Propane

Another pathway for the chemical synthesis of lactic acid concerns the use of propene ($CH_3CH_2CH_3$). This alkene is oxidised to α-nitropropionic acid (Vaidya et al. 2005) by using nitric acid (HNO_3) in presence of oxygen (Eq. 2.6). Subsequently, the obtained acid has to be converted into lactic acid by hydrolysis (Eq. 2.7).

$$H_3C{-}CH_2{-}CH_3 + HNO_3 \xrightarrow{\text{oxydation}/O_2} H_3C{-}CH{-}NO_2{-}COOH \quad (2.6)$$

$$H_3C{-}CH{-}NO_2{-}COOH + H_2O \xrightarrow{\text{hydrolysis}} H_3C{-}CHOH{-}COOH + HNO_3 \quad (2.7)$$

2.2.2 *Lactic Acid Fermentation*

The mass production of lactic acid by using fermentation became widely used after the discovery of *Lactobacillus sp*. by the French chemist Loius Pasteur in 1856. *Lactobacillus* bacteria are able to produce lactic acid from carbohydrates such as glucose and lactose, and they are even living in our gastrointestinal system (Carr et al. 2002). Fermentation is a biochemical process in which carbohydrate molecules, e.g. glucose, are converted into energy, lactate, and other by-products depending on the type of microorganism involved in the fermentation process (John et al. 2007). For these reasons, there are two basic fermentation processes (Sect. 5.1): the homolactic fermentation (prevailing product: lactic acid), and a heterolactic fermentation (the final product is a mixture containing mainly lactic acid, other organic acids, and ethyl alcohol). Both mechanisms are shown in Figs. 2.2 and 2.3 (Fugelsang and Edwards 2007). Other fermentation types can occur depending on the fermentation raw materials and conditions (Ikushima et al. 2009; Wasewar et al. 2002; Wee et al. 2005).

2.2.2.1 Solid-state Fermentation

In relation to solid-state fermentation, microbial growth and fermentation take place at the surface of solid substrates such as wheat, soya bean, and cheeses. Such substrates are more convenient for a large number of filamentous fungi and a few bacteria (Chisti 1999; Jelen 2003; Kim et al. 2006).

2.2.2.2 Submerged Fermentation

In this fermentation, the substrate for microbial growth is the liquid solution placed in large tanks called ‘fermenters’ or ‘bioreactors’ (Chisti 1999; Wee et al. 2006). Submerged fermentation can be subdivided in three categories:

- Batch fermentation. Substrates and required raw materials for fermentation and the desired microbial growth are placed into a bioreactor; incubation is allowed to proceed on condition that operating parameters such as pH and thermal values are defined. During fermentation, nothing is added except oxygen in case of aerobic microorganisms. After each process, the product is collected and the

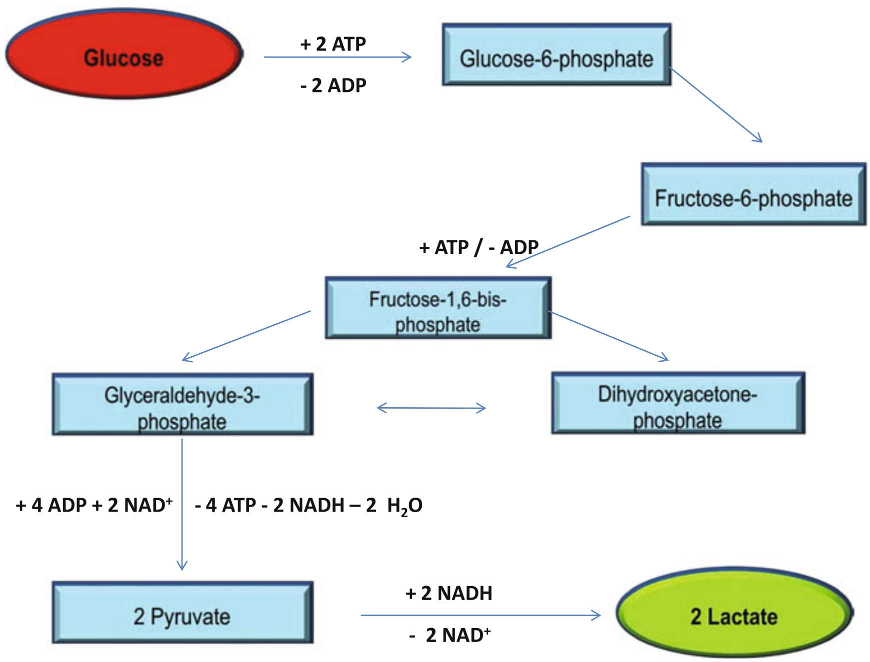

Fig. 2.2 LAB and lactic acid synthesis: the homofermentative mechanism (Embden–Meyerhof–Parnas pathway), from one single glucose molecule to two lactate units. *ATP* means: adenosine triphosphate; *ADP* is for: adenosine diphosphate; *NAD*$^+$ and *NADH* correspond to nicotinamide adenine dinucleotide reduced and non-reduced forms

fermenter is cleaned; then, another batch can be prepared and the process may restart
- Fed-batch fermentation. Substrates and raw materials are added in small amounts during the fermentation process. Both batch and fed-batch procedures are considered as 'closed' fermentation systems, differently from 'open' systems such as continuous fermentation (Portno 1968)
- Continuous fermentation. The addition of substrates and raw materials is performed continuously during the process. Consequently, continuous fermentation is considered as an open system: The introduction of new raw materials is allowed, differently from 'closed' systems (Portno 1968).

The highest concentration of lactic acid is normally obtained in batch and fed-batch cultures (discontinuous processes), whereas the highest productivity is observed in continuous fermentation processes because of longer temporal periods (working cycles).

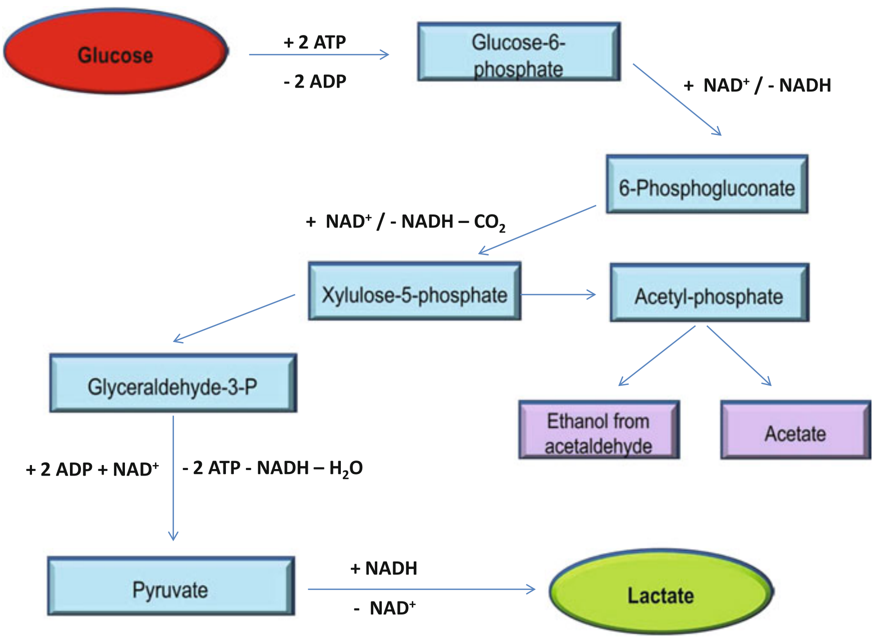

Fig. 2.3 LAB and lactic acid synthesis: the heterofermentative mechanism, from one single glucose molecule to one lactate unit. *ATP* means: adenosine triphosphate; *ADP* is for: adenosine diphosphate; *NAD*$^+$ and *NADH* correspond to nicotinamide adenine dinucleotide reduced and non-reduced forms. This mechanism gives also ethanol (from acetaldehyde) and acetate

2.2.2.3 Anaerobic Fermentation

This fermentation process involves anaerobic microorganisms; the air into fermenters is replaced by carbon dioxide, hydrogen, nitrogen, or a suitable mixture of these gases.

2.2.2.4 Aerobic Fermentation

Fermentation process can be also carried out in presence of aerobic microorganisms under aerobic conditions. Raw materials and conditions used for lactic acid production, e.g. purity, pH, and temperature, are critical parameters for the further purification of obtained and impure lactic acid (Krishna et al. 2012). Monosaccharides (e.g. glucose) and disaccharides (e.g. sucrose, maltose, and lactose) are common substrates for this process (Lunelli et al. 2010, 2011). Monosaccharide and disaccharide are end products of starch hydrolysis by application of enzymes such as glucoamylases and α-amylases or by chemical hydrolysis, since most microorganisms cannot utilise polysaccharides such as starch without hydrolysis. The choice of substrate and other conditions are dependent on microorganisms used in fermentation (Fukushima et al. 2004).

Life forms used in industrial fermentation can be bacteria such as *Escherichia coli* and *Lactobacillus* spp., or fungal organisms such as *Rhizopus* spp. In relation to food industries, bacteria involved in fermentation are named as 'lactic acid bacteria' (LAB) such as genera *Lactobacillus, Streptococcus, Leuconostoc*, and *Pediococcus*. In this ambit, conditions such as temperature, pH, aeration, and agitation are important parameters and they can vary depending on the type of LAB; so, these conditions have to be carefully set (Carr et al. 2002; Chooklin et al. 2011; Ge et al. 2010; Coelho et al. 2011; Narayanan et al. 2004; Romani et al. 2008; Secchi et al. 2012). LAB reach the maximum productivity only within specific temperature and pH ranges, while the fermentation process is associated with the production of lactic acid as well as other organic acids which lower the pH of fermentation media (or the broth). It is necessary to maintain optimum pH values at a constant value during fermentation by addition of alkali such as hydroxides or calcium carbonate, or ammonia. Calcium hydroxide, $Ca(OH)_2$, can react with carbohydrates such as glucose (Vaidya et al. 2005) with the production of calcium lactate, $(H_3C–CHOH–COO^-)_2\ Ca^{2+}$, and water (Eq. 2.8).

$$C_6H_{12}O_6 + Ca(OH)_2 \xrightarrow{\text{fermentation/enzymes}} (H_3C-CHOH-COO^-)Ca^{2+} + 2H_2O \tag{2.8}$$

Calcium lactate has to be filtered and separated from the obtained aqueous solution, treated with H_2SO_4 to be hydrolysed, and turned into lactic acid and calcium sulphate (Eq. 2.9).

$$2(H_3C-CHOH-COO^-)Ca^{2+} + H_2SO_4 \xrightarrow{\textit{hydrolysis}} 2H_3C-CHOH-COOH + CaSO_4 \tag{2.9}$$

Obtained lactic acid is separated by filtration of calcium sulphate; subsequently, purification and esterification with methanol are needed to obtain methyl lactate which undergoes hydrolysis to pure lactic acid (Narayanan et al. 2004; Vaidya et al. 2005) as shown in Eqs. 2.4 and 2.5. The output of fermentation is an aqueous lactic acid solution which is subsequently concentrated by evaporation (Komesu et al. 2013; Martins et al. 2012, 2013).

LAB utilise either the well-known Embden–Meyerhoff–Parnas pathway of glucose metabolism to obtain lactic acid as the major end product, or use pathways of pentose metabolism resulting in the formation of lactic acid plus other products such as acetic acid, ethanol, and carbon dioxide.

A limited number of non-LAB microorganisms are capable to produce larger amounts of lactic acid from common carbon sources if compared with LAB. The best known of these life forms is *Rhizopus oryzae* which can be used for commercial lactic acid production since it can convert several sugars. The average lactic acid yield seems to be approximately around 93.8 g per l, while other LAB (*E. faecalis*) are reported to produce higher amounts and different Lactobacilli can produce lactic acid in the range 21.8–90.0 with average amounts of 60.3 g per l. Anyway, these microorganisms may be really different when speaking of productivity values in terms of grams per litre in one single hour (Abdel-Rahman et al. 2011; Wee et al. 2006).

Unlike the chemical synthesis, fermentation processes for the production of lactic acid can give selectively one of the two lactic acid stereoisomers or their racemic mixture depending on the bacteria species selection (Martinez et al. 2013). Industrial production of lactic acid, especially where pure optical isomers are required, is presently carried out predominantly by fermentation processes. In summary, the production process can be subdivided into two steps:

1. The real production of lactic acid by fermentation of a carbohydrate source.
2. The downstream processing of the fermentation broth to obtain pure lactic acid.

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