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Ettore Baglio

Chemistry and Technology of Honey Production

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Ettore Baglio

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Ettore Baglio
Industrial Consultant
Catania
Italy

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Chapter 1
The Industry of Honey. An Introduction

Abstract The word 'honey' means the natural sweet substance produced by bees (*Apis mellifera*) from the nectar of plants. The nectar composition, mainly consisting of sucrose, glucose, and water, may be assumed constant for each species of plant, and it directly affects the composition of the honey and the strategy of related industries. In general, the composition of honey involves carbohydrates, water, nitrogenous substances, and minerals with the additional presence of vitamins, organic acids, flavour aromatic substances, and polyphenols. From the legal viewpoint, a good guideline for the chemical composition of commercially available honeys can be the Council Directive 2001/110/EC of 20 December 2001 relating to honey. In addition, honeys are evaluated by the normal consumer by means of physical-chemical indicators related to its chemical composition: colour, density, rheology, and refraction index. Finally, honey has high energy density profiles: this product is a suitable component supply especially for the sportsman, the elderly, and school-age children. However, the extreme variability of commercial honeys should be taken into account.

Keywords European Union · Fructose · Glucose · Honey · Refraction index Sucrose · Viscosity

Abbreviations

5-hydroxymethylfurfural	HMF
International Union of Pure and Applied Chemistry	IUPAC

1.1 Honey. Definition and Simplified Chemical Composition

Basically, the word 'honey' means the natural sweet substance produced by bees (*Apis mellifera*) from the nectar of plants. Nectar is a sugary secretion produced by special glands called 'nectaries' at the base of the flowers (Contessi 2005). The

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composition of the nectar, mainly consisting of sucrose, glucose, and water (up to 90%), may be assumed constant for each species of plant, and it directly affects the composition of the honey (Persano Oddo et al. 1997).

In general, the composition of honey involves carbohydrates (average amount 82.3%), water (17.2%), nitrogenous substances (0.3%), and minerals (0.03–1%), with the additional presence of vitamins, organic acids, flavour aromatic substances, and polyphenols. A good guideline for the chemical composition of commercially available honeys can be the Council Directive 2001/110/EC of 20 December 2001 relating to honey (Council of the European Union 2002).

1.2 Honey. Chemical Composition and Criteria According to the Council Directive 2001/110/EC

From the legal viewpoint, several chemical data concerning commercial honeys can be found in the European Council Directive 2001/110/EC of 20 December 2001 relating to honey (Council of the European Union 2002).

Six different chemical analytes are defined in this Directive (Annex II) when speaking of main chemical criteria for commercial honeys on the European market. These categories, and the main representative chemicals for each of these groups, are discussed in the following sections and in Chap. 3. It has to be noted that these analytes are described in detail in the Codex document CODEX STAN 12-1981 (Codex Alimentarius Committee on Sugars 2001).

The first group of analytes concerns sugars only (Fig. 1.1). According to the Directive 2001/110/EC, Annex II, honey 'as it is' or any similar product used as

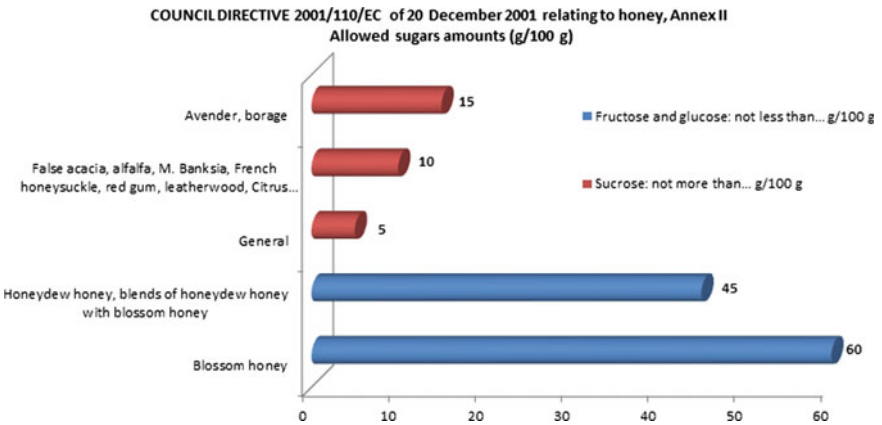

Fig. 1.1 Allowed limits for sugars in commercial honeys, according to the European Directive 2001/110/EC, Annex II (Council of the European Union 2002). These sugars are: glucose and fructose—the main sugar components of honey—and sucrose. The suggested limits depend on the peculiar honey or honey typology

ingredient in other foods intended for human consumption should not contain less than 60 g/100 g of the sum of glucose and fructose when speaking of blossom honey. With reference to 'honeydew honey, blends of honeydew honey with blossom honey', the same group has a minimum allowed limit of 45 g/100 g (Council of the European Union 2002). Glucose and fructose (Fig. 1.2) are the main sugar components of honey; consequently, the estimation of the sum of the above-mentioned sugars is extremely important from the commercial viewpoint.

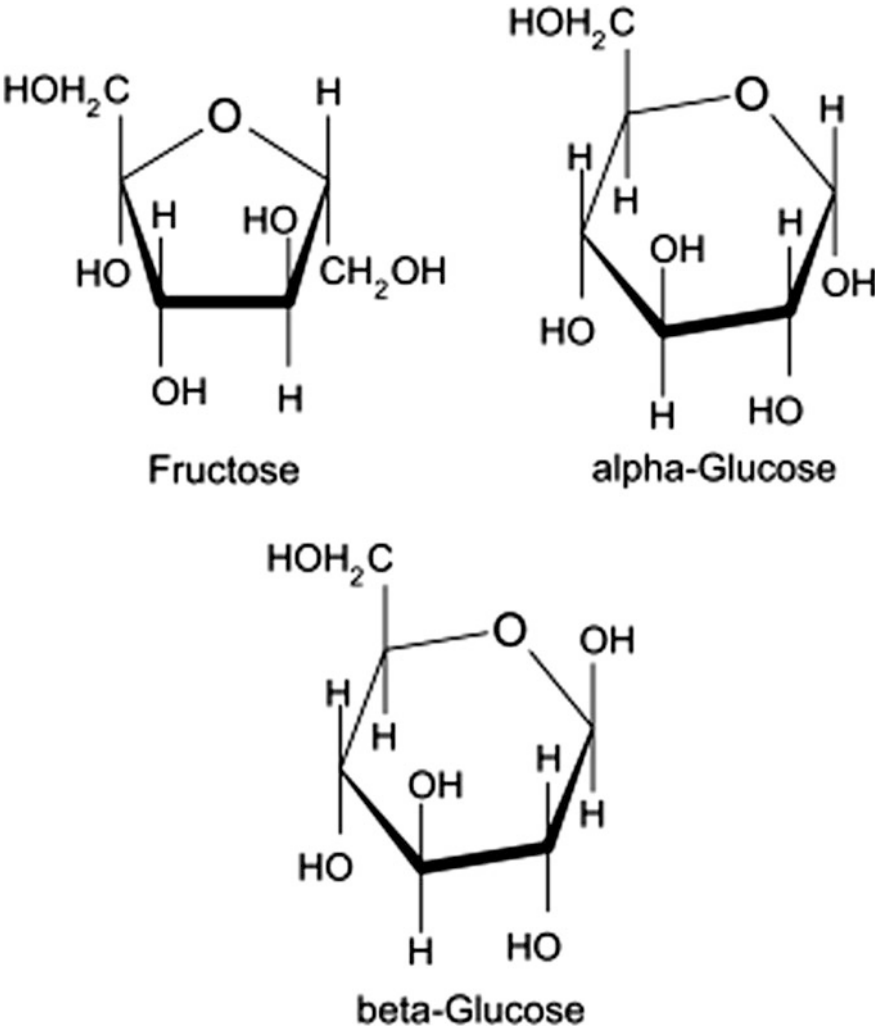

Fig. 1.2 Chemical structure of fructose and glucose (α- and β-types), the main sugars normally found in honeys. BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html), has been used for drawing this structure

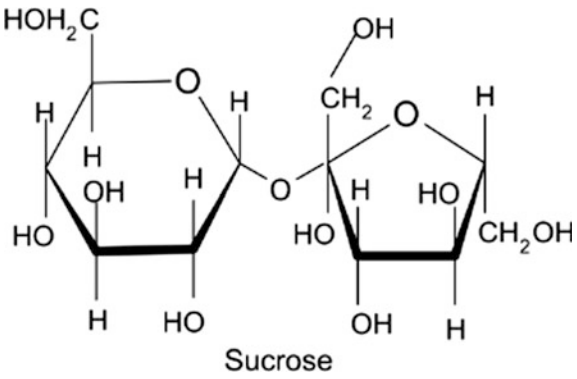

Fig. 1.3 Chemical structure of sucrose. This disaccharide, also named saccharose, with molecular formula $C_{12}H_{22}O_{11}$, is the combination of fructose and glucose. Because of its clear origin from the main monosaccharides in honeys, the related amount may be considered in some relationship with the quantity of these sugars. BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html), has been used for drawing this structure

However, the category of sugars contains another important analyte: sucrose. This disaccharide, also named saccharose,[1] with molecular formula $C_{12}H_{22}O_{11}$, is the combination of fructose and glucose (Fig. 1.3). Because of its clear origin from the main monosaccharides in honeys, the related amount may be considered in some relationship with the quantity of these sugars. In relation to commercial honeys as intended in the Directive 2001/110/EC, Annex II, sucrose cannot exceed 5 g/100 g when speaking of general products, with several exceptions (Fig. 1.1). In fact, this maximum limit may be surpassed in two situations. Sucrose cannot exceed 10 g/100 g when speaking of honeys obtained from the following floral species:

- *Robinia pseudoacacia*
- *Medicago sativa*
- *Banksia menziesii*
- *Hedysarum*
- *Eucalyptus camadulensis*
- *Eucryphia lucida* and *Eucryphia milliganii*
- *Citrus* spp.

In addition, saccharose cannot exceed 15 g/100 g when speaking of honeys obtained from the following floral species (Council of the European Union 2002):

- *Lavandula* spp.
- *Borago officinalis*.

[1]In accordance with the International Union of Pure and Applied Chemistry (IUPAC), the chemical IUPAC name of this compound is (2R,3R,4S,5S,6R)-2-[(2S,3S,4S,5R)-3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol.

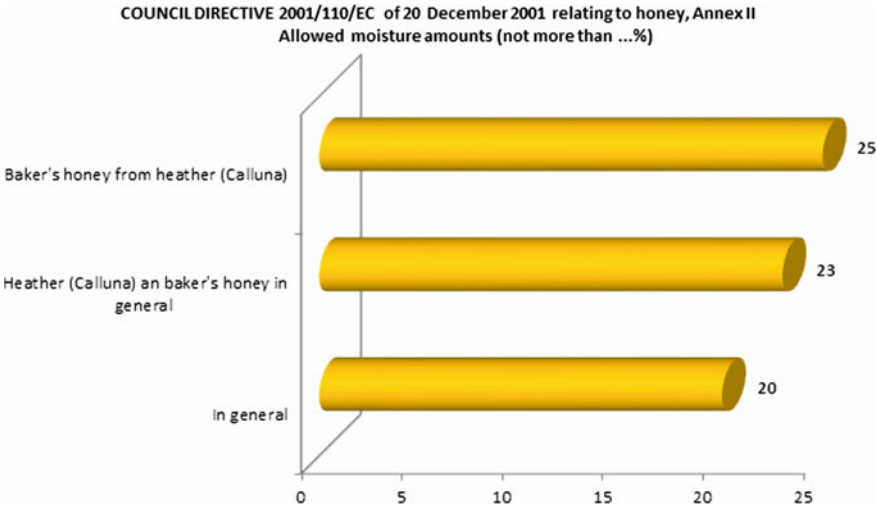

Fig. 1.4 Allowed limits for moisture content in commercial honeys, according to the European Directive 2001/110/EC, Annex II (Council of the European Union 2002)

The second monocategory concerns moisture only (Fig. 1.4). According to the Directive 2001/110/EC, Annex II, honeys 'as it is' or any similar product used as ingredient in other foods intended for human consumption should not contain more than 20% on the total honey mass, with two important exceptions. This amount can exceed the 20% limit up to 23% when speaking of honeys obtained from *Calluna* (*Ericaceae* family) and the so-called baker's honey [a peculiar product suitable for industrial uses and/or as ingredient for other products (Adriana and Purcărea 2011)]. Moreover, should baker's honey be obtained from *Calluna*, sucrose may reach 25% (Council of the European Union 2002).

The third monocategory concerns 'water-insoluble content' only (Fig. 1.5). Actually, the group concerns many possible substances; however, the simplified group has been proposed for commercial classification purposes. According to the Directive 2001/110/EC, Annex II, honeys 'as it is' or any similar product used as ingredient in other foods intended for human consumption should not contain more than 0.1 g/100 g of honey, although pressed honey may contain no more than 0.5 g/100 g (Council of the European Union 2002).

The fourth criterion does not concern chemical substances: it is related exclusively to electrical conductivity as a discriminant indicator for classification purposes. According to the Directive 2001/110/EC, Annex II, honeys 'as it is' or any similar product used as ingredient in other foods intended for human consumption should not show more than 0.8 mS/cm as electrical conductivity (Council of the European Union 2002), although honeys obtained from the following species have to show not less than 0.8 mS/cm (blends of these honeys are not comprised in the exceptions) or are exempted (no limits):

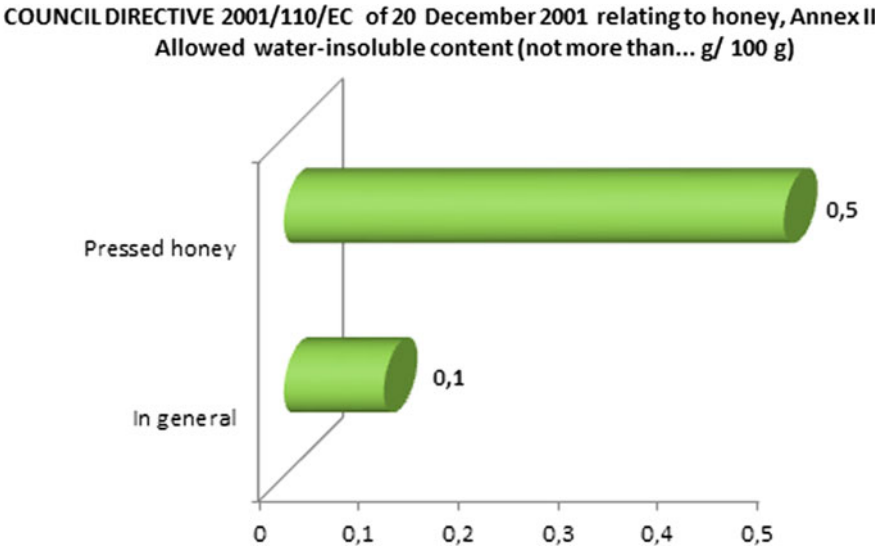

Fig. 1.5 Allowed limits for water-insoluble content in commercial honeys, according to the European Directive 2001/110/EC, Annex II (Council of the European Union 2002)

- Honeydew and chestnut honey and blends of these except with the below-mentioned honeys: the minimum allowed limit is 0.8 mS/cm
- *Arbutus unedo*
- Bell heather (*Erica*)
- Eucalyptus
- Lime (*Tilia* spp.)
- *Calluna vulgaris*
- Manuka or jelly bush (*leptospermum*)
- Tea tree (*Melaleuca* spp.)

The fifth category concerns only free acidity contents (Fig. 1.6). According to the Directive 2001/110/EC, Annex II, honeys 'as it is' or any similar product used as ingredient in other foods intended for human consumption should not contain more than 50 milli-equivalents acid per 1000 g, although an exception exists: in relation to 'baker's honey' only, the maximum limit can reach no more than 80 milli-equivalents acid per 1000 g (Council of the European Union 2002).

Finally, the last group concerns two important indicators for the assessment of honey quality (Chap. 3): diastase activity and 5-hydroxymethylfurfural (HMF) content. These values should be considered together, as required in the Annex II of Directive 2001/110/EC, when speaking of HMF, first indent (general limits).

In relation to the first index, actually a measure of enzymatic performances related to the diastase molecule, and according to the Directive 2001/110/EC, Annex II, 'honeys with low natural enzyme content (e.g. citrus honeys) and an HMF content of not more than 15 mg/kg' should not have a diastase activity level

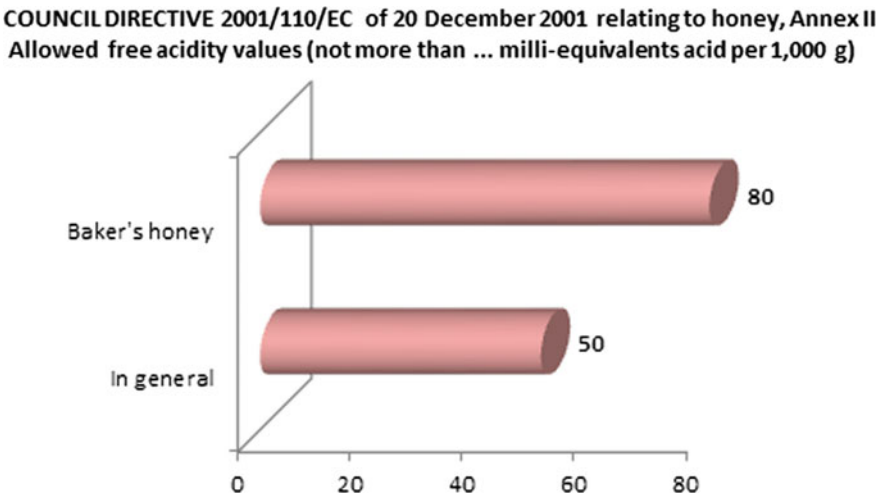

Fig. 1.6 Allowed limits for free acidity in commercial honeys, according to the European Directive 2001/110/EC, Annex II (Council of the European Union 2002)

below 3 (Schade scale), while other honeys have to show the same indicator ≥ 8 (with the exception of baker's honey).

With concern to the second indicator, related to Maillard reaction (Chap. 3), and according to the Directive 2001/110/EC, Annex II, 'honeys of declared origin from regions with tropical climate and blends of these honeys' should not contain more than 80 mg/kg of HMF, although the general maximum limit is 40 mg/kg (with the exception of baker's honey).

After this regulatory premise, the honey composition can be discussed in detail.

1.2.1 Honey Composition. Carbohydrates

The average amount of carbohydrates in honeys does not exceed normally 82.3%. About 90% of the total sugar content in honey is represented by two simple sugars, fructose and glucose (Contessi 2005) with 40 and 30% quantities, respectively. Amounts and proportions of individual sugars are highly variable depending on the botanical origin of honey (Persano Oddo et al. 1997). Some honeys such as citrus, heather, eucalyptus sunflower, and dandelion honeys have a fructose/glucose ratio between 0.99 and 1.20. Other honeys, such as rhododendron, thyme, and honeydew honeys, have a ratio ranging from 1.30 to 1.40, while acacia and chestnut honeys show the clear abundance of fructose if compared to glucose with a related ratio between 1.59 and 1.67 (Persano Oddo et al. 1997). Also, other sugars such as

disaccharides (sucrose $\leq 5\%$, maltose, and isomaltose) are found in honey, although in modest quantities. In relation to trisaccharides and higher sugars, the following molecules should be mentioned: melesitose, erlose, raffinose, coibiose, dextrantriose, and melibiose (Gonnet and Vache 1984).

1.2.2 Honey Composition. Water

Honey is normally operculated by bees: in this condition, it contains 17–18% of water, but sometimes the aqueous component can be found up to 21% with easy fermentation processes (Contessi 2005).

1.2.3 Honey Composition. Nitrogenous Substances

The main part of nitrogen-based compounds found in honeys is represented by free amino acids such as valine, isoleucine, cysteine, phenylalanine, tryptophan, asparagine, proline, and lysine. In particular, lysine appears to be the most abundant amino acid in honeys (Cotte et al. 2004; Petrov 1971). Biological enzymes, produced by glandular secretions of bees, are particularly important in the group of proteins.

The main enzymes in honey are sucrase (also named invertase)—responsible for the breakdown of sucrose—and diastase (or amylase) that breaks down starch into glucose. The presence of these enzymes assumes a particular importance for the evaluation of heat treatment for honey and the correlated preservation. Another enzyme, glucose oxidase, is responsible for the recognised antibacterial activity of honeys. Catalase and phosphatase should also be considered.

1.2.4 Honey Composition. Mineral Substances

In relation to the European Union, nectar honey cannot contain more than 0.6% of mineral substances, while honeydew—alone or in mixture with nectar honey—can contain up to 1%, in accordance with the Council Directive 2001/110/EC of 20 December 2001 (Council of the European Union 2002). In general, the following mineral elements are found in commercial honeys: potassium, sodium, calcium, iron, manganese, phosphorus, copper, and sulphur (chlorine is also found as halogen partner).

1.2.5 Minor Honey Components. Vitamins

Honey contains a few vitamins with negligible amounts (generally measurable as micrograms per 100 g of sampled honey). These vitamins comprehend the B-group (B_1, B_2, and B_6) and the following compounds: vitamin C, PP, K, pantothenic acid, and vitamin P.

1.2.6 Minor Honey Components. Organic Acids

In general, commercially available honeys have pH values between 3.4 and 6.2 depending on the original nectars. Honeys from citrus, acacia, heather, eucalyptus, sunflower, rhododendron, and thyme have pH values between 3.6 and 4.0. Arbutus, dandelion, and thyme honeys are also reported to have pH values between 4.2 and 4.5, while pH for chestnut and honeydew honey should be between 5.2 and 5.5 (Cardoso and Silva 2016; Persano Oddo et al. 1997). The acidity of honey is correlated to the presence of numerous organic and inorganic acids and lactones. Hydrochloric and phosphoric acids are included as inorganic acids; gluconic, acetic, butyric, citric, formic, lactic, malic, oxalic, succinic, and pyroglutamic organic acids are also found.

1.2.7 Minor Honey Components. Aroma Compounds

Aromatic substances are responsible for the characteristic aroma of honeys, depending on the botanical and geographical origin of plant species; however, processing and storage steps are reported to affect the final quality and obtained yields of the final product (Gheldof and Engeseth 2002; Wang et al. 2004; Turkmen et al. 2006). Volatile compounds, including hexanol, acetone, 2-butanone, 2-propanol, and 2 pentanone, have a critical role in the evaluation of the floral honey. The distribution of aromatic compounds and flavonoids was used to determine the botanical origin of honey according to different authors (Anklam 1998; Anupama et al. 2003; Mateo and Bosch-Reig 1998; Serrano et al. 2004).

1.2.8 Minor Honey Components. Polyphenols

Several recent studies have been carried out to assess the presence and profile of polyphenolic flower honeys. Among the major flavonoids the following molecules were found: quercetin, hesperetin, chrysin, pinocembrin, luteolin, apigenin, myricetin, and campeferol. At the same time, the following phenolic acids were

identified: caffeic, coumaric, ferulic, ellagic, and chlorogenic acids (Gheldof et al. 2002).

Several researchers have also reported a strong correlation between honey colour and the polyphenol content (Beretta et al. 2005; Frankel et al. 1998). It was also shown that the antioxidant activity in honey is strongly correlated to the total polyphenol content and therefore to the observed colour (Blasa et al. 2006; Gheldof and Engeseth 2002; Meda et al. 2005).

1.3 Physical Properties of Honeys

In general, honey has numerous physical properties, to a large extent related to its chemical composition. These features combine to determine the important organoleptic properties which can be examined and critically analysed by the normal consumer (Contessi 2005).

1.3.1 Colour

The colour, the more pronounced feature of honeys among physical properties, depends on the presence of plant pigments including carotene, xanthophylls, anthocyanins, flavonoids, polyphenols, as well as amino acids and mineral salts. The natural colour of honey presents many shades, from pale yellow to amber, from dark amber to almost black through the reddish tint (Terrab et al. 2004).

Some studies have shown a correlation between honey colour and other parameters such as floral origin, processing methods, temperature, and storage time (Crane 1984; Feller-Demalsy et al. 1989; Free and Williams 1983; Gonzales et al. 1999). The darkening causes of honey during processing and storage can be briefly identified with Maillard reactions, caramelisation of the sugars, and oxidation of polyphenols.

The degree of darkening depends on the temperature and/or on the retention time (Gonzales et al. 1999). Different subjective methods are used for the colorimetric evaluation: the most used of these systems is the visual Lovibond comparator which measures the colour in Pfund units (Gonzalez and de Lorenzo 2002; Swanson and Lewis 1991–1992), although these methods' comparison does not establish small colour differences. Furthermore, the visual evaluation of food depends not only on the colour, but also on other apparent features (Hutchings 1999). For these reasons, methods using reflectance measurements or transmittance through spectrophotometers and colorimeters are widely used at present in the scientific community (Aubert and Gonnet 1983; Bertoncelj et al. 2007; Castro et al. 1992; Negueruela et al. 2000; Terrab et al. 2004). The colour of honey is normally expressed through the parameters of the CIELAB colour space proposed by the Commission International de l'Eclairage.

1.3.2 Density

Basically, the density of a substance is expressed as the ratio between weight and volume for a constant temperature. In relation to honey, density is between 1.39 and 1.44 at 20 °C, that is to say that a litre of honey weighs from 1390 to 1440 g. Since the density of water, equal to 1 kg/l, is much lower, the greater the water percentage, the smaller the honey density. As a result, the water amount in honey may be approximately determined by means of a simple density measure.

1.3.3 Viscosity

The viscosity is the internal resistance of fluids against an external force. The honey, due to a high sugar concentration, has high viscosity values. Consequently, the assessment of rheological properties should be taken into account during processing steps, from the extraction to the final packaging. In fact, during honey processing, heating is aimed at the reduction of measurable viscosity, with the dissolution of crystals, the concentration of the product, and its microbiological and physical stabilisation (Singh and Bath 1998). Special rooms are used for honey heating: circulation of hot air, or a water bath thermostated at a temperature between 35 and 50 °C for 4/7 days is required. Anyway, different honeys may mean also different durabilities: the simple variation of rheological properties may depend on mechanical processing procedures with the consequent correlated stress, according to the second Parisi's Law of Food Degradation (Barbera and Gurnari 2017; Parisi 2002; Steinka et al. 2017).

1.3.4 Refraction Index

Refraction is the phenomenon by which a ray of light undergoes a deviation and a speed change when passing from one medium to another medium. The light propagates with different speeds depending on media; these differences define the refractive index of the peculiar medium. From a numerical viewpoint, refractive index is simply the ratio between the speed of propagation of light in air and the speed of propagation of light in the considered medium. With reference to honey as a medium, the refractive index varies in a practically linear way at the same temperature depending on the moisture degree. As a consequence, the water amount of honeys may also be evaluated with a simple refraction index measure (Contessi 2005).

1.4 Biological and Nutritional Features of Honeys

Honey has high energy density profiles; because of the main components (simple sugars: glucose and fructose), it is easy to digest. Thus, honey provides an immediate energy boost to consumers; for this reason, such a food product becomes a suitable component supply especially for the sportsman, the elderly, and school-age children.

Honey provides 300 kcal per 100 g against about 400 kcal for sucrose or table sugar. In addition, it shows higher sweetening power if compared with table sugars and sucrose. In fact, should the sweetening power of sucrose be equal to 100, the fructose and glucose powers would be calculated as 173 and 74, respectively.

Some studies show that the combination of 'nutrition-health' honey can be really interesting. White and co-workers have shown antibacterial activity both in honey as such or in diluted solutions. This activity, attributed for a long time to a substance of unknown nature defined by the generic name of 'inhibin', would be due to the action of the enzyme glucose oxydase, which produces hydrogen peroxide and gluconic acid from glucose in particular dilution conditions (White et al. 1963). According to this research, the accumulation of hydrogen peroxide would give honey interesting antibiotic properties. This mechanism would be the basis of antibacterial activity explicated by honey on wounds.

Some authors (Kajiwara et al. 2002; Ustunol and Gandhi 2001) have shown that the consumption of honey favours the development of bifidobacteria. Consequently, prebiotic properties are shown and this food can contribute to a good digestion. Therapeutic virtues attributed to honey are still numerous; honey would act favourably on various disorders of the circulatory, respiratory, and digestive systems, with good results in relation to liver and teething babies. The list may be longer, but many statements would need a proper and timely clinical trial for support. Moreover, the extreme variability of the food product does not allow to generalise results related to several experiments conducted on a honey without well-assessed compositional features. The honey is not a simple aqueous solution of sugars because of the variety of 'secondary' constituents, which together contribute to the extreme variability commonly observed for honeys.

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Chapter 2
Honey: Processing Techniques and Treatments

Abstract The industry of honeys is not a simple sequential chain of processing operations, although the normal consumer may consider it in this way. Each processing step is the answer to peculiar problems concerning the physicochemical and biological features of different honeys. This chapter discusses the honey production by the technological and chemical viewpoints. The production flow involves six different steps—initial extraction, dehumidification, liquefaction and mixture, heating, pasteurisation, crystallisation, and final packaging—although different industries can alter related conditions depending on various factors, including economic reasons. All steps have to be carefully considered and performed; otherwise, the quality of produced honeys, including also honey for medical purposes, could be negatively affected. Physicochemical and microbiological features of the final product can be assured on condition that each process is well carried out; in addition, certain precautionary measures should be taken before the final commercialisation.

Keywords Crystallisation · Dehumidification · Extraction · Heating · Honey production · Liquefaction · Pasteurisation

2.1 The Industry of Honey. Description of Processing Steps

The industry of honeys is not a simple sequential chain of processing operations, although the normal consumer may consider it in this way, at first sight. It should be noted that each processing step, from the initial extraction to the packaging of the final food product, is the answer to peculiar problems concerning the physicochemical and biological features of different honeys.

This chapter is dedicated to the description of honey production. In general, the following integrated steps correspond to the flow-chart description of such a process (Fig. 2.1):

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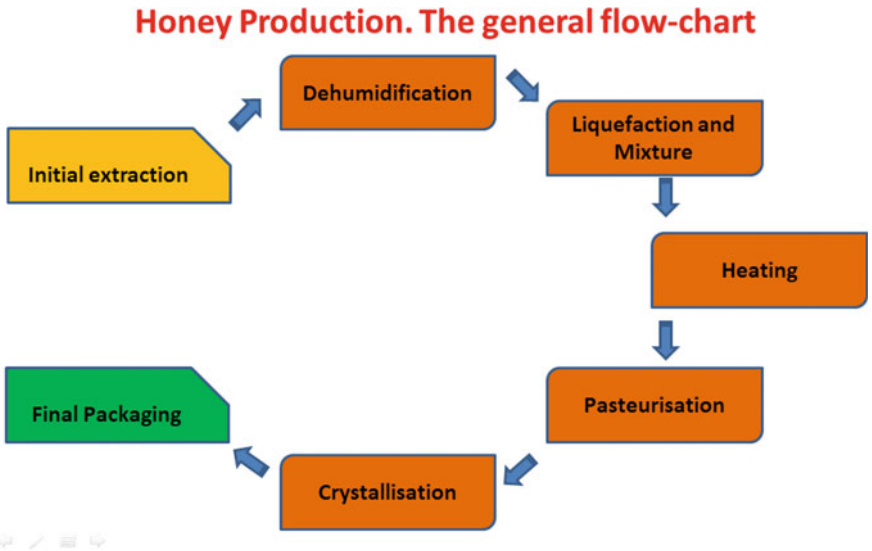

Fig. 2.1 The production flow of commercial honeys involves six different steps, from the initial extraction to the final packaging. It should be considered that different industries can perform their processes in a different way depending on various factors, including economic reasons

(a) Initial extraction
(b) Dehumidification
(c) Liquefaction and mixture
(d) Heating
(e) Pasteurisation
(f) Crystallisation
(g) Final packaging.

The following sections correspond to the description of each step, with related procedures and correlated reasons.

2.2 Initial Extraction

After the initial harvest, the material (e.g. honeycombs, frames) is introduced into the so-called honey extractor: a container able to remove honey by means of the centrifugal force. The operation has to be carried out into special rooms, with possibility of heating. At the exit from the extractor, the honey is (a) collected by gravity in tanks placed often on the floor (wax is separated from honey) and (b) sent to the decanters with the aid of pumps from the same floor. The extraction must be performed by a desired degree of purification with the aim of eliminating wax

particles and air bubbles, which are possibly mixed with honey during extraction. The purification is carried out with two different techniques: decanting and filtration.

By the safety viewpoint, it should also be considered that extraction procedure (with the collection and other processing steps) may affect negatively the quality of produced honeys, with special reference to honey for medical purposes. Consequently, physicochemical and microbiological features of the final product can be assured on condition that a certain number of precautionary measures are taken before the final commercialisation.

2.3 Dehumidification

Certain honeys obtained with peculiar plant species (Rape, Calluna, Chestnut, etc.) may sometimes contain high water percentages, affecting its conservation; consequently, a dehumidification step is needed, and relative humidity values should be lower than 18.5–18.0% (Kuehl 1988; Oliveira 2007).

Should the number of honeycombs to be treated be very small, the procedure would easily be performed as follows: honeycombs may be piled up in a very dry and warm room, sucking air from the base with ordinary vacuum cleaner and funnelling it outside. On the other hand, the same operation is not possible when speaking of many batches. Should this be the situation, it would instead be necessary to introduce a current of hot air produced by a generator into the so-called hot rooms where honeycombs are left; also, rooms would be thermostatically maintained at a constant temperature. The temperature must be between 32 and 35 °C; anyway, it cannot exceed 38 °C; otherwise, honey may lose its basic features. The treatment should be prolonged for a period of 12–36 h depending on the contained humidity.

By the macroscopic viewpoint, the main difference is naturally the increase in dry content with augment of reducing sugars and apparent sucrose. On the other time, the increase in certain quality indicators such as hydroxymethylfurfural (produced as a result of Maillard reaction) and diastase activity (Chap. 3) may be observed because of two main reasons (Carvalho et al. 2009; da Silva et al. 2016):

(a) The obvious augment of honey concentration, and
(b) Possible thermally favoured reactions at temperatures such as 35.5 °C.

2.4 Liquefaction and Mixture

The physical state of extracted honey may often require a specific liquefaction step, depending on the solidification or stickiness of this intermediate. However, because of the low thermostability of certain honey components (enzymes, vitamins, etc.) and possible harmful and irreversible modifications after heating, liquefaction must

take place at temperatures that do not exceed 40 °C in the shortest possible time (Mousa 1999, 2001), although the use of different liquefaction or liquefaction/pasteurisation cycles (times and temperatures) is reported (Escriche et al. 2014). The main problem is correlated with the excessive thermal values used for liquefaction (and pasteurisation): generally, honey quality may be heavily affected. On the chemical level, one can affirm that a certain amount of volatile molecules can be lost in these conditions, with the concomitant reduction of enzymatic power (Bogdanov and Martin 2002). Normally, containers are placed in a water bath or in 'hot rooms'. The beekeepers (or companies) that have to manage different honey deliveries, need to proceed with a dedicated mixing step in order to obtain a uniform final product (parameters are colour, texture, and moisture). The mixing step is carried out in containers where the honey is put after being fluidised. A rotating axis is placed at the centre of the container: this axis drags helical blades placed at various heights allowing a uniform mixing of the mass.

2.5 Heating

It has to be noted that heating treatments can affect severely basic honey features from the organoleptic viewpoint. Honey heating entails a progressive browning and a more or less obvious loss of volatile substances that characterise the aroma. The following substantial modifications have been observed:

- Change in the crystal structure
- Increase in the amount complex sugars and concomitant reduction of the simple ones
- Augment of total acidity
- Partial activation of enzymes
- Increase in hydroxymethylfurfural (HMF) amount.

It should be also noted that the viscosity of honey takes on values ranging between 5000 and 40,000 cycles per second, and it decreases when temperature increases up to 40 °C; should this thermal value be exceeded, the viscosity would decrease slowly, with a final increase at elevated temperatures. This singular behaviour suggests that heating temperatures should not exceed 40 °C even for phase filtering. Moreover, it should be considered that the thermal conductivity of honey corresponds to 1/6 of the correspondent feature for water.

The most common heating and processing instruments for honey production are the so-called traditional bain marie (it has to be suitably thermostated) and hot rooms (with forced air circulation by means of adequate thermoconverters). Also, jacket-equipped tubs with continuous hot-water circulation and a central stirrer can be used. Finally, larger-size companies are accustomed to use size heat exchangers.

2.6 Pasteurisation

Pasteurisation is a heating process: honey particles, such as pollen grains, should aggregate themselves around microscopic air bubbles and small crystals acting as aggregation nuclei. This process could favour honey crystallisation (Sect. 2.7). Normally, thermal values should be rapidly raised up to 72 °C; this temperature is maintained for about 120 s. Subsequently, a rapid cooling of honey masses is required (Bogdanov and Martin 2002).

It has to be noted that the pasteurisation of honeys, unlike similar processes carried out on the majority of food products, is not performed for food safety purposes, including also prolonged shelf-life, but with the aim of meeting commercial needs. As an example, a honey should maintain its typical liquid state on the shelves as long as possible: a similar behaviour has to be reached by means of pasteurisation treatments. Anyway, all pasteurised honeys are subjected to be irreversibly modified during time, according to the first Parisi's Law of Food Degradation (Barbera and Gurnari 2017; Parisi 2002).

2.7 Crystallisation

Crystallisation is probably the most important physical features for the characterisation of honeys by the commercial viewpoint. The crystallisation process involves the formation of glucose monohydrate crystals in different quantity, shape, and arrangement depending on processing conditions.

In general, the longer the processing time, the more voluminous the obtained crystals. Different honeys have a different tendency to crystallise depending on the composition (the lower the water content and the higher amount in glucose, the greater the tendency to crystallise), but also depending on the storage temperature (maximum allowed value: 14 °C).

The crystallised honey is unfortunately still considered with suspicion by the average consumer; however, the crystallisation is a natural process and it is normally seen in natural honeys. In fact, the extracted honey tends to be a supersaturated solution of sugars in water; as a result, exceeding amounts of various sugars are naturally released from the liquid solution as crystals after some time. There are several factors influencing the complex crystallisation phenomenon:

(a) The amount of glucose and fructose
(b) Possible impurities.

With relation to the quantity of main sugars, glucose is the most interested sugar to undergo such a similar transformation because it is less soluble in water than fructose. As a consequence, honeys with high fructose percentage crystallise slowly or remain totally uncrystallised.

With concern to possible impurities, an important and necessary prerequisite of crystallisation is the presence of adequate condensation nuclei, including single glucose crystals, dust or pollen grains, air microbubbles.

Anyway, the speed and the type of crystallisation are influenced by the number of condensation nuclei: high numbers of aggregation centres will lead to a fast and fine crystallisation, and a sparse numbers of nuclei will lead to a slow and coarse crystallisation. This phenomenon takes place at temperatures between 5 and 25 °C; should thermal values be lower than 5 °C, honey viscosity would increase with unsatisfactory crystallisation (masses slow down during the crystal growth), while the formation of crystals is slowed down at temperatures >25 °C (formed crystals are destroyed). Should thermal values exceed 78 °C, the destruction of formed crystals would be complete: as a result, honey can no longer crystallise.

For these reasons, peculiar techniques have been developed to guide the natural tendency of honey to crystallise and obtain fully crystallised finished products: this result means stable, homogeneous, and creamy texture with good hedonistic performances by the customer viewpoint.

The most immediate method consists in the mixing of liquid honey with completely crystallised honey in varying proportions depending on the temperature and viscosity of the product (generally, ratios should be 9–1). Should some available honeys have a moisture content that would allow the growth of yeasts, crystallisation process would need to be performed after pasteurisation at 65 °C for 5–10 min. Moreover, in order to obtain an optimum crystallisation, operating temperatures should be recommended in the range 24–28 °C: the aim is to favour honey mixing without the incorporation of air bubbles, with the concomitant introduction of melt crystals.

After packaging (Sect. 2.8), honey must be stored at 14 °C in a few days, so that the crystallisation process can be completed until the final result: a fine granulation of the product. The drawback to this type of procedure is the formation of whitish outcrops at the surface, in correspondence of englobed air bubbles. The visual result is the consequence of water evaporation and drying of glucose crystals that appear white. This aesthetic defect may be avoided with the separation of honey crystals and the concomitant creamy consistency. For these reasons, honey is introduced in drums and placed in hot chambers at a temperature of 28–30 °C before wrapping step.

Subsequently, the honey is passed in a homogeniser with the aim of separating crystals; the resulting mass is introduced in jars. Should homogenisation be absent, the passage from hot rooms to the packing step would still give acceptable results, being sufficient handling of the honey to separate crystals. However, the crystallisation—a very delicate process—may present defects in terms of crystal structure, size, and crystal shape. One can obtain a coarse and non-homogeneous crystallisation due to prolonged processing times. In this situation, obtained crystals have angular, rough or sharp shapes. On the other hand, a compact crystallisation is the result of a very quickly process; it can occur spontaneously in honeys with high glucose/water ratio.

The honeys with very compact crystalline structure tend to present stains retraction, namely 'white veins' on the walls of the vessel, on the honey surface or in correspondence of air bubbles. This purely aesthetic defect may be avoided heating honey at 30 °C for 24–48 h. The separation into phases is the defect of more burden crystallisation: it occurs in honeys with high humidity degree, or in those kept at high temperatures for a too-long storage; in these conditions, crystals precipitate to the bottom while a solid surface is obtained instead of a liquid layer.

It should be remembered that crystallised honeys may be used to produce the so-called 'creamed honey' by means of the Dyce method (Bogdanov and Martins 2002). In detail, liquid honeys may be mixed with crystallised hones with the aim of allowing the increase in crystal dimensions (suggested temperature: 14 °C).

2.8 Final Packaging

With reference to the final packing, small quantities of honey can be easily packaged placing the vat and decant honey at least 50 cm from the ground. The vat must be equipped with a large cutting tap or ball which will be placed under the vessel to be filled. The vessel must be placed under a calibrated balance (the weight of the empty vessel has to be considered 'zero') to control labelled net weight.

For medium or large amounts of honey, automatic dosing pumps, adjustable from 25 to 2500 g, can be used: the complete packaging step becomes easier and faster. Higher quantities would require further machines able to withdraw, fill and seal the jars automatically.

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Chapter 3
Overheating Indexes and Honey Quality

Abstract Honey is certainly one of sugar-based foods, which requires the littlest number of technological steps before entering the market. It could be paradoxically affirmed that the quality of the product 'honey' may be deemed the higher if used manufacturing tools and processes (before its final packaging) are extremely limited in number. A very important parameter for the commercial evaluation of honey producers is the so-called honey freshness: this parameter means the ability to maintain original chemical–physical and sensorial features over time. Because of the negative effects of needed heat treatments on certain sensorial honey features honeys, adequate indexes are needed. The most used of these indicators are the amount of 5-hydroxymethylfurfural and the enzymatic activity of diastase. However, new possible indexes for overheating and quality honey—invertase activity, amount of 1,2-dicarbonyl compounds, furosine, etc.—have been proposed in the last few years (Bogdanov et al. in Bee World 80(2):61–69, 1999).

Keywords Ageing · Diastase · Furosine · Honey freshness 5-hydroxymethylfurfural · Methylglyoxal · Overheating

Abbreviations

AGE	Advanced glycation end
AOAC	Association of Official Analytical Chemists
3-DG	3-deoxyglucosulose
DA	Diastase activity
HMF	5-hydroxymethylfurfural
CIE	International Commission on Illumination

3.1 Honey Processing Modifications. An Introduction

Between foods of natural origin mainly consisting of sugar, honey is certainly one of those foods, which requires the littlest number of technological steps before entering the market. It could be paradoxically affirmed that the quality of the

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product 'honey' may be deemed the higher if used manufacturing tools and processes before its final packaging are extremely limited in number. A very important parameter for the commercial evaluation of honey producers is the so-called honey freshness.

The freshness of honeys should be considered and defined as the ability to maintain original chemical–physical and sensorial features over time; consequently, bad freshness performances may mean that the result of technological processing steps has not been conducted properly.

During honey processing, heating is aimed at the reduction in viscosity, the dissolution of the crystals, the concentration of the product, and at the microbiological and physical stabilisation of the final product (Singh and Bath 1998).

As a result, technologists would need adequate indexes for assessing the extent of heat treatment and the 'degree of ageing' of a honey. The most used indexes are the quantitative estimation of two peculiar molecules: the 5-hydroxymethylfurfural (HMF) and diastase (Bogdanov et al. 1987; Tosi et al. 2008), although the latter has been heavily criticised (White 1992, 1994). Other proposals have been also reported in recent years.

3.2 Honey Heating and Storage Indexes. 5-Hydroxymethylfurfural

By a general viewpoint, extracted honey should not contain HMF (Fig. 3.1) unless bees were not administered syrups and/or aged or blackened honeys during the formation of supers. It may be also affirmed that HMF levels in fresh (intended as raw or unheated) honeys are low; it has been reported that maximum amounts could be 25 mg/kg or <15 mg/kg in association with high invertase activity (Bogdanov and Martins 2002; Duisberg and Hadorn 1966). On the other side, honeys stored for one year may exhibit notable HMF amounts (Askar 1984; Khalil et al 2010).

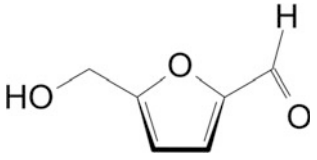

Fig. 3.1 Molecular structure of 5-hydroxymethylfurfural (HMF). This compound has been proposed as a useful index for assessing the extent of heat treatment and the 'degree of ageing' of a honey (Bogdanov et al. 1987; Tosi et al. 2008). By a general viewpoint, extracted honey should not contain HMF with some exception. In addition, HMF levels in raw or unheated honeys should be below 25 mg/kg or <15 mg/kg (Bogdanov and Martins 2002; Duisberg and Hadorn 1966), while honeys stored for one year may exhibit notable HMF amounts (Askar 1984; Khalil et al 2010). Some exception has been reported at room temperature (Khalil et al. 2010; Cosentino et al. 1996; Langridge 1977). BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html) has been used for drawing this structure

Anyway, low HMF levels are expected in many honeys produced in the western Europe because of low or absent heating damages, if compared with imported (and long-durability) honeys (Bogdanov and Martins 2002; Bogdanov et al. 1987; Ramírez Cervantes et al. 2000). The same has been reported for DA values (da Silva et al. 2016). However, some research has demonstrated that HMF may be found in high amounts even in honeys stored at room temperature (Khalil et al. 2010; Cosentino et al. 1996; Langridge 1977).

HMF is the intermediate product of two reactions: (a) the dehydration of hexoses to acids and (b) the decomposition of 3-deoxyglucosulose (3-DG) in the Maillard reaction under heating and storage conditions (Belitz and Grosch 1999; Bogdanov and Martins 2002; Henle et al. 1998; Morales 2008; da Silva et al. 2016; Tornuk et al. 2013; Ulloa et al. 2010; Velásquez Cifuentes 2013). At low pH values, glucose and fructose undergo enolisation with formation of 1,2-endiol. The last intermediate turns into the main degradation product, HMF, through the elimination of two water molecules, cyclisation, and subsequent loss of a further water molecule of water (Fig. 3.2). Consequently, HMF is obtained by means of the reduction of hexoses, while the same reaction on pentoses would produce furfural (Belitz and Grosch 1999; Moreira et al. 2010).

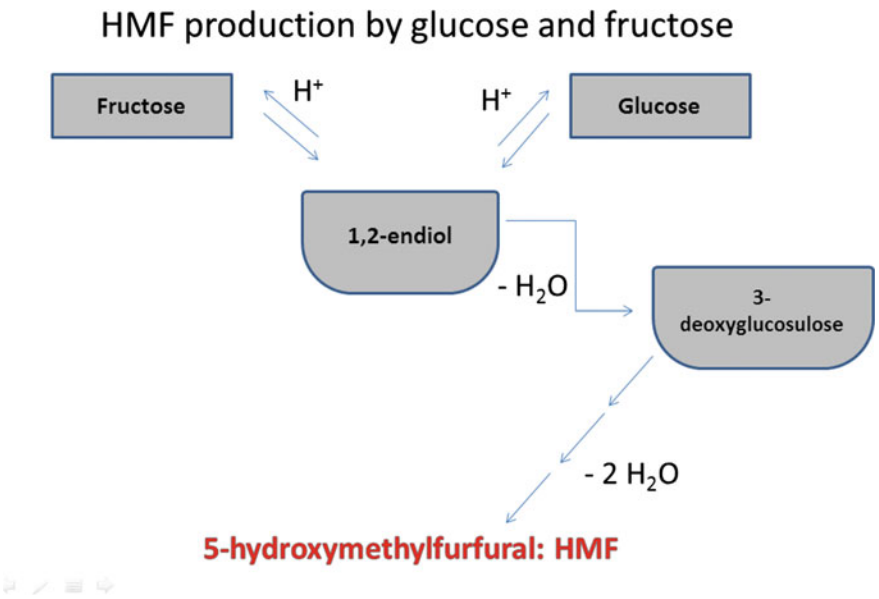

Fig. 3.2 HMF is the intermediate product of two reactions: the dehydration of hexoses to acids and the decomposition of 3-deoxyglucosulose (3-DG) in the Maillard reaction under heating and storage conditions. At low pH values, glucose and fructose undergo enolisation with formation of 1,2-endiol. The last intermediate turns into HMF, through the elimination of two water molecules, cyclisation, and subsequent loss of a further water molecule of water. Consequently, HMF is obtained by means of the reduction of hexoses, while the same reaction on pentoses would produce furfural

The maximum content of HMF established in the Directive 2001/110/EC (Council of the European Union 2002) is 40 mg/kg for all honeys except those from regions with tropical climate where maximum limit is 80 mg/kg. These limits, originally proposed in 2001 (Codex Alimentarius Committee on Sugars 2001), must be guaranteed in the European Union until the labelled sell-by date of the honey.

In detail, HMF amounts in honeys should not exceed 40 mg/kg of finished product after processing and/or blending steps (Morales 2008; Ulloa et al. 2010), except for honeys from countries or areas with tropical temperatures and their mixtures (the allowed limit should be 80 mg/kg maximum). From the kinetic viewpoint, it has been reported that these differences may be explained with low speed values at low temperatures (maximum reported thermal values: 30 °C; maximum HMF increase in honeys should be 1 mg/kg per month in these conditions). In addition, it has been reported that HMF quantities should not increase in normal conditions within 12 months from the production, while remarkable augments should be observed between one and two storage years (Ajlouni and Sujirapinyokul 2010; Velásquez Cifuentes 2013). Anyway, the botanical origin and characterisation of different honeys may notably influence evolutive HMF profiles and other honey features in function of storage temperatures (Krpan et al. 2009; Velásquez Cifuentes 2013; Vorlova and Čelechovská 2002). As an example, honeys obtained from *girasol* and *pino* plants may show different HMF amounts depending on thermal values, ranging from 29.2 mg/kg for *girasol* and 1.95 mg/kg for *pino* at 35 °C to 226.35 mg/kg for *girasol* and 43.4 mg/kg for *pino* at 75 °C (Karabournioti 2010).

Another reflection should be made with reference to the influence of processing and blending steps on HMF (and diastase) allowed levels. From the legal viewpoint, the restriction is not valid for raw materials and intermediate honeys; only processed and/or blended honeys should be analysed in this way. Similar HMF limits are also guaranteed in Argentina and Canada, although this approach is not always considered in other countries (Fallico et al. 2008). In addition, the Directive 2001/110/EC establishes a third limit of 15 mg/kg for those honeys having diastase values between 3 and 8 Schade units (Council of the European Union 2002).

The content of HMF in honey depends on chemical features and the floral origin (Fallico et al. 2004; Singh and Bath 1997, 1998; Zappalà et al. 2005), heating temperature and time values (Bath and Singh 1999), and storage conditions (Sancho et al. 1992). Interestingly, it has been reported that HMF production depends also on the reduced or increased crystallisation of sugars. In detail, heating processes may slow down the production of sugar crystals into honey masses with consequent water diminution (in other words, reduced aqueous retention), lower water activity amounts, accelerated sugar degradation, HMF increase, and colorimetric modifications in reduced times (Bulut and Kilic 2008; Salamanca Grosso et al. 2001; Subovsky et al. 2004; Terrab et al. 2004; Tuyuc Chex 2013; Velásquez Cifuentes 2013). In addition, it should be considered that blossom and honeydew honeys are quite different when speaking of approximate composition. In fact, apparent reducing sugars are extremely abundant in the first honey typologies, while

honeydew products contain notable amounts of non-reducing carbohydrates including maltotriose and raffinose (Bogdanov et al. 1999, 2002). This simple discrimination between blossom and honeydew honeys is based only on the composition of reducing and non-reducing sugars. It may be inferred that similar differences may have some influence when speaking of reduced or increased crystallisation of sugars, and related heating effects. The variegated composition of blossom and honeydew honeys may be also observed and indirectly evaluated by means of measures such as electrical conductivity and the determination of mineral contents (Bogdanov et al. 1999; Horn and Lüllmann 1992; Vorwohl 1964).

The kinetics of HMF development in flower honeys and its dependence on the pH of examined samples has been studied (Fallico et al. 2004). It has been reported that the proposed HMF limit established by Directive 2001/110/EC is too restrictive for some types of flower honeys (citrus, eucalyptus), while it is too permissive for others (chestnut) (Fallico et al. 2006). Ferrer and co-workers have also shown irregular HMF levels in milk-based baby products after a two-year monitoring study at temperatures comprised between 20 and 37 °C (Ferrer et al. 2005).

In addition, the HMF degradation kinetics in monofloral honeys stored at temperatures between 25 and 50 °C has been studied (Fallico et al. 2008). Obtained results showed that HMF degradation is favoured at low temperatures if compared to the related formation. Therefore, HMF seems to be a good indicator for evaluating thermal damage, while the same meaning may not be assured when speaking of honey preservation. In addition, HMF is reported to be a good indicator for authenticity issues, if used with other indexes such as DA and invertase activity (Bogdanov and Martins 2002; Bogdanov et al. 1997, 1999; da Silva et al. 2016; Lord et al. 1988).

It should be also considered that HMF has been linked with the detection of levulinic and formic acids in honeys. These acids may be originated from HMF by means of subsequent reactions with the addition of water molecules. Therefore, free acidity of the produced honey may be increased (da Silva et al. 2016).

From the safety viewpoint, it should be remembered that HMF can have important effect on the human health because of suspect mutagenicity (Khalil et al. 2010; Surh et al. 1994; Janzowski et al. 2000).

Anyway, it has to be considered that HMF cannot be used as a good overheating indicator without other indexes such as the presence of organic acids, the quantitative composition of sugars, and other parameters. Should these data be absent, it could be only suggested that high-HMF honeys have been not adequately heated or badly stored (da Silva et al. 2016). For this reason, some studies have also considered HMF detection (the AOAC 980.23 norm is specifically recommended) with a more specific characterisation of the sampled honey by means of the determination of different analytes (Codex Alimentarius Committee on Sugars 2001; Kasperová et al. 2012; Tuyuc Chex 2013):

(a) Moisture content (by means of the AOAC 969.38 method or similar systems)
(b) Water activity (by means of AOAC 978.18 norm or similar methods)

(c) pH evaluation (by means of AOAC 973.41 method or similar systems)
(d) Colorimetric evaluations in the CIELab colour space system (International Commission on Illumination 1976)
(e) Sugar determination (by means of AOAC 982-14 or equivalent systems).

This strategy might allow a certain discrimination between honeys from different geographical areas in the same country also. The chemometric approach has been proposed when speaking of certain Spanish honeys, although related results have been questioned (Bogdanov and Martins 2002; Gómez Bárez et al. 2000; González Paramás et al. 2000; Lopez et al. 1996; Pena Crecente and Herrero Latorre 1993; Sanz et al. 1995). With relation to moisture, it has been reported that honeys subjected to evaporation (and consequent water loss) may have notable HMF values with some exception (Khalil et al 2010; Tumin et al. 2005). With concern to pH values, it has been also suggested that low values could justify antimicrobial properties for certain honeys (Khalil et al. 2010; Tan et al. 2009).

By the analytical viewpoint, HMF is evaluated by means of the AOAC Official Method 958.09. This analytical approach concerns the simple ultraviolet spectrophotometrical determination of HMF in a treated honey solution after use of Carrez solutions I and II, filtering, water dilution, and addition of a sodium bisulphite solution. The spectrophotometric determination is carried out at 284 and 336 nm; obtained result is calculated as mg of HMF per 100 g of initial honey (AOAC 2016; Perez-Arquillué et al. 1994; White 1979).

3.3 Honey Heating and Storage Indexes. Diastase and Invertase

The diastase activity (DA) measures the amount of enzymes—the major enzyme of honey being diastases, α- and β-amylases—and this value is variable from honey to honey and for the same type of honey. Substantially, the enzymatic action is focused on the digestion of starch with the aim of obtaining disaccharides and trisaccharides together (da Silva et al. 2016). Because of the known thermolability of diastases, the correlated DA index may be used with the aim of evaluating the destruction of related enzymes and consequently the overheating level of honeys, although some doubt has been recently reported (Tosi et al. 2008). In general, the higher the DA value, the higher the durability (da Silva et al. 2016). This reflection is extremely important because honeys are subjected to be irreversibly modified during time, according to the first Parisi's Law of Food Degradation (Barbera and Gurnari 2017; Parisi 2002). In general, diastase values appear to be in the range 2.1–6.1.2 with average content equal to 20.8 while pH optimal values are reported to be 5.0–5.3 (Belitz et al. 2009). On the other side, many analysed raw and retail honey samples can show DA values >8 (Lüllmann 1997); this fact and other considerations have been often taken into account when speaking of DA activity as a valid heating index.

Acacia honey has a DA index that varies between 3 and 15, while citrus honey has lower DA (and this value represents one of its peculiar features). Should DA be low, the related honey would have been heated at high temperature; alternatively, this product should be judged 'old'. It can be used as a convenient overheating index (Codex 2001). Basically, diastases are only one class of enzymes normally found in honeys, including also invertase (also named α-glucosidase or sucrose), acid phosphatase, glucose oxydase, and α- and β-glucosidases (da Silva et al. 2016; Persano Oddo et al. 1999). On the other side, and similarly to HMF, this indicator should be carefully used as a pure overheating index because DA values may depend also on various variables correlated with the peculiar behaviour of involved bees and the possible artificial feeding with compounds such as commercial glucose (da Silva et al. 2016; Persano Oddo et al. 1999). In fact, some difference between heating effects on HMF and DA indexes have been observed (Bogdanov and Martins 2002; Dyce 1975).

Diastase is an enzyme naturally present in fresh honey (Persano Oddo et al. 1999): it can be degraded during time and/or by means of thermal treatments (Sanchez et al. 2001). White evaluated amounts of diastase and HMF during honey processing: according to obtained results, He reported that diastase is not a good indicator for evaluating honey quality, therefore proposing the use of HMF only when speaking of honey overheating and freshness evaluation (White 1994).

Fallico and co-workers have also shown that the prediction of shelf life for honeys based on diastase estimation does not improve results, but in fact increases the uncertainty of proposed models and provides a slightly longer durability (Fallico et al. 2008). Certain correlations have been reported when speaking of DA and invertase on the one hand, and of β-glucosidase and invertase on the other side, suggesting that more research could be useful (Huidobro et al. 1995; Persano Oddo et al. 1999). Substantially, the observed variability in the correlation of DA and invertase activities should need a more detailed explanation: apparently, related ratio values appear to be between 0.1 and 2.0, suggesting that enzymatic activity may be dependent on the following factors at least (Brouwers 1982, 1983; Fluri et al. 1982; Huang and Otis 1989; Huang et al. 1989; Persano Oddo et al. 1999):

(1) Age of interested bees
(2) Nectar collection period
(3) Pollen consumption
(4) Sugar concentration in nectars.

On these bases, it has been also reported that invertase might be a more useful heating index if compared with DA; the same concept has been repeated when speaking of high-quality honey products (Dustmann 1993; Dustmann et al. 1985; Gonnet 1965; Persano Oddo et al. 1999; White et al. 1964). DA has been often criticised for its apparent variability (Persano Oddo et al. 1990; Thrasyvoulou 1968; White 1967).

Anyway, HMF seems to remain the most reliable heating index in honeys if compared with DA, invertase, other enzymes, and other numbers such as DA/invertase ratio (Persano Oddo et al. 1999).

By the analytical viewpoint, an interesting recommended method is the AOAC Official Method 958.09 concerning 'diastatic activity of honey'. Substantially, this method concerns the spectrophotometric determination (wavelengths: 660 nm or 600 nm) of diastase activity in acetate-buffered starch–honey solution after incubation (no heating); the result (determination at the end-point measured in minutes) is calculated as the amount (in millilitres) of 1% solution starch hydrolysed by diastase for one gram of sampled honey in 60 min (AOAC 2016; White 1964; White and Pairent 1959).

3.4 Honey Heating and Storage Indexes. Alternative Approaches

Both HMF and diastase are used as quality indexes by more than 80 years; however, other thermal treatment indicators have been reported and proposed such as invertase, furosine (Fig. 3.3), and 2-furoylmethyl amino acids already used in other foods (Bogdanov and Martins 2002; Sanz et al. 2003; Villamiel et al. 2001). Hidalgo and Pompei have also studied the kinetics of formation of furosine and HMF in tomato-based products, showing that HMF tends to decrease during storage at room temperature (Hidalgo and Pompei 2000). Consequently, the use of furosine as a freshness indicator in combination with HMF has been proposed recently (Cifuentes 2012; Yamaguchi et al. 2012).

The determination of these compounds involves a laborious and lengthy preparation of honey samples before analytical protocols (Sanz et al. 2003; Villamiel et al. 2001). For these reasons, the assessment of reliable quality indicators should be evaluated by means of reliable molecules. An interesting and recent approach may be to assess the presence of 1,2-dicarbonyl compounds including 3-DG.

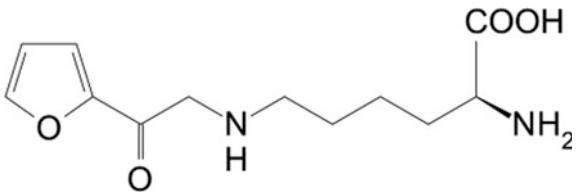

Fig. 3.3 Molecular structure of furosine. This compound has been proposed as a freshness indicator for honeys in combination with HMF (Cifuentes 2012; Yamaguchi et al. 2012). However, the related analytical determination involves a laborious and lengthy preparation of honey samples before analytical protocols (Sanz et al. 2003; Villamiel et al. 2001). For this reason, HMF and diastase activity are generally recommended as 'ageing' and overheating indexes in honeys. BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html) has been used for drawing this structure

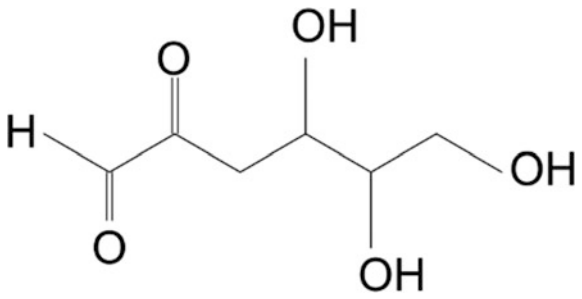

Fig. 3.4 Molecular structure of 3-deoxyglucosulose (3-DG), also called 3-deoxy-*D*-erythrohexos-2-ulose. This compound has been identified as one of intermediate molecules in the Maillard reaction from hexoses (glucose and fructose) to HMF (Fig. 3.2). 3-DG may increase notably in heated honeys with other peculiar substances such as hydroxymethylfurfuraldehyde, differently from methylglyoxal. Consequently, 3-DG has been proposed as useful heating and storage index in honeys (Mavric 2007; Stephens and Schlothauer 2009). According to some authors, 3-DG could have some antioxidant property in selected foods (Moon et al. 2002). With relation to advanced glycation end (AGE) products, the AGE-6 molecule is reported to be produced with the active role of 3-DG (Bastos et al. 2012; Hidalgo and Zamora 2017; Nursten 2007; Sato et al. 2006). BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html) has been used for drawing this structure

3-DG (Fig. 3.4) is an intermediate product of the Maillard reaction: in detail, it is produced from the degradation of Amadori products. In addition, it is also the HMF precursor during the complex reaction of sugars dehydration (Belitz and Grosch 1999; Henle et al. 1998; Marceau and Yaylayan 2009). Weigel and co-workers have determined 3-DG content in Millefiori honeys: obtained results have been found up to 100 times higher than HMF values (Weigel et al. 2004).

The presence of 1,2-carbonyl compounds (glyoxal, methylglyoxal, and diacetyl) has been also found in fermented and cooked products including cookies, wine, beer, and butter (Arribas-Lorenzo and Morales 2010; Barros et al. 1999; Bednarski et al. 1989; Hayashi and Shibamoto 1985; Homoki-Farkas et al. 1997; Kasai et al. 1982; Kwok et al. 2016; Nagao et al. 1986). These molecules are produced by means of enzymatic reactions during fermentation processes (Barros et al. 1999; Degen et al. 2012; Hayashi and Shibamoto 1985). According to some authors, the antioxidant power of soy sauce would be positively correlated to 3-DG content (Moon et al. 2002). When speaking of products such as honey, carbohydrates may be degraded under more or less drastic heating processes with the production of 1,2-carbonyl compounds such as glyoxal, methylglyoxal, and 3-deoxyglucosone (Adams et al. 2008). Glyoxal is normally produced by means of lipid peroxidation, sugar oxidation, or as part of Amadori products (Bastos et al. 2012; Thornalley et al. 1999); other possible pathways may be explored.

With exclusive relation to honey, methylglyoxal (Fig. 3.5) has been found and studied in Manuka honey because of the potential antibacterial activity (Adams et al. 2008). This compound, found in Manuka honey with glyoxal and 3-DG, may be potentially able to increase antimicrobial power of honeys without reference to

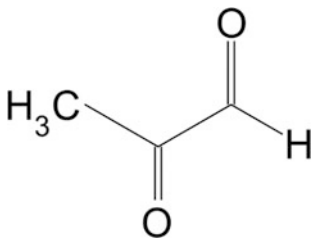

Fig. 3.5 Molecular structure of methylglyoxal. This compound has been found and studied in Manuka honey because of the potential antibacterial activity against methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and vancomycin-resistant enterococci (Adams et al. 2008; Bastos et al. 2012; Thornalley et al. 1999). Methylglyoxal may be potentially able to increase antimicrobial power of honeys. The importance of this compound is also correlated with its production as one of Maillard reaction derivatives in foods with high sugar content. With relation to AGE products, the AGE-4 molecule is reported to be produced with the active role of methylglyoxal (Bastos et al. 2012; Hidalgo and Zamora 2017; Nursten 2007; Sato et al. 2006). More research is still needed with relation to positive honey properties as a result of methylglyoxal presence, and the possible use of this compound as heating and storage index. BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html) has been used for drawing this structure

hydrogen peroxide activity (Oelschlaegel et al. 2012); glucose oxidase can produce this substance from glucose and water (Adams et al. 2008). The importance of this compound is also correlated with its production as one of Maillard reaction derivatives in foods with high sugar content. According to recent researches, this substance, very active against methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and vancomycin-resistant enterococci, can be found in Manuka honey in remarkable amounts. In addition, glyoxal and methylglyoxal can produce advanced glycation end (AGE) products by means of the reaction with side chains of three amino acid residues or different pathways (Henle 2005; Fu et al. 1996; Li et al. 2014; Singh et al. 2001; Uribarri et al. 2010). Moreover, lipoxidation end products can be obtained by means of different reactions (Adams et al. 2008). Interestingly, 3-DG may increase notably in heated honeys with other peculiar substances such as hydroxymethylfurfuraldehyde, differently from methylglyoxal. Apparently, it can be assumed that methylglyoxal is not influenced by chemical reactions in stored honeys while 3-DG is undoubtedly influenced, with a specific recommendation as useful heating and storage index (Mavric 2007; Stephens and Schlothauer 2009).

With relation to AGE products, it should be considered that their production involves probably the transformation of peculiar proteins by means of the active role of glyoxal, methylglyoxal, and 3-DG. These α-oxoaldehydes seem to have a particular role when speaking of AGE products; briefly, methylglyoxal and remaining oxoaldehydes may be considered AGE crosslinks, as recently reported (Thornalley et al. 1999). Up to six different AGE products have been identified, and a possible pathway for their production has been recently proposed. Interestingly, three of these molecules, AGE-4, AGE-5, and AGE-6, are reported to be produced

with the active role of methylglyoxal, glyoxal, and 3-DG, respectively (Bastos et al. 2012; Hidalgo and Zamora 2017; Nursten 2007; Sato et al. 2006).

The biochemical origin of methylglyoxal is reported to be the dihydroxyacetone molecule: this compound, normally found in nectars of some *Leptospermum* species in abundant amounts, is dehydrated; the final Manuka honey contains notable methylglyoxal quantities and low dihydroxyacetone levels. Actually, different enzyme-mediated and non-enzymatic pathways have been considered when speaking of this molecule, including (Adams et al. 2009; Federoňko and Königstein 1969; Riddle and Lorenz 1968; Wang and Ho 2012):

(a) Acid-catalysed reaction on trioses
(b) Dehydration in acetic acid solutions of dihydroxyacetone and all isomeric glyceraldehyde forms (*D*- and *L*-isomers)
(c) Possible reaction between dihydroxyacetone and glycine in one of Maillard reaction steps, with methylglyoxal production as a simple intermediate of the pathway
(d) Conversion of dihydroxyacetone–phosphate by means of methylglyoxal synthase enzyme.

It has also been reported that methylglyoxal amounts are quite low in fresh Manuka honeys, while are extremely low in non-Manuka honeys (reported quantities do not exceed 491 mg/kg of fresh product). On the other side, storage at 37 °C is considered a key factor when speaking of observed augments, and this reflection could be related to thermal reactions apparently responsible for methylglyoxal production (Majtan 2010; Weigel et al. 2004).

Interestingly, methylglyoxal is also reported to react with certain proteins, deoxyribonucleic and ribonucleic acids; in addition, some toxicity problems could be detected (Carter et al. 2016; Kalapos 2008; Mavric et al. 2008; Norton et al. 2015; Williams et al. 2014). On the other hand, positive properties of certain Manuka honeys have been demonstrated in relation with the presence of methylglyoxal, including antioxidant features (anti-inflammatory effects), although not all *Leptospermum* plants are able to produce this molecule and active honey at the same time. In general, methylglyoxal has been found be between 100 and 1200 ppm in certain honeys, but more research is still needed with relation to positive honey properties as a result of methylglyoxal presence (Carter et al. 2016; Irish et al. 2011; Kalapos 1999; Windsor et al. 2012).

With specific reference to Manuka honey, another remarkable bioactive compound is *D*-glucono-δ-lactone (Fig. 3.6) (Campuzano et al. 2007; Cantarelli et al. 2008; Kretavičius et al. 2010; Malika et al. 2005; Mato et al. 1997, 2006; Patel and Cichello 2013; Pulcini et al. 2004; Serrano et al. 2004). This compound is able to reduce Manuka honey pH; in addition, some antimicrobial properties and enhanced durability have been ascribed to this honey because of the presence of *D*-glucono-δ-lactone (Rupesh et al. 2014). Substantially, this compound seems important when speaking of honey preservation, acidity, and the correct identification of botanical species (Pulcini et al. 2004), while the usefulness as heating or

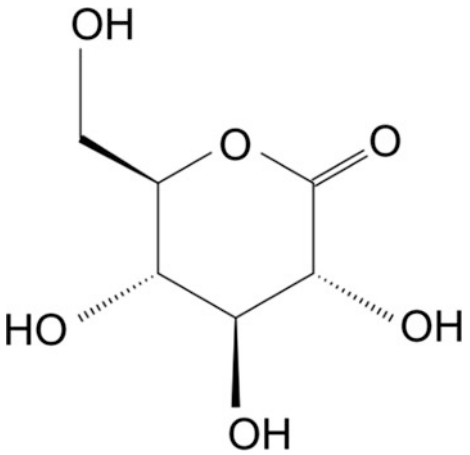

Fig. 3.6 Molecular structure of *D*-glucono-δ-lactone. This molecule is able to reduce Manuka honey pH; it is reported to have bioactive properties and justify enhanced durability in this honey (Rupesh et al. 2014). On the other hand, the usefulness as heating or storage index does not seem important so far when speaking of honey products. BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html) has been used for drawing this structure

storage index is not apparently discussed in this ambit. Anyway, more research is needed in general with relation to the use of these compounds as possible overheating and freshness indexes.

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