Food Chemistry 196 (2016) 309–323

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Review

Honey: Chemical composition, stability and authenticity

Priscila Missio da Silva *, Cony Gauche, Luciano Valdemiro Gonzaga, Ana Carolina Oliveira Costa, Roseane Fett

Department of Food Science and Technology, Federal University of Santa Catarina, Florianópolis, SC, Brazil

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 21 August 2015
Accepted 15 September 2015
Available online 16 September 2015

Keywords:
Honey
Parameters of identity and quality
Authenticity
Stability
Degradation
Chemical transformation
Furan compounds
Maillard reaction
Volatile compounds

ABSTRACT

The aim of this review is to describe the chemical characteristics of compounds present in honey, their stability when heated or stored for long periods of time and the parameters of identity and quality. Therefore, the chemical characteristics of these compounds were examined, such as sugars, proteins, amino acids, enzymes, organic acids, vitamins, minerals, phenolic and volatile compounds present in honey. The stability of these compounds in relation to the chemical reactions that occur by heating or prolonged storage were also discussed, with increased understanding of the behavior regarding the common processing of honey that may compromise its quality. In addition, the identity and quality standards were described, such as sugars, moisture, acidity, ash and electrical conductivity, color, 5-HMF and diastase activity, along with the minimum and maximum limits established by the Codex Alimentarius.

Contents

* Corresponding author at: Department of Food Science and Technology, Laboratory of Food Chemistry, Federal University of Santa Catarina, Admar Gonzaga 1346, 88034-000 Itacorubi, Florianópolis, SC, Brazil.
E-mail address: priscilamissio@yahoo.com.br (P.M. da Silva).

http://dx.doi.org/10.1016/j.foodchem.2015.09.051
0308-8146/

1. Introduction

Honey is a natural food, mainly composed of sugars and other constituents such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances. It is rich in flavonoids and phenolic acids that exhibit a wide range of biological effects and act as natural antioxidants (Alqarni, Owayss, & Mahmoud, 2012). The composition, color, aroma and flavor of honey depend mainly on the flowers, geographical regions, climate and honeybee species involved in its production, and are also affected by weather conditions, processing, manipulation, packaging and storage time (Escuredo, Dobre, Fernández-González, & Seijo, 2014; Tornuk et al., 2013).

Moreover, honey is a food that undergoes many changes in its composition during storage. Thus, these are expected changes that usually occur due to different chemical reactions, including fermentation, oxidation and thermal processing, thereby modifying honey constituents (Moreira, Maria, Pietroluongo, & Trugo, 2010). For example, 5-hydroxymethylfurfural (5-HMF), which is a *Maillard* reaction product, can be formed when honey is submitted to heat treatment or a long storage time (Tornuk et al., 2013), becoming volatile and toxic, depending on its concentration. In addition, 5-HMF can also be formed by the dehydration of sugars in an acidic environment, such as honey (Barra, Ponce-Díaz, & Venegas-Gallegos, 2010; Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2007; Wang, Juliani, Simon, & Ho, 2009).

The limited availability and high price of honey have provided a heightened interest in its adulteration. The identity and quality parameters of honey are considered useful for detecting these possible adulterations, and also for confirming the hygiene conditions for the manipulation and storage of honey (Puscas, Hosu, & Cimpoiu, 2013).

Honey is a viscous, aromatic, sweet food that is consumed and enjoyed by people around the world. For this reason, it requires certain standards and norms that guarantee its identity and quality so that consumers may safely consume honey, and the same shall have free circulation in the internal market and access to the external market (Codex Standard for Honey, 2001). The most common forms of honey tampering are the addition of cheap sweeteners (such as cane sugar or refined beet sugar, corn syrup, high fructose or maltose syrup) and honeybees fed with sucrose (Puscas et al., 2013).

This review aims to describe the characteristics of the compounds present in honey to expand knowledge about the stability of chemical compounds in order to identify markers that could attest to the chemical stability of honey as a food and the quality safety measure for consumers. In addition, this review describes the parameters that confirm the quality and authenticity of honey and finds similar characteristics in the different honeys produced throughout the world, using requirements such as purity, maturity and deterioration, as defined by the Codex Alimentarius.

2. Chemical composition and reaction products

Honey is a food that contains about 200 substances (Escuredo, Míguez, Fernández-González, & Seijo, 2013), and consists mainly of sugars, water, and other substances such as proteins (enzymes), organic acids, vitamins (especially vitamin B6, thiamine, niacin, riboflavin and pantothenic acid), minerals (including calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), pigments, phenolic compounds, a large variety of volatile compounds, and solid particles derived from honey harvesting (Alqarni et al., 2012; Ciulu et al., 2011; Pontes, Marques, & Câmara, 2007). In order to better understand each component present in honey and their behavior in prolonged storage, the topics listed below describe each compound present in honey, outlining their chemical structures, their importance as a structural constituent of honey and studies that show the stability of these components during storage time.

2.1. Sugars

Monosaccharides represent about 75% of the sugars found in honey, along with 10–15% disaccharides and small amounts of other sugars. The sugars present in honey are responsible for properties such as energy value, viscosity, hygroscopicity and granulation (Kamal & Klein, 2011).

Sugar composition depends mainly on the honey's botanical origin (the types of flowers used by the bees), geographical origin, and is affected by climate, processing and storage (Escuredo et al., 2014; Tornuk et al., 2013). The concentration of fructose and glucose, as well as the ratio between them, are useful indicators for the classification of monofloral honeys (Kaskoniene, Venskutonis, & Ceksteryte, 2010). In almost all types of honey, fructose is the carbohydrate in greatest proportion, except in some honeys such as rape (*Brassica napus*) and dandelion (*Taraxacum officinale*), wherein the fraction of glucose may be higher than the fraction of fructose (Escuredo et al., 2014), and consequently these honeys, generally, have a rapid crystallization.

The sugar profile of honey has been studied by scientists throughout the world. In these profiles, many sugars were detected, such as fructose, glucose, sucrose, rhamnose, trehalose, nigerobiose, isomaltose, maltose, maltotetraose, maltotriose, maltulose, melezitose, melibiose, nigerose, palatinose, raffinose, erlose and others (Fuente, Ruiz-Matute, Valencia-Barrera, Sanz, & Castro, 2011).

Sugars in honey are represented by monosaccharides, glucose and fructose, followed by disaccharides, sucrose, maltose, turanose, isomaltose, maltulose, trehalose, nigerose, kojibiose and trisaccharides maltotriose and melezitose. Disaccharides and trisaccharides like sucrose and maltotriose are hydrolyzed enzymatically to monosaccharides. Sucrose consists of one molecule of fructose linked with glucose through α-1,4 binding. It is hydrolyzed by the enzyme invertase, yielding an equimolar mixture of hexoses (Kamal & Klein, 2011).

Maltotriose consists of three glucose units (α-1,4 glycosidic bonds), which are hydrolyzed by enzymes to maltose. Maltose is also hydrolyzed by enzymes, but in this case, the enzyme is α-glucosidase, resulting in two glucose molecules (Soldatkin et al., 2013).

Sugars and other components of honey may change during storage. Rybak-Chmielewska (2007) analyzed both stabilized (at a temperature of 100 °C for 15 min – enzyme inactivated) and non-stabilized honeys stored for 24 weeks. Sucrose concentration in honeys that were not stabilized decreased 14% in honey stored at 4 °C, and 79% in honey stored at room temperature (20 °C). For

other sugars, such as trehalose and isomaltose, percentages showed no significant changes during prolonged storage. The fructose content increased 4% and glucose content increased 1.1% compared to their initial value at 4 °C; however, at a temperature of 20 °C, the fructose content increased 7% and the glucose content increased 8.8%. In stabilized honeys, the percentages of sugars varied 0.1% higher in sucrose, trehalose and melezitose + erlose or 0.1% lower in turanose. Comparing the honeys at different temperatures of 4 and 20 °C, the variation in levels of individual sugars was 0.2%. Over time, in addition to the chemical changes, there are physical changes in honey, such as darker color and change in flavor.

When honey is heated or stored for a long time, pentoses and hexoses decompose in a slow enolization and a fast β-elimination of three molecules of water to form undesirable compounds such as furans (Chernetsova & Morlock, 2012). The main furans formed are furfural, which is derived from pentoses, and 5-hydroxymethylfurfural (5-HMF), derived from hexoses such as glucose and fructose (Moreira et al., 2010). These are the main degradation products of sugars and their occurrence in foods is usually related to non-enzymatic browning reactions, i.e., *Maillard* reaction, sugar degradation in an acidic medium and caramelization. Actually, these furans have been used as markers for the heat treatment of food (Moreira et al., 2010). The furfuryl alcohol is also an indicator of thermal treatment and storage conditions. Thus, these compounds are not considered to be good markers of floral honey, although they may indicate a possible loss of freshness due to exposure to high temperatures or prolonged storage (Barra et al., 2010; Castro-Vázquez et al., 2007).

In addition to the previously mentioned compounds, other products of sugar degradation, such as 2-acetylfuran (Wang et al., 2009), isomaltol (Ota, Kohmura, & Kawaguchi, 2006), 3,5-dihydroxy-2-methyl-5,6-diidropiran-4-one and maltol (Jelen, 2012) are formed when submitted to heat in the presence of amino acids, contributing to the change in color, taste and odor of honey.

2.2. Proteins

The protein content of honey varies according to the species of the honeybees. *Apis cerana* honey contains from 0.1% to 3.3% protein, while *Apis mellifera* honey contains between 0.2% and 1.6% protein (Won, Li, Kim, & Rhee, 2009). Proteins and amino acids in honeys are attributed both to animal and vegetal sources, including fluids and the nectar secretions of the salivary glands and pharynx of honeybees (Escuredo et al., 2013; Sak-Bosnar & Sakac, 2012), but the main source of protein is the pollen.

Amino acids are responsible for 1% (w/w) of the constituents of honey and their relative proportions depend on the origin of the honey (nectar or honeydew) (Hermosín, Chicón, & Cabezudo, 2003). The most abundant amino acid in honey and pollen is proline (Iglesias et al., 2006). Besides proline, other amino acids present in honey include glutamic acid, aspartic acid, glutamine, histidine, glycine, threonine, β-alanine, arginine, α-alanine, γ-aminobutyric acid, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, tryptophan, phenylalanine, ornithine, lysine, serine, asparagine and alanine (Hermosín et al., 2003; Keckes et al., 2013; Rebane & Herodes, 2010), with the most common of these being glutamic acid, alanine, phenylalanine, tyrosine, leucine and isoleucine (Girolamo, D'amato, & Righetti, 2012).

Proline originates mainly from the salivary secretions of honeybees (*Apis mellifera* L.) during the conversion of nectar into honey. In honey, proline represents a total of 50–85% amino acids (Iglesias et al., 2006; Truzzi, Annibaldi, Illuminati, Finale, & Scarponi, 2014). Proline has been used as a criterion for the evaluation of the maturation of honey, and in some cases, adulteration with sugar. A minimum value of 180 mg kg^{-1} of proline is accepted as the limit value for pure honey (Hermosín et al., 2003; Manzanares, García, Galdón, Rodríguez, & Romero, 2014).

A small fraction of the proteins present in honey are enzymes such as invertase, the α-and β-glucosidase, catalase, acid phosphatase, diastase, and glucose oxidase (Sak-Bosnar & Sakac, 2012; Won, Lee, Ko, Kim, & Rhee, 2008). Diastases are a group of amylolytic enzymes that include α- and β-amylases. α-Amylase hydrolyzes starch chains in the α-D-(1→4) linkages, producing dextrin. β-Amylase hydrolyzes starch chain at the end, leading to the formation of maltose (Sak-Bosnar & Sakac, 2012). Another enzyme present in honey is glucose oxidase. It converts glucose into δ-gluconolactone, which is hydrolyzed to gluconic acid. Besides δ-gluconolactone, glucose oxidase also produces hydrogen peroxide, which has bactericidal action (Moreira, Maria, Pietroluongo, & Trugo, 2007).

The conditions of storage and processing may lead to the formation of undesirable products due to the reaction of the carboxylic group on the reducing end of sugars and the free amino groups of amino acids and proteins (*Maillard* reaction). The *Maillard* reaction occurs as an initial step to the formation of Amadori compounds, which then form melanoidins, during the storage of foods with high sugars or amino acids, such as honey. Amadori compounds are derived from the amino acids lysine, proline, γ-aminobutyric acid and arginine (Iglesias et al., 2006). These amino acids are present in honey and therefore can trigger the *Maillard* reaction.

Iglesias et al. (2006) analyzed the free amino acids of different honey nectar, honeydew and honey blend stored at room temperature and unpasteurized over 24 months of storage. The authors found that the content of the amino acids: aspartic acid, β-alanine and proline increased during the first 6 months of storage. Proline concentration decreased after 6 months of storage, while the concentration of aspartic acid decreased after 12 months of storage. β-Alanine concentration did not change between 6 and 24 months. This result may suggest that while β-alanine is involved in the *Maillard* reaction, its reactivity is lower than that of other amino acids. The increase of aspartic acid and proline during the first months of storage can be explained by the presence of enzymes such as protease and/or peptidase in honey, because these amino acids are present in pollen. The concentration of free amino acids decreased over the first nine months of storage, while no significant difference was observed in the 15 months of storage.

Quantitative and qualitative changes of protein content were related to protein–polyphenol complex formations in honey stored for six months at different temperatures (Brudzynski, Sjaarda, & Maldonado-Alvarez, 2013). These authors stated that the polyphenols present in honey can be easily oxidized to quinones, and perform a key role in the interaction with proteins. These interactions were intensified when honey was stored at high temperatures, by modifying the protein structure and size, leading to covalent bonds between proteins and quinones derived from oxidized polyphenols.

2.3. Organic acids

According to many authors, all honeys have a slight acidity, as a result of approximately 0.57% organic acids (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). These organic acids are derived from sugars by enzymes secreted by honeybees when transforming the nectar into honey or when obtained directly from nectar (Cherchi, Spanedda, Tuberoso, & Cabra, 1994).

Organic acids are also used to discriminate the honeys according to their botanical and/or geographical origin. These acids are related to the color and flavor of honey and its chemical properties such as acidity, pH and electrical conductivity (Mato, Huidobro, Simal-Lozano, & Sancho, 2006).

Some organic acids from different regions of the world are present in honey as acids: aspartic acid, butyric, citric, acetic, formic, fumaric, galacturonic, formic, gluconic, glutamic, glutaric, butyric, glyoxylic, 2-hydroxybutyric, α-hydroxyglutaric, isocitric, α-ketoglutaric, lactic, malic, malonic, methylmalonic, 2-oxopentanoic, propionic, pyruvic, quinic, shikimic, succinic, tartaric, oxalic and others (Cherchi et al., 1994; Mato et al., 2006; Nozal, Bernal, Gómez, Higes, & Meana, 2003).

The predominant acid in honey is gluconic acid. Its presence in honey originates from glucose oxidase, which honeybees provide during ripening (Karabagias et al., 2014). Besides gluconic acid, citric acid is also present in honey, and the concentration of these two substances is used as a reliable parameter to differentiate floral honey from honeydew (Mato et al., 2006).

Levulinic and formic acids are also present in honey. They can be derived from 5-HMF in successive reactions, then link with two water molecules, producing one molecule of levulinic acid and one molecule of formic acid, thereby increasing the concentration of free acidity in honey (Cavia, Fernández-Muino, Alonso-Torre, Huidobro, & Sancho, 2007).

Cavia et al. (2007) evaluated the free acidity of 35 Spanish honeys which had not been heated during 30 months. All samples were stored at room temperature and analyzed every 5 months. The free acidity remained practically constant during the first 15 months of storage, with a slight tendency to increase. After 20 months, most of the honey samples showed a constant increase in free acidity, which is provided by free acids in honey and can vary widely. Many authors have reported the increase of acidity over time, as well as during fermentation because honey sugars and alcohols transform into acids by the action of honey yeasts (Cavia et al., 2007).

When storing food, heat treatment should be efficient to ensure food safety, because if contamination does occur, the result of sugar fermentation is the formation of volatile acids (C2–C12), which can damage the quality of some products. In addition, organic acids contribute to sensory properties such as color and flavor as components of foods (Jurado-Sánchez, Ballesteros, & Gallego, 2011).

2.4. Vitamins

Honey contains small amounts of vitamins, especially the vitamin B complex, which are from the pollen grains in suspension. Vitamins found in honey include thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8 or H) and folic acid (B9). Vitamin C is also present. Those vitamins present in honey are preserved due to the low pH of honey (Bonté & Desmoulière, 2013).

Vitamin C is found in almost all types of honey and has been evaluated mainly due to its antioxidant effect. The determination of vitamin C is an unstable indicator, because it is very vulnerable to chemical and enzymatic oxidation and has an accelerated rate of change due to various factors such as light, oxygen or heat (León-Ruiz, Vera, González-Porto, & Andrés, 2013).

The first study of vitamins in honey was performed initially by biological assay methods. Only after 1940, chemical methods were introduced for the determination of ascorbic acid (Ciulu et al., 2011). Ciulu et al. (2011) validated a rapid and simple method for High Performance Liquid Chromatography-Reverse Phase (HPLC-RP) to simultaneously determine 5 water-soluble vitamins (Vitamin B2: riboflavin, vitamin B3: nicotinic acid, vitamin B5: pantothenic acid, vitamin B9: folic acid and vitamin C: ascorbic acid). The method was effective in the analysis of 28 honey samples (mainly from Sardinia, Italy), from 12 different botanical origins.

The commercial filtration of honey may cause a reduction in vitamin content due to the almost complete removal of pollen. Another factor that causes loss of vitamins in honey is the oxidation of ascorbic acid by the hydrogen peroxide produced by glucose oxidase (Ciulu et al., 2011).

2.5. Minerals

Various groups of chemical compounds have been detected in different types of honey. These chemical groups include macro and microelements minerals such as potassium, magnesium, calcium, iron, phosphorus, sodium, manganese, iodine, zinc, lithium, cobalt, nickel, cadmium, copper, barium, chromium, selenium, arsenic, and silver that are found in different honeys (Alqarni et al., 2012) comprising those groups that are important for the human diet (Madejczyk & Baralkiewicz, 2008).

The mineral content in honey ranges from 0.04% in light honeys to 0.2% in dark honeys (Alqarni et al., 2012). Honey reflects the chemical components of the plants from which the honeybees collect their food, so the content of trace elements present in honey depends on the type of soil in which the plant and nectar were found (Escuredo et al., 2013; Madejczyk & Baralkiewicz, 2008) and may indicate the botanical origin of a specific honey (Alqarni et al., 2012). Some studies classify honeys botanically based on the estimation of their mineral content. Nalda, Yagu, Calva, and Gómez (2005) identified the minerals of different honeys from Spain and found a higher concentration of manganese in honey from the flowers of heather and ling. Ajtony, Bencs, Haraszi, Szigeti, and Szoboszlai (2007) evaluated the mineral content of honey samples from Hungary and found the lowest concentrations of cadmium, chrome, copper and lead were observed in linden honey, as for other types of honey these elements showed the highest content.

Potassium is the most abundant element, corresponding generally to one third of the total mineral content found in honey (Alqarni et al., 2012; Yücel & Sultanoglu, 2013). In smaller quantities, honey also contains sodium, iron, copper, silicon, manganese, calcium and magnesium. Macro elements (such as potassium, calcium, and sodium) and trace minerals (such as iron, copper, zinc, and manganese) perform a fundamental function in biological systems: maintaining normal physiological responses, inducing the overall metabolism, influencing the circulatory system and reproduction, and as catalysts in various biochemical reactions (Alqarni et al., 2012). Some heavy metals, such as arsenic, lead, mercury and cadmium, are toxic if the maximum limit is exceeded. However, maximum residue levels of these potentially toxic elements in honey have not been established. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have jointly proposed acceptable levels of 15 $\mu g\ kg^{-1}$ for arsenic, 25 $\mu g\ kg^{-1}$ for lead, 5 $\mu g\ kg^{-1}$ for mercury and 7 $\mu g\ kg^{-1}$ for cadmium. The increased concentration of trace elements in honey samples near industrial areas was observed in most cases. Therefore, the quantification of trace toxic mineral elements in honeys becomes important for the effects of human health, safety and

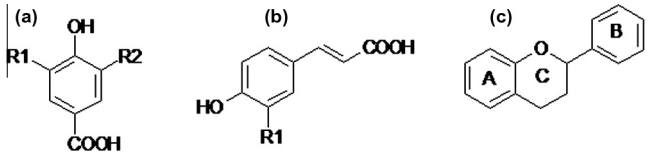

Fig. 1. Chemical structure of phenolic acids and flavonoids. (a) Hydroxybenzoic acid: þ-hydroxybenzoic, R1 = H, R2 = H; gallic acid, R1 = OH, R2 = OH. (b) Hydroxycinnamic acids: þ-coumaric acid, R1 = H; caffeic acid, R1 = OH; ferulic acid R1 = OCH3. (c) General chemical structure of the flavonoids.

environmental biomonitoring (Ajtony et al., 2007; Bilandzic et al., 2011).

Mineral elements, in contrast to vitamins and amino acids, are not subject to degradation by exposure to heat, light, oxidizing agents, extreme pH or other factors that affect organic nutrients. In essence, the minerals are indestructible (Damodaran, Parkin, & Fennema, 2010), a very important fact, because these minerals are components of essential enzymes for a number of metabolic reactions in the human body and also play an important role in body functions, so they must be supplied through diet (Pohl, Stecka, Greda, & Jamroz, 2012).

2.6. Phenolic compounds

Phenolic compounds are a chemically heterogeneous group, with approximately 10,000 compounds, which are grouped into different classes according to their basic chemical structure. They can be divided into non-flavonoids (phenolic acid) and flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidin, isoflavones and chalcones) (Andersen & Markham, 2006). These compounds have an aromatic ring with one or more hydroxyl groups in their structures, which can vary from a simple to a complex molecule phenolic polymer of high molecular weight (Pyrzynska & Biesaga, 2009).

Phenolic acids constitute an important class of phenolic compounds with bioactive functions typically found in vegetable products and foods, and are secondary metabolites required for normal operation of naturally occurring plants. They are compounds that act as antioxidants, eliminating free radicals and inhibiting lipid oxidation. They can be divided into two subgroups according to their structure: the hydroxybenzoic and hydroxycinnamic acids (Challacombe, Abdel-Aal, Seetharamana, & Duizer, 2012).

Hydroxybenzoic acids have a general structure C1–C6 (Fig. 1a), derived from benzoic acid. Variations in this structure can be found in methylation and hydroxylation of the aromatic ring (Tsao, 2010). Acids which are derived from hydroxybenzoic acids include þ-hydroxybenzoic, vanillic, syringic, salicylic (2-hydroxybenzoate), gallic and ellagic. These compounds may be present in their soluble form in cells, combined with sugars or organic acids, or forming together with cells linked with lignins (Rice-Evans & Packer, 2003).

Hydroxycinnamic acids have a general structure of C3–C6 (Fig. 1b) and display differences in the originating ring substituents such as phenolic acids differ from þ-coumaric, caffeic, ferulic and sinapic acids. These compounds occur normally in their conjugated form as esters of hydroxy acids such as tartaric acid and shikimic as well as in pure form (Rice-Evans & Packer, 2003).

Flavonoids have a C6–C3–C6 nuclear structure, involving two benzene rings connected by a pyran ring (Fig. 1c). Substitutions on the rings result in major classes of flavonoids. They represent the largest group of plant phenolic compounds, which represents more than 50% of all natural phenolic compounds. They are widely distributed in the seeds, bark, leaves, and flowers of plants and trees. In plants, these compounds provide protection against ultraviolet radiation, pathogens and herbivores. Plants have several polyphenolic derivatives with high structural diversity and complexity, and when honeybees collect nectar, these bioactive compounds can be transferred from plants to honey (Silici, Sagdic, & Ekici, 2010).

Various authors have studied the profile of phenolic compounds in honey. They have found vanillic acid, caffeic acid, syringic acid, þ-coumaric acid, ferulic acid, quercetin, kaempferol, myricetin, pinobanksin, pinocembrin, chrysin, ellagic acid, galangin, 3-hydroxybenzoic acid, chlorogenic acid, 4-hydroxybenzoic acid, rosmarinic acid, gallic acid, hesperetin, benzoic acid and others (Alvarez-Suarez et al., 2012; Trautvetter, Koelling-Speer, & Speer, 2009).

Phenolic compounds present in honey have been used as floral markers and have also increased the amount of interest directed to the study of antioxidant activity attributed to these compounds due to their ability to scavenge or reduce the formation of free radicals (reactive oxygen species – ROS). Some studies show that flavonoids could protect lipids against oxidation of the cell membrane (Sghaier et al., 2011).

The main functional components of honey are flavonoids. They can significantly contribute to the total antioxidant activity of honey, bringing beneficial effects for human health (Alvarez-Suarez et al., 2012). The antioxidant activity of flavonoids in most cases depends on the number and position of hydroxyl groups and other substituents and the glycosylation of flavonoid molecules. The presence of certain hydroxyl groups in the flavonoid rings enhances antioxidant activity. Substitution patterns in the A ring and B ring, and the 2,3-double bond (unsaturated) and 4-oxo group in the C ring also affect the antioxidant activity of flavonoids. The glycosylation of flavonoids decreases their antioxidant activity when compared to the corresponding aglycones (Sghaier et al., 2011).

Phenolic compounds, as well as other organic compounds as previously discussed, are degraded depending on the environmental conditions to which they are subjected. Escriche, Kadar, Juan-Borrás, and Domenech (2014) evaluated the impact of industrial heat treatment on the phenolic compounds of Spanish honeys, subjected to liquefaction (45 ± 1 °C/48 h) and liquefaction + pasteurization (80 ± 0.05 °C/4 min). Phenolic compounds found in these honeys were caffeic and þ-coumaric acids and flavonoids naringenin, hesperetin, pinocembrin, chrysin, galangin, quercetin and kaempferol. A significant decrease in the concentration of galangin, kaempferol, myricetin and þ-coumaric acid was observed after heat treatment, being more relevant to the myricetin after pasteurization.

Truchado, Ferreres, Bortolotti, Sabatini, and Tomás-Barberán (2008) evaluated the stability of flavonoid glycosides in honey and concluded that the common structural characteristic of flavonoids kaempferol-7-O-robinoside, kaempferol-7-O-rhamnoside and kaempferol-7-O-rhamnosyl (1→2) rhamnosyl (1→2) hexosyl (1→2) rhamnoside is the presence of a free hydroxyl in position 3 (Fig. 1c). This gives certain instability to these compounds under slight alkaline conditions and high sensitivity to oxidation in the presence of slight oxidizing agents such as hydrogen peroxide, which is present in honey, being responsible for the degradation verified in the flavonoids analyzed.

2.7. Volatile compounds

The honey flavor is produced by complex mixtures of volatile compounds, which may differ depending on the nectar, processing conditions, origin and storage. Unifloral honeys have a distinctive flavor of the plant, due to the presence of certain volatile organic compounds from nectars (Castro-Vázquez et al., 2007).

Volatile compounds in honey have their origin in different sources, such as the transference of volatile compounds from the plant, for example. On the other hand, honeybees can also produce or convert plant constituents in other compounds with volatile properties. Moreover, these compounds can be affected by post-harvest processing, from the presence of micro-organisms (Barra et al., 2010; Iglesias et al., 2006).

More than 400 different compounds have been identified in the volatile fraction of the honey, and some are used as markers of commercial honeys, such as 3,9-epoxy-1-þ-mentadieno, t-8-þ-menthan-oxide-1,2-diol and cis-rose, which have been proposed as markers of lemon honey; diketones, sulfur compounds and alkanes are characteristic of eucalyptus honey, while hexanal and hep-

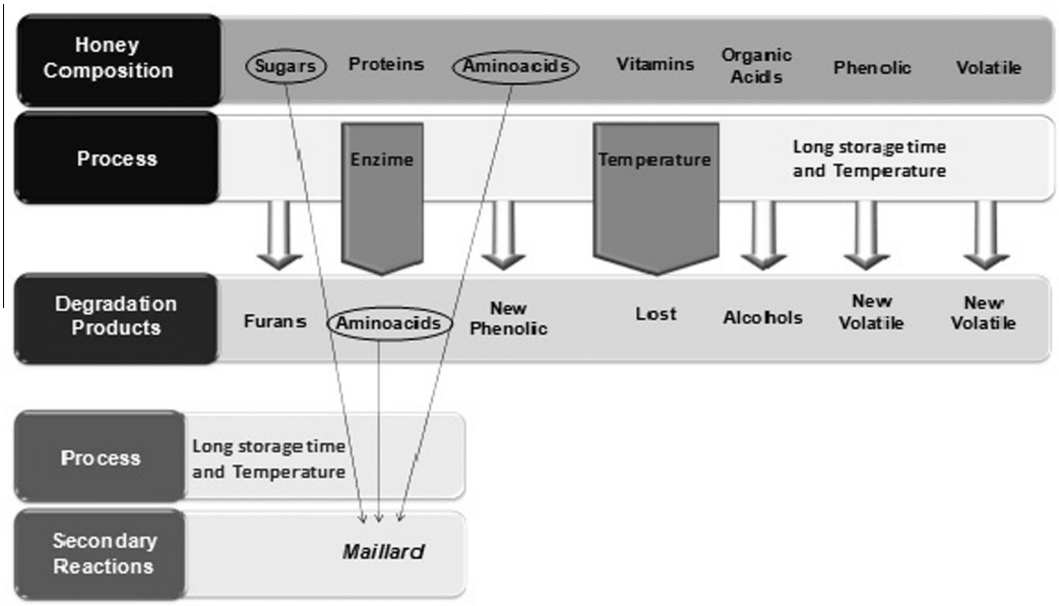

Fig. 2. Compounds present in honey, the processes that influence the stability of honey, degradation products and secondary reactions that may occur.

tanal are the main compounds in the aroma of lavender honeys (Castro-Vázquez et al., 2007; Radovic et al., 2001).

Complex mixtures of the volatile compounds of different chemical families are present in honey at very low concentrations, which generally belong to monoterpenes, C13-norisoprenoid, sesquiterpenes, benzene derivatives and, to a lower content of superior alcohols, esters, fatty acids, ketones, terpenes and aldehydes (Pontes et al., 2007).

The length of the carboxylic acids carbon chains provides different flavors that can range from spicy to rancid. The short chain carboxylic acids, such as acetic acid, have a spicy aroma and flavor, while the butanoic and hexanoic acid present in butter provides a rancid aroma (Barra et al., 2010).

Alcohols are a large and important class of compounds present in honey. Methyl groups with alcohols, such as 3-methyl-3-butene-1-ol and 2-methyl-2-buten-1-ol, provide freshness to honey (Castro-Vázquez et al., 2007). There are also studies that show that the derivatives of linalool originate from flowers visited by honeybees, concluding that these compounds are found only in specific honeys (Moreira et al., 2010).

Radovic et al. (2001) analyzed 43 samples of honey from different countries, among them were Denmark, Germany, Italy, France, Holland, Spain, Portugal and England, and found linear and branched aldehydes, ketones, and short-chain alcohols in almost all of the honeys. The major volatile compounds detected by headspace analysis in such honeys were furfural, benzaldehyde and acetone.

Some studies have reported modification in the volatile compounds profile during the storage of honey, as described in the study by Moreira et al. (2010), where the honey volatiles were evaluated during 6 months of storage. The disappearance of 17-pentatriacontene in cashew honey was observed as early as the third month of monitoring. However, Kaskoniene, Venskutonis, and Ceksteryte (2008) found that the concentration of octane increases with storage time. These studies indicate that the evaluation of the volatile profile of honey, depending on the storage time and/or the industrial process involved, should be performed meticulously, seeking to monitor the formation or loss of the characteristic volatile compounds in the honey evaluated.

As described above, the chemical compounds present in honey may change over time during storage. The knowledge about these changes is very important to understand how honey behaves during the storage period.

Fig. 2 shows the compounds present in honey, the processes that influence the stability of honey, the products' degradation, and the secondary reactions that may occur.

Honey is composed of different chemical compounds, including sugars, proteins, amino acids, vitamins, organic acids, phenolic compounds and volatile compounds (Fig. 2). Through processes such as the specific enzymes present in honey, high temperature and long storage time, honey may change and degrade into new products such as furans, amino acids, alcohols and new phenolic compounds and new volatile compounds. These can also be affected by the production of secondary reactions, such as the *Maillard* reaction, when the compounds of furan derivatives are heated or stored for a long time.

3. Stability of the compounds present in honey

Honey is a food containing several chemical compounds and as such, it is expected that variations in their composition may occur during storage. Some of these changes influence the nutritional and sensory characteristics that may be associated with reactions such as the *Maillard* reaction, catalyzed by heating. Moreover, due to commercial aspects, sometimes honey is stored for almost a year before consumption (Moreira et al., 2007, 2010).

The browning of food under heat or during storage (in foods containing reactive groups such as NH_2 and C=O) is the result of a chemical reaction between a reducing sugar and a primary amino group. The flavors and colors formed may be either desirable or undesirable. They can be formed slowly during storage, or more rapidly at high temperatures (Damodaran et al., 2010; Ordóñez, 2005).

In the *Maillard* reaction, the reducing sugars react reversibly with an amine to form a Schiff base, which may cause a ring to form a glucosamine. The isomerization of aldoses generates aldosilamines while the isomerization of ketoses generates ketosilamines. The Schiff base suffers rearrangement, forming products of Amadori (a key reaction for browning). Amadori compounds are the first intermediate sequence of browning reactions. The aldosilamines have internal restructuring (Amadori rearrangement) and

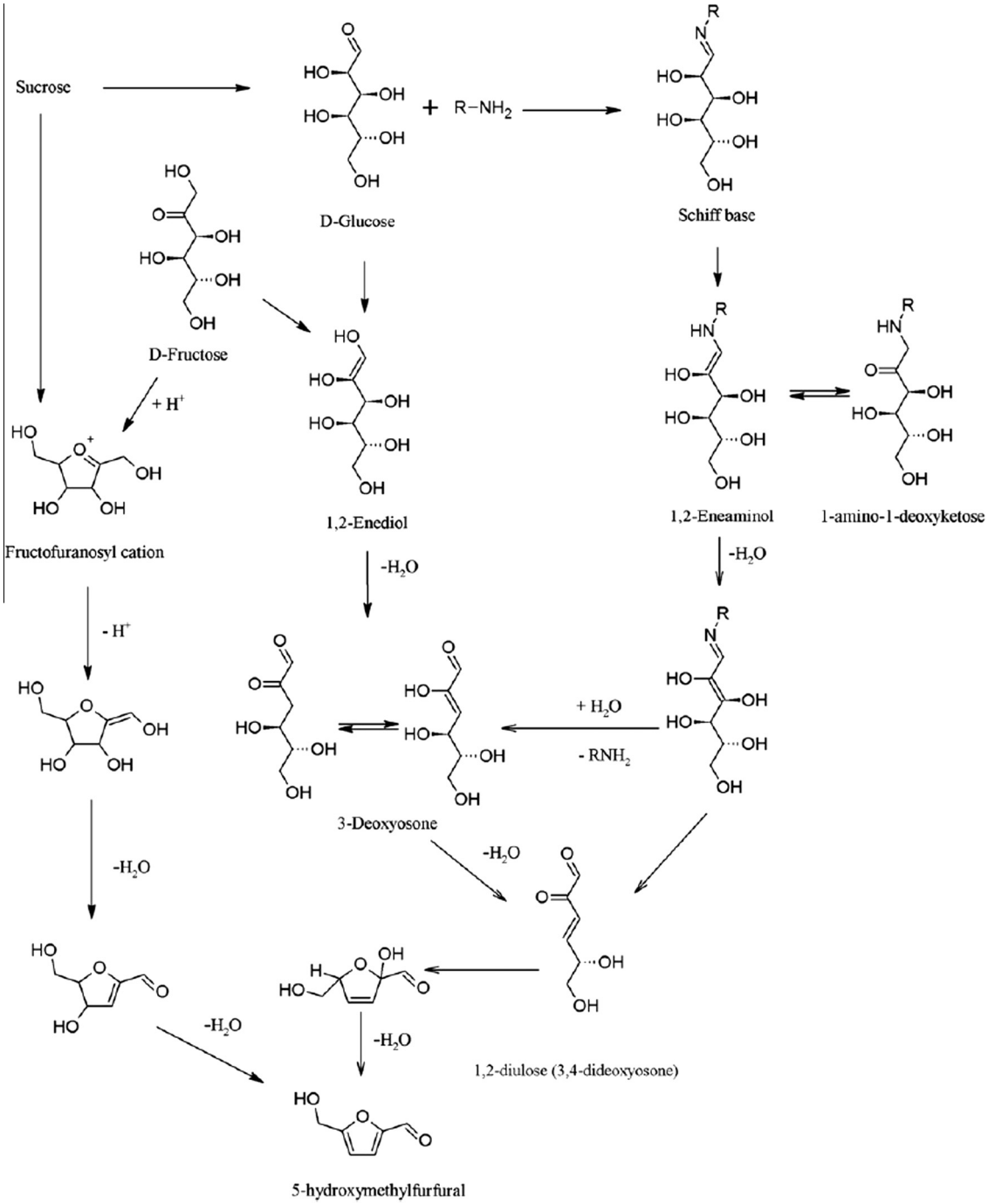

Fig. 3. Proposed scheme for the formation of 5-hydroxymethylfurfural.

are converted into ketosamines, while ketosilamines are transformed into aldosilamines (Heyns restructuring or reverse Amadori) (Damodaran et al., 2010; Oetterer, Regitano-D'arce, & Spoto, 2006; Ordóñez, 2005).

The following reactions give a dehydrated intermediate, especially if the pH $\leqslant$ 5. Occasionally, this forms a furan derivative that originated from a hexose, known as 5-HMF and/or a derivative composed of a pentose, such as furfural (Damodaran et al., 2010; Ordóñez, 2005).

5-HMF is formed as an intermediate in the *Maillard* reaction and in the direct dehydration of sugars under acidic conditions applied during the heat treatment of foods or during prolonged storage (Barra et al., 2010; Castro-Vázquez et al., 2007; Wang et al., 2009). 3-Deoxyosone is known as a key component in the formation of 5-HMF, and derives from 1,2 enolization and dehydration of glucose or fructose. Further dehydration and cyclization of 3-deoxyosone increase the content of 5-HMF. Fig. 3 illustrates an alternative scheme for the formation of 5-HMF in foods, showing the main pathway, under heat and low humidity, and the reaction involving the formation of a highly reactive cation fructofuranosyl, which can be rapidly converted to 5-HMF in low pH (pH < 5), as in the case of honey (Capuano & Fogliano, 2011).

The *Maillard* reaction promotes a reduction in the nutritional value of the food, because the destruction of essential amino acids occurs, such as lysine ε-amino group, which reacts with sugars and other amino acids such as L-arginine and L-histidine (Damodaran

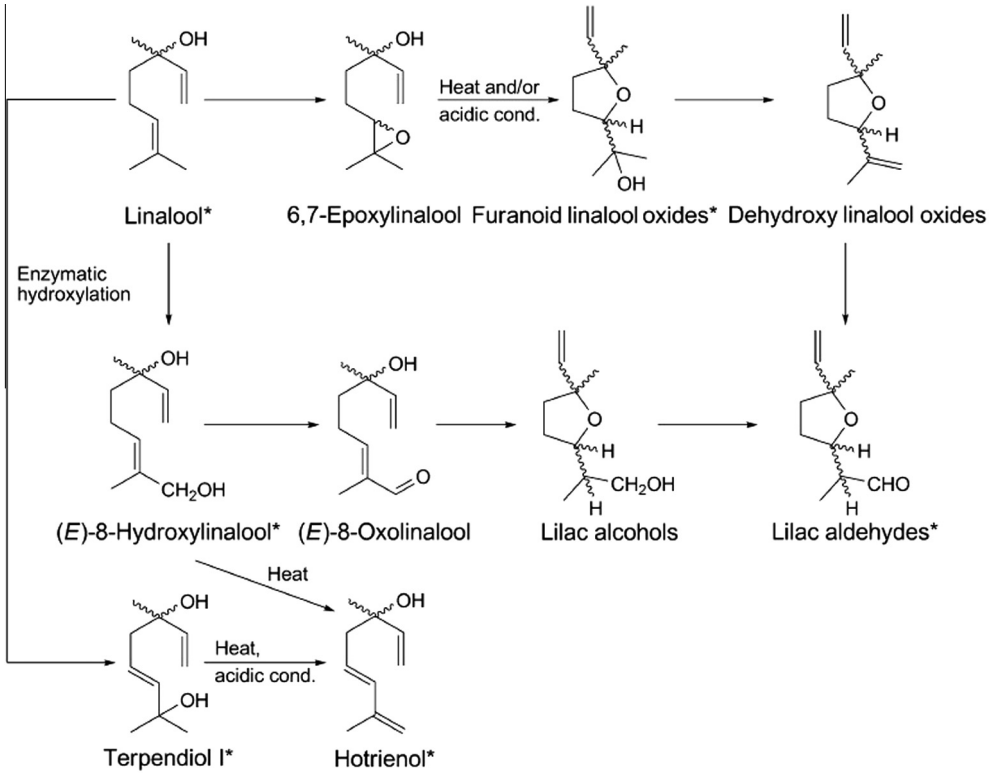

Fig. 4. Relations between terpenes.

et al., 2010; Ordóñez, 2005). As in the *Maillard* reaction, Strecker degradation contributes to the loss of amino acids; however, it does not cause the food to darken (Oetterer et al., 2006).

A dark color in honey may develop during storage and may also be related to storage temperature and the composition of the honey. In some cases, the honey is subjected to heat treatment to inhibit or retard the crystallization process, or to block the development and growth of micro-organisms, such as preventing the contamination of the fresh product and also during filling or extraction, because honey must have an adequate fluidity (Vaikousi, Koutsoumanis, & Biliaderis, 2009).

Hydrocarbons have low reactivity but undergo a concentration decrease, which is most likely due to volatilization. Generally, the concentration of these compounds decreases with increasing molecular weights of the hydrocarbons. The oxidative processes that may occur during storage transform hydrocarbons into smaller molecules such as alcohols, which can be volatilized rapidly. The increased concentration of alcohols during storage may be due to their formation via lipid oxidative degradation or the reduction processes catalyzed by aldehyde reductase from honeybees or contaminating microorganisms (Moreira et al., 2010).

Kaskoniene et al. (2008) detected ethanol in samples of honey from Lithuania. The presence of ethanol may be related to the development of yeasts in carbohydrate foods. The hotrienol (3,7-dimethyl-1,5,7-octatrien-3-ol) can be generated in honeys subjected to heat treatment, while its precursor, (E)-2,6-dimethyl-6-acetoxy-2,7-octadienal (Barra et al., 2010), or other compounds such as terpendiol I, 8-hydroxylinalool and lilac aldehydes can be formed by isomerizing 8-hidroxilinalool lilac alcohol followed by oxidation (Fig. 4) (Kus, Jerkovic, Tuberoso, & Sarolic, 2013).

Kaskoniene et al. (2008) suggest that various aldehydes and ketones can be formed by the oxidation of fatty acids in honey, particularly linoleic and linolenic acids, which may develop a rancid flavor. The hexadecanoic acid may be formed due to oxidation of the aldehyde during storage of hexadecanal (Moreira et al., 2010).

Some of the organic acids, ketones and benzenes such as 2-hydroxy-2-propanone, butanoic acid, benzyl alcohol or 2-phenylethanol found in fresh honey gradually increase in concentration with increasing temperature and storage time (Barra et al., 2010). However, low molecular weight acids such as propanoic acid and 2-methylpropanoic disappeared during 6 months of storage, most likely because they are short carbon chains, facilitating volatilization (Moreira et al., 2010).

Kaskoniene et al. (2008) reported that despite the samples of Lithuanian honey showing the same chemical classes of compounds, qualitative and quantitative changes of these compounds became evident only after 3 months of storage. Volatile compounds in caraway and white clover honeys analyzed by headspace, decreased to 70% after three months of storage, while in some other honeys, the percentage of the loss of volatiles was less expressive. For the monofloral rape honeys analyzed, the content increased from 14.7% to 29.8%. This was perhaps due to the direct chemical changes of the honeys' composition, such as the formation of new compounds and the loss of some volatile components. Physical changes such as consistency, rheological properties and crystallization were also evaluated in this study and can have a remarkable influence on the release of volatile compounds.

Kus et al. (2013) evaluated the profile of volatile compounds of two types of honeys from Poland and Spain. They noted that honey is characterized by a high percentage of compounds derived from shikimic acid, terpenes, and some norisoprenoid and some compounds such as coumaran and methyl-1H-indole-acetate. Fig. 5 illustrates the possible relationships between the compounds derived from shikimic acid. Benzoic acid is possibly formed by cleavage of the side chain of cinnamic acid or by the oxidation of benzaldehyde via reduction of the benzyl alcohol. However, 2-

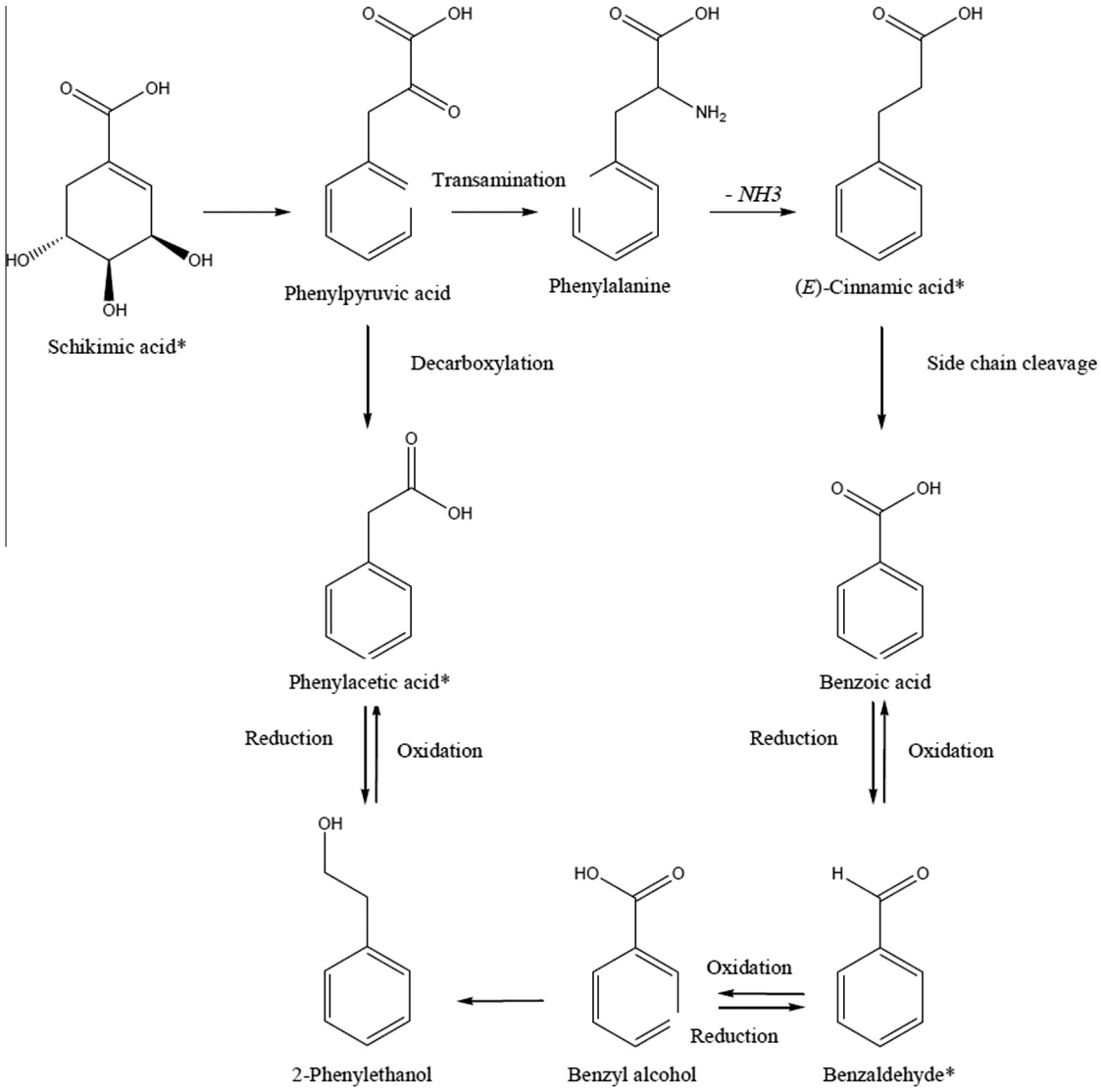

Fig. 5. Compounds derived from shikimic acid.

phenylacetic acid can be formed by decarboxylation of phenylpyruvic acid or by oxidation of 2-phenylethanol.

4. Parameters of identity and quality of honey

The laws regarding honey are developed by considering the requirements to standardize the processing of products, and to ensure equal conditions and full transparency in their development and marketing. The authenticity of honey is defined internationally by the Codex Alimentarius, which establishes the identity and the essential quality requirements of honey intended for direct human consumption. These standards are applied to honey produced by bees and cover all styles of honey presentations, which are processed and ultimately intended for human consumption (Codex Standard for Honey, 2001).

The purpose of these laws is to establish the identity and minimum quality requirements for honey. Among other factors, these regulations take into account the sensory and physicochemical properties of honey by setting the color and the minimum or maximum amount related to maturity, purity and deterioration parameters for honeys. With respect to maturity, the regulation evaluates sugar content and moisture; for purity, it analyses ash content, electrical conductivity and insoluble solids in water; and for maturity, it verifies HMF content, acidity and diastase activity (Codex Standard for Honey, 2001).

4.1. Sugars

As previously discussed, honey is rich in sugars. These are produced by honey-bees from nectar sucrose, which is transformed through the action of enzymes such as α- and β-glucosidase, the α- and β-amylase and β-fructosidase. Monosaccharides are the most common carbohydrates found in honey, ranging from 65% to 80% of total soluble solids. Fructose (about 38.5%) and glucose (about 31.0%) are the compounds present in a higher concentration in honey. The average ratio of fructose and glucose is 1.2:1, but this ratio depends largely on the source of the nectar from which the honey was extracted. This ratio is used to evaluate the crystallization of the honey, due to glucose's lower solubility in water as compared to fructose (Escuredo et al., 2014; Fuente et al., 2011; Tornuk et al., 2013).

According to the standards of the Codex Alimentarius Committee on Sugars (2001), the minimum amount of reducing sugars is 60 g 100 g^{-1} for floral honey (Codex Standard for

Honey, 2001). In general, the sugar composition of honey is affected by the types of flowers used by the bees, as well as regions and climate conditions (Tornuk et al., 2013).

Besides the reducing sugars analysis, the amount of sucrose is a very important parameter in evaluating the honeys' maturity. The sucrose content in honey is analyzed with the purpose of identifying any improper manipulation of honey, and high levels may indicate a variety of adulterations, such as adding cheap sweeteners like cane sugar or refined beet sugar; early harvest, indicating that the sucrose was not completely transformed into glucose and fructose; or prolonged artificial feeding of honeybees with sucrose syrups, resulting in high commercial profits (Escuredo et al., 2013; Puscas et al., 2013; Tornuk et al., 2013). Due to these factors the Codex Alimentarius Committee on Sugars (2001) stipulates a maximum value of 5 g of total sugar in 100 g of floral honey (Codex Standard for Honey, 2001).

Readers are encouraged to take a look at a well written review by Camiña, Pellerano, and Marchevsky (2012), which focuses on the geographical and botanical classification of honeys around the world, but also provides a great overview of different analytical techniques combined with multivariate analysis to verify honeys' authenticity through sugar evaluation. More recently, the adulteration of honeys was monitored by $^{13}C/^{12}C$ analysis using an isotope ratio mass spectrometer in combination with an elemental analyzer (EA-IRMS) (Tosun, 2013). This technique was useful in detecting the adulteration of honeys by the addition of corn-sugar cane syrups, but fails to detect the adulteration of beet sugar syrups.

In order to rapidly and efficiently detect the presence of adulterants, like sugar syrups in honeys, Chen et al. (2014) applied the three-dimensional fluorescence spectra technology, aiming to replace the previous techniques. It was considered time-consuming, expensive and required a high degree of technical knowledge for data interpretation. Multivariate analysis combined with this technique proved to be a potential tool to detect adulterated honey in rice syrup. HPLC can also be used as a fast technique to detect adulteration in honeys as presented by Wang et al. (2015), without the requirement of multivariate analysis. HLPC chromatograms indicated a separate peak at 15.25 min retention time indicative of the presence of syrup in honey samples, with a detectable syrup content near 2.5%.

Besides sugar content determination, water content is also an effective parameter to evaluate the quality of honeys. The authentication of honeys from different countries was evaluated by multivariate analysis in commercial, adulterated and artificial honeys. The sum or ratio of sugars (glucose and fructose) and the glucose: water ratio were found to be more specific and better indicators of honey quality than any other parameter evaluated (Kukurová, Karovicová, Kohajdová, & Biliková, 2008).

4.2. Moisture and water activity (A_w)

Water is the second largest constituent of honey. Its content may vary from 15 to 21 g 100 g^{-1} depending on the botanical origin of the honey, the level of maturity achieved in the hive, processing techniques and storage conditions (Yücel & Sultanoglu, 2013).

The moisture content is one of the most important characteristics, influencing physical properties of honey such as viscosity and crystallization, as well as other parameters: color, flavor, taste, specific gravity, solubility and conservation (Escuredo et al., 2013).

Escuredo et al. (2013) evaluated the water content of 187 honeys harvested in Northwest Spain and the samples' contents ranged from 16.9% to 18.0%, averaging 17.6%. These honeys are in accordance with the Codex Alimentarius Committee on Sugars (2001) stipulating that the moisture content in honey should not exceed 20 g 100 g^{-1} (Codex Standard for Honey, 2001).

Karabagias et al. (2014) found the water content of 39 pine honey samples in Greece, and the samples showed values between 10.50% and 20.50%. The percentage of moisture in honey can also vary in regions with high relative humidity, or depending on the season, as honey is more likely to suffer a fermentation process in the rainy season rather than the dry season. The moisture in honey can also increase during the processing operations of the product, as well as the inadequate storage conditions, because honey is hygroscopic and absorbs moisture from the atmosphere (Karabagias et al., 2014).

The crystallization of glucose in honey leads to the reduction of soluble solids resulting in the dilution of the amorphous solution, which therefore increases the A_w. Honey usually has A_w between 0.50 and 0.65, and A_w values above 0.60 represent a critical threshold for microbial stability. Although there are no limits imposed by the standards, it is known that the value of A_w is very important, because honey contains osmophilic yeasts that can cause fermentation, forming ethyl alcohol and carbon dioxide, thereby changing the quality of the honey (Escuredo et al., 2013; Tornuk et al., 2013; Yücel & Sultanoglu, 2013).

4.3. Free acidity and pH

Although the pH limit has not yet been described by the Regulatory Committees, a pH level between 3.2 and 4.5 and the natural acidity of the honey inhibit the growth of micro-organisms, as the optimum pH for most organisms is between 7.2 and 7.4 (Karabagias et al., 2014; Suárez-Luque, Mato, Huidobro, Simal-Lozano, & Sancho, 2002). On the other hand, the pH determination could also be also correlated with other authenticity parameters to verify adulterations. The addition of high fructose corn syrup in Brazilian honey resulted in a significant increase of pH values compared to pure honey (Ribeiro et al., 2014).

Free acidity is an important parameter related to the deterioration of honey. It is characterized by the presence of organic acids in equilibrium with lactone, internal esters and some inorganic ions such as phosphates, sulfates and chlorides (Moreira et al., 2007). The Codex Alimentarius Committee on Sugars (2001) permits a maximum value of 50.00 meq kg^{-1} for free acidity. Higher values may be indicative of fermentation of sugars into organic acids. However, the presence of different organic acids, geographical origin and harvest season can affect the honeys' acidity (Codex Standard for Honey, 2001; Tornuk et al., 2013). In Portugal, for example, monofloral honeys were evaluated, and depending on the geographical region, as well as the botanical origin, the values obtained were higher than the established limit (Alves, Ramos, Gonçalves, Bernardo, & Mendes, 2013).

4.4. Ash and electrical conductivity

Ash content is a measure of quality that evaluates the mineral content present in honey. The mineral content may be indicative of environmental pollution and geographical origin, because the content depends on the type of soil used for the flowers from which the nectar was collected (Karabagias et al., 2014; Suárez-Luque, Mato, Huidobro, & Simal-Lozano, 2005). It can be also used as a parameter to evaluate the nutritional value of honeys. Usually, the major mineral content contribution is from potassium (its content usually varying between 200 and 900 ppm), followed by other minerals in lower quantities. This affirmation was proved by analyzing honey samples from different regions of Portugal, whose values were corroborated with other literature data (Alves et al., 2013). Potassium content determination can be combined with other techniques already presented to verify the honeys' authenticity. Lime honey adulterated with glucose, for example, showed potassium content below 200 ppm and changes in the electrical

conductivity values, as compared to pure honey (Nikolova, Panchev, Sainov, Gentscheva, & Ivanova, 2012).

Although the Codex Alimentarius Committee on Sugars (2001) does not provide a standard value for this parameter, studies have shown the average ash content in honey is 0.17% (w/w), ranging between 0.02% and1.03% (w/w) (Chakir, Romane, Barbagianni, Bartoli, & Ferrazzi, 2011). Mineral content is related to the color and flavor of honey, with a higher mineral content leading to a darker and stronger flavor (Escuredo et al., 2013; Karabagias et al., 2014), which are attractive features for its consumption. There is usually a positive correlation between the color, mineral content and electrical conductivity of honey (Karabagias et al., 2014).

The electrical conductivity of honey is related to the ash content (mineral content) and acidity, revealing the presence of ions, organic acids and proteins (Yücel & Sultanoglu, 2013); thus, the higher their content, the higher the resulting conductivity. It is an indicator often used in the quality control of honey that can be used to distinguish floral honeys from honeydew honeys (Karabagias et al., 2014). Despite electrical conductivity changes when the amount of the plant pollen decreases, the determination of the geographical origin of honeys could not be based entirely on this measurement in the study performed in honeys from different regions of Lithuania. But the authors showed a strong correlation between pollen content and electrical conductivity of monofloral honeys (Kaskoniene et al., 2010). As this parameter is directly related to the ash content, it was recently included in the Codex Alimentarius standards, replacing the determination of the ash in honey. The standards recommend a maximum value of 800.00 mS cm^{-1} (Codex Standard for Honey, 2001; Kaskoniene et al., 2010).

4.5. Color

Color is the first attractive attribute of honey, and as such is very important for commercialization. It is an important parameter in the quality, acceptance and preference of consumers.

Boussaid et al. (in press) evaluated six samples of honeys from different regions of Tunisia and found lightness values (L^*) from 36.64 to 51.37. The lightness of honey plays an important role due to consumer preferences. The analyzed honeys also contained the colors orange, yellow and green. The rosemary honey showed the green color, which are the negative values of a^* while mint honey had the highest redness followed by eucalyptus honey. The thyme, orange, eucalyptus horehound and mint honeys showed values between 10 and 20 for component b^*.

Honey experts know that its color can vary from light tones to almost black amber tones, with the most common being bright yellow, reddish or greenish. In many countries, the price of honey is related to its color. Lightly colored honeys generally have a higher value, although dark honeys are appreciated in certain regions (Tuberoso et al., 2014), showing that the general acceptance of the honeys' color by consumers can vary widely (Gámbaro, Ares, Giménez, & Pahor, 2007).

Color is one of the parameters that varies most, and is mainly determined by its botanical origin. It also depends on its ash content, the temperature at which the honey remains in the hive and storage time (Gámbaro et al., 2007). The Codex Alimentarius Committee on Sugars (2001) stipulates that the color of honey should be nearly colorless to dark brown.

4.6. 5-Hydroxymethylfurfural (5-HMF)

5-HMF content is presented as indicative of honey deterioration. Tornuk et al. (2013) evaluated twenty samples of Turkish flower honey HPLC/DAD and the content varied between 0 and 4.12 mg kg^{-1}, which can be considered fresh honey. It meets the standards established by the Codex Alimentarius Committee on Sugars (2001) that sets a maximum value of 40.00 mg kg^{-1} for the mixture or processed honey and a maximum value of 80.00 mg kg^{-1} if the honey and blends of these honeys contain a declared origin from regions with a tropical climate.

Rizelio et al. (2012) developed a fast MECK (Micellar electrokinetic chromatography) method for the determination of 5-HMF in honey samples from Brazil. Among the samples analyzed, two had values above the stipulations by the Codex Alimentarius Committee on Sugars (2001), 82.80 mg kg^{-1} and 127.25 mg kg^{-1}.

Normally 5-HMF is formed by the decomposition of monosaccharides or the *Maillard* reaction, when honey is heated or stored for a long time. As the heat treatment temperature and the storage time increase, the concentration of 5-HMF increases significantly. However, 5-HMF alone cannot be used to determine the severity of the heat treatment, because other factors can influence the levels of 5-HMF, such as the sugar profile, presence of organic acids, pH, moisture content, A_w and floral source. Therefore, the 5-HMF content gives only an indication of overheating or inadequate storage conditions. In addition, 5-HMF can also be formed at low temperatures, even under acidic conditions, for subsequent dehydration reactions of sugars (Barra et al., 2010; Castro-Vázquez et al., 2007; Tornuk et al., 2013; Wang et al., 2009).

High 5-HMF content in honeys may also be an indication of falsification by adding invert syrup, because 5-HMF can be produced by heating sugars in the presence of an acid to the inversion of sucrose (Capuano & Fogliano, 2011; Yücel & Sultanoglu, 2013).

4.7. Diastase activity

Diastases (α-and β-amylases) are enzymes naturally present in honey. Diastase content depends on the floral and geographical origins of the honey. Its function is to digest the starch molecule in a mixture of maltose (disaccharide) and maltotriose (trisaccharide). They are sensitive to heat (thermolabile) and consequently are able to indicate overheating of the product and the degree of preservation (Ahmed et al., 2013). Similar to 5-HMF, the diastatic activity can be used as an indicator of aging and increase temperature because the diastatic activity may be reduced during storage or when the product is subjected to heating above 60 °C (Yücel & Sultanoglu, 2013).

The diastatic activity corresponds to the activity of the enzyme present in 1 g of honey, which can hydrolyze 0.01 g of starch in 1 h at 40 °C, expressed as the diastase number in Göthe units (Ahmed et al., 2013). The current law stipulates a minimum value of 8.00 Göthe units. However, honeys with naturally lower diastase activity tolerate a minimum of 3 Göthe units if honeys have up to 15 mg kg^{-1} of 5-HMF (Codex Standard for Honey, 2001). The content of diastase activity in honey may vary. The differences in enzyme content present in honeys may vary depending on the age of the bees, the nectar collection period, the physiological period of the colony, the large quantity of nectar flow and its sugar content because a high flow of concentrated nectar leads to a lower enzyme content and pollen consumption (Oddo, Piazza, & Pulcini, 1999).

Honeys with a lower enzyme content are produced from young nectars in early spring. They have a low enzyme concentration caused by a low concentration of nectar and higher sugar content with reduced activity of the bees during their growth (Vorlova & Pridal, 2002). Anton and Denisow (2014) evaluated the nectar crossing the floral sexual phases. The authors noted that the complete absence of glucose *Aconitum lycoctonum* nectar can be the result of the differences in enzyme activity, including nectar invertase, which probably differs from *Aconitum carmichaelii*. According to the author, metabolic and/or enzymatic pathways can vary even between phylogenetically related genera and such seemingly larger differences than previously thought.

Table 1
Compilation of articles on honeybees *Apis mellifera* L. from different continents regarding the identity and quality parameters.

Continents/countries	Sugars (g 100 g^{-1})			Moisture (g 100 g^{-1})	Free acidity (meq kg^{-1})	Electrical conductivity (μS cm^{-1})	Color (Pfund scale)	5-Hydroxymethylfurfural (5-HMF) (mg kg^{-1})	Diastase activity (Göthe units)	References
	Fructose	Glucose	Sucrose							
European/Spain	37.75–41.40	28.80–37.30	0.15–1.43	15.40–17.38	20.10–35.20	0.24–0.99	Light amber–dark amber	5.36–15.00	11.50–45.80	Manzanares et al. (2014)
European/Spain, Romania, and Czech Republic	39.30–49.20	26.80–38.30	0.60–2.20	15.30–17.50	–	0.17–0.80	Water white–light amber	3.30–23.40	8.70–19.10	Juan-Borrás, Domenech, Hellebrandova, and Escriche (2014)
European/Greece	–	–	–	10.47–20.47	22.31–41.54	0.81–1.75	*L*: 60.78–72.74 (dark honeys); *a*: −5.05 to −1.95; *b*: 13.35–30.30	–	–	Karabagias et al. (2014)
African/Morocco	39.44–42.42	29.25–33.08	0.47–1.86	14.64–18.59	10.69–30.74	0.31–1.12	White–dark amber	7.16–30.43	6.05–19.10	Chakir et al. (2011)
African/Tunisia	35.78–37.84	31.07–36.58	n. d. –4.60	17.27–19.80	7.11–27.20	0.39–0.89	*L*: 36.64–51.37 (dark honeys); *a*: −0.67 to 4.41; *b*: 6.06–17.67	12.07–27.43	–	Boussaid et al. (in press)
Asian–European/ Turkey	29.80–44.49	25.93–35.98	2.85–8.44	7.99–17.40	3.86–30.42	–	*L*: 8.88–18.54 (dark honeys); *a*: 2.64–8.04; *b*: 11.50–23.56	0.00–4.12	–	Tornuk et al. (2013)
Asian/Indian	43.30–65.50		0.40–8.80	17.20–21.60	–	0.33–0.94	*L*: 26.3–36.8 (dark honeys); *a*: 0.12–4.9; *b*: 0.7–14.4	–	–	Saxena, Gautam, and Sharma (2010)
American/Argentina	67.70–73.5		0.40–5.6	14.10–18.80	9.00–36.8	0.12–0.68	Extra white–dark amber	4.00–26.30	–	Isla et al. (2011)
American/Uruguay	–		–	16.60–18.61	–	0.41–0.99	Extra light amber–amber	5.25–13.40	–	Corbella and Cozzolino (2006)
American/Brazil	33.30–38.60	21.00–26.35	0.12–0.50	17.10–20.50	23.60–45.50	–	–	2.80–7.40	10.55–12.40	Moreira, Maria, Pietroluongo, and Trugo (2007)

Another honey with low diastase activity occurs when honeybees are fed artificially. Guler et al. (2014) conducted a study in which the bees were fed with a commercial glucose. The authors found that bees may not be fed glucose in excessive amounts, as this may have promoted an enzyme deficiency (especially diastase) which are used to convert glucose and fructose.

As these honeys contain low diastase activity, it is essential that they contain a maximum of 15 mg kg^{-1} of 5-HMF, because as diastase activity is low, it is necessary to prove that honey has not undergone heat treatment or prolonged storage.

4.8. Physical–chemical characteristics of honeys A. mellifera L. from different continents

The identity and quality parameters of honey are analyzed by several authors in honey samples from different continents worldwide. The quality and authenticity of food perform an important role for both consumers and producers around the world. For honey, authenticity is related to the determinations of geographical and floral origins and the detection of unauthorized substances.

Table 1 shows a compilation of articles on the physico-chemical characteristics of honeys from *Apis mellifera* L. from different continents. As seen in this table, the honeys from different continents presented similar values and they are in accordance with the law for reducing sugars. European, Asian and American honeys presented values above those permitted by the Codex Alimentarius Committee on Sugars (2001) for moisture content, but Asian honeys also had the lowest moisture content, with a value of 7.99 g 100 g^{-1}. Regarding free acidity, the honey of all continents had values below those allowed by the Codex Alimentarius Committee on Sugars (2001). The African and Asian continents had the lowest free acidity values compared to European and American continents. All continents described in Table 1 presented values above those permitted by the Codex Alimentarius Committee on Sugars (2001) for electrical conductivity. The European, American and Asian continents presented darker honeys, however, the American continent also presented the lighter honeys. Regarding 5-HMF, the African and American continents showed the highest values, but even so, every continent showed 5-HMF values below the level stipulated by law. Concerning diastase activity, although few articles reported their values, the European continent had the highest values in the presence of this enzyme and the African continent presented honeys with values below those stipulated by law.

5. Conclusions

Currently, most studies that provide information on *Apis mellifera* L. honeybees are directly related to the identity and quality parameters, there are not many studies that analyze the stability of chemical compounds present in the honey during storage. Therefore, further research is needed to inform a control system that evaluates maintenance of the characteristics and levels of these compounds provided by honey to human nutrition and health. The approach for chemical markers that can help in the maintenance of honey can facilitate the control of possible degradation. The degradation process can occur due to prolonged storage conditions and can provide a robust data concerning shelf life, thereby increasing the levels of security in quality, generating reliability for consumers, and ensuring honey consumption devoid of toxic compounds for human health.

Acknowledgments

The authors wish to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Santa Catarina (FAPESC) for fellowships and financial support. The authors would also like to thank Prof. Dr. Graciele da Silva Campelo Borges and Prof. Dr. Marcel Piovezan by the substantial contributions for this manuscript.

References

Ahmed, M., Djebli, N., Aissat, S., Khiati, B., Meslem, A., & Bacha, S. (2013). In vitro activity of natural honey alone and in combination with curcuma starch against *Rhodotorula mucilaginosa* in correlation with bioactive compounds and diastase activity. *Asian Pacific Journal of Tropical Biomedicine, 3*, 816–821.

Ajtony, Z., Bencs, L., Haraszi, R., Szigeti, J., & Szoboszlai, N. (2007). Study on the simultaneous determination of some essential and toxic trace elements in honey by multi-element graphite furnace atomic absorption spectrometry. *Talanta, 71*, 683–690.

Alqarni, A. S., Owayss, A. A., & Mahmoud, A. A. (2012). Mineral content and physical properties of local and imported honeys in Saudi Arabia. *Journal of Saudi Chemical Society, 5*, 618–625.

Alvarez-Suarez, J. M., Giampieri, F., González-Paramás, A. M., Damiani, E., Astolfi, P., Martinez-Sanchez, G., et al. (2012). Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. *Food and Chemical Toxicology, 50*, 1508–1516.

Alves, A., Ramos, A., Gonçalves, M. M., Bernardo, M., & Mendes, B. (2013). Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. *Journal of Food Composition and Analysis, 30*, 130–138.

Andersen, Ø. M., & Markham, K. R. (2006). Flavonoids chemistry, biochemistry and applications. In *Separation and quantification of flavonoids*. New York: Taylor & Francis.

Anton, S., & Denisow, B. (2014). Nectar production and carbohydrate composition across floral sexual phases: Contrasting patterns in two protandrous *Aconitum* species (Delphinieae, Ranunculaceae). *Flora, 209*, 464–470.

Barra, M. P. G., Ponce-Díaz, M. C., & Venegas-Gallegos, C. (2010). Volatile compounds in honey produced in the central valley of Ñuble Province, Chile. *Chilean Journal of Agricultural Research, 70*, 75–84.

Bilandzic, N., Dokic, M., Sedak, M., Kolanovic, B. S., Varenina, I., Koncurat, A., et al. (2011). Determination of trace elements in Croatian floral honey originating from different regions. *Food Chemistry, 128*, 1160–1164.

Bonté, F., & Desmoulière, A. (2013). Le miel: Origine et composition. *Actualités Pharmaceutiques, 531*, 18–21.

Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsıa, F., Ferrari, G., et al. (in press). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry*.

Brudzynski, K., Sjaarda, C., & Maldonado-Alvarez, L. (2013). A new look on protein–polyphenol complexation during honey storage: Is this a random or organized event with the help of dirigent-like proteins? *PLoS One, 8*, 1–9.

Camiña, J. M., Pellerano, R. G., & Marchevsky, E. J. (2012). Geographical and botanical classification of honeys and apicultural products by chemometric methods. A review. *Current Analytical Chemistry, 8*, 408–425.

Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *Food Science and Technology, 44*, 793–810.

Castro-Vázquez, L., Díaz-Maroto, M. C., & Pérez-Coello, M. S. (2007). Aroma composition and new chemical markers of Spanish citrus honeys. *Food Chemistry, 103*, 601–606.

Cavia, M. M., Fernández-Muino, M. A., Alonso-Torre, S. R., Huidobro, J. F., & Sancho, M. T. (2007). Evolution of acidity of honeys from continental climates: Influence of induced granulation. *Food Chemistry, 100*, 1728–1733.

Chakir, A., Romane, A., Barbagianni, N., Bartoli, D., & Ferrazzi, P. (2011). Major and trace elements in different types of Moroccan honeys. *Australian Journal of Basic and Applied Sciences, 5*, 223–231.

Challacombe, C. A., Abdel-Aal, E. M., Seetharamana, K., & Duizer, L. M. (2012). Influence of phenolic acid content on sensory perception of bread and crackers made from red or white wheat. *Journal of Cereal Science, 56*, 181–188.

Chen, Q., Qi, Shuai., Li, H., Han, X., Ouyang, Q., & Zhao, J. (2014). Determination of rice syrup adulterant concentration in honey using three-dimensional fluorescence spectra and multivariate calibrations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 131*, 177–182.

Cherchi, A., Spanedda, L., Tuberoso, C., & Cabra, P. (1994). Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. *Journal of Chromatography A, 669*, 59–64.

Chernetsova, E. S., & Morlock, G. E. (2012). Assessing the capabilities of direct analysis in real time mass spectrometry for 5-hydroxymethylfurfural quantitation in honey. *International Journal of Mass Spectrometry, 314*, 22–32.

Ciulu, M., Solinas, S., Floris, I., Panzanellia, A., Pilo, M. I., Piu, P. C., et al. (2011). RP-HPLC determination of water-soluble vitamins in honey. *Talanta, 83*, 924–929.

Codex Alimentarius Committee on Sugars (2001). Codex standard 12, revised Codex Standard for Honey. *Standards and Standard Methods, 11*, 1–7.

Corbella, E., & Cozzolino, D. (2006). Classification of the floral origin of Uruguayan honeys by chemical and physical characteristics combined with chemometrics. *Food Science and Technology, 39*, 534–539.

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2010). *Química de alimentos de Fennema* (4th ed.). Artmed.

Escriche, I., Kadar, M., Juan-Borrás, M., & Domenech, E. (2014). Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry, 142*, 135–143.

Escuredo, O., Dobre, I., Fernández-González, M., & Seijo, M. C. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry, 149*, 84–90.

Escuredo, O., Míguez, M., Fernández-González, M., & Seijo, M. C. (2013). Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry, 138*, 851–856.

Fuente, E., Ruiz-Matute, A. I., Valencia-Barrera, R. M., Sanz, J., & Castro, I. M. (2011). Carbohydrate composition of Spanish unifloral honeys. *Food Chemistry, 129*, 1483–1489.

Gámbaro, A., Ares, G., Giménez, A., & Pahor (2007). Preference mapping of color of Uruguayan honeys. *Journal of Sensory Studies, 22*, 507–519.

Girolamo, F. D., D'amato, A., & Righetti, P. G. (2012). Assessment of the floral origin of honey via proteomic tools. *Journal of Proteomics, 75*, 3688–3693.

Guler, A., Kocaokutgen, H., Garipoglu, A. V., Onder, H., Ekinci, D., & Biyik, S. (2014). Detection of adulterated honey produced by honeybee (*Apis mellifera* L.) colonies fed with different levels of commercial industrial sugar (C_3 and C_4 plants) syrups by the carbon isotope ratio analysis. *Food Chemistry, 155*, 155–160.

Hermosín, I., Chicón, R. M., & Cabezudo, M. D. (2003). Free amino acid composition and botanical origin of honey. *Food Chemistry, 83*, 263–268.

Iglesias, M. T., Martian-Alvarez, P. J., Polo, M. C., Lorenzo, C., Gonzalez, M., & Pueyo, E. N. (2006). Changes in the free amino acid contents of honeys during storage at ambient temperature. *Journal Agricultural and Food Chemistry, 54*, 9099–9104.

Isla, M. I., Craig, A., Ordoñez, R., Zampini, C., Sayago, J., Bedascarrasbure, E., et al. (2011). Physico chemical and bioactive properties of honeys from Northwestern Argentina. *Food Science and Technology, 44*, 1922–1930.

Jelen, H. (2012). *Food flavours: Chemical, sensory and technological properties*. Taylor & Francis Group. <http://books.google.com.br/books?id=LPKQuJLzn5MC&pg=PA99&lpg=PA99&dq=1,2+eneaminol&source=bl&ots=XvEJnzf_zF&sig=1baQMgbjZ5x_mnfsx_YvmDV_ehQ&hl=ptBR&sa=X&ei=8gelUsTSD47qkAfqj4D4DQ&ved=0CDQQ6AEwAQ#v=onepage&q=1%2C2%20eneaminol&f=true> Accessed on 10.09.14.

Juan-Borrás, M., Domenech, E., Hellebrandova, M., & Escriche, I. (2014). Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and *Tilia* honeys. *Food Research International, 60*, 86–94.

Jurado-Sánchez, B., Ballesteros, E., & Gallego, M. (2011). Gas chromatographic determination of 29 organic acids in foodstuffs after continuous solid-phase extraction. *Talanta, 84*, 924–930.

Kamal, M. A., & Klein, P. (2011). Determination of sugars in honey by liquid chromatography. *Saudi Journal of Biological Sciences, 18*, 17–21.

Karabagias, I. K., Badeka, A., Kontakos, S., Karabournioti, S., & Kontominas, M. G. (2014). Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. *Food Chemistry, 146*, 548–557.

Kaskoniene, V., Venskutonis, P. R., & Ceksteryte, V. (2008). Composition of volatile compounds of honey of various floral origin and beebread collected in Lithuania. *Food Chemistry, 111*, 988–997.

Kaskoniene, V., Venskutonis, P. R., & Ceksteryte, V. (2010). Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania. *Food Science and Technology, 43*, 801–807.

Keckes, J., Trifkovic, J., Andric, F., Jovetic, M., Tesic, Z., & Milojkovic-Opsenica, D. (2013). Amino acids profile of Serbian unifloral honeys. *Journal of the Science of Food and Agriculture, 93*, 3368–3376.

Kukurová, K., Karovicová, J., Kohajdová, Z., & Biliková, K. (2008). Authentication of honey by multivariate analysis of its physico-chemical parameters. *Journal of Food and Nutrition Research, 47*, 170–180.

Kus, P. M., Jerkovic, I., Tuberoso, C. I. G., & Sarolic, M. (2013). The volatile profiles of a rare apple (*Malus domestica* Borkh.) honey: Shikimic acid-pathway derivatives, terpenes, and others. *Chemistry & Biodiversity, 10*, 1638–1652.

León-Ruiz, V., Vera, S., González-Porto, A. V., & Andrés, M. P. S. (2013). Analysis of water-soluble vitamins in honey by isocratic RP-HPLC. *Food Analytical Methods, 6*, 488–496.

Madejczyk, M., & Baralkiewicz, D. (2008). Characterization of Polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. *Analytica Chimica Acta, 617*, 11–17.

Manzanares, A. B., García, H., Galdón, B. R., Rodríguez, E. R., & Romero, C. D. (2014). Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. *Food Science and Technology, 55*, 572–578.

Mato, I. S., Huidobro, J. F., Simal-Lozano, J. S., & Sancho, M. T. (2006). Rapid determination of nonaromatic organic acids in honey by capillary zone electrophoresis with direct ultraviolet detection. *Journal of Agricultural and Food Chemistry, 54*, 1541–1550.

Moreira, R. F. A., Maria, C. A. B., Pietroluongo, M., & Trugo, L. C. (2007). Chemical changes in the non-volatile fraction of Brazilian honeys during storage under tropical conditions. *Food Chemistry, 104*, 1236–1241.

Moreira, R. F. A., Maria, C. A. B., Pietroluongo, M., & Trugo, L. C. (2010). Chemical changes in the volatile fractions of Brazilian honeys during storage under tropical conditions. *Food Chemistry, 121*, 697–704.

Nalda, M. J. N., Yagu, J. L. B., Calva, J. C. D., & Gómez, M. T. M. (2005). Classifying honeys from the Soria Province of Spain via multivariate analysis. *Analytical and Bioanalytical Chemistry, 382*, 311–319.

Nikolova, K., Panchev, I., Sainov, S., Gentscheva, G., & Ivanova, E. (2012). Selected physical properties of lime bee honey in order to discriminate between pure honey and honey adulterated with glucose. *International Journal of Food Properties, 15*, 1358–1368.

Nozal, M. J., Bernal, J. L., Gómez, L. A., Higes, M., & Meana, A. (2003). Determination of oxalic acid and other organic acids in honey and in some anatomic structures of bees. *Apidologie, 34*, 181–188.

Oddo, L. P., Piazza, M. G., & Pulcini, P. (1999). Invertase activity in honey. *Apidologie, 30*, 57–65.

Oetterer, M., Regitano-D'arce, M. A. B., & Spoto, M. H. F. (2006). *Fundamentos de ciência e tecnologia de alimentos* (1st ed.). Barueri/SP: Manole.

Ordóñez, J. A. (2005). *Tecnologia de alimentos – Componentes de alimentos e processos* (Vol. 1). Porto Alegre: Artmed.

Ota, M., Kohmura, M., & Kawaguchi, H. (2006). Characterization of a new Maillard type reaction product generated by heating 1-deoxymaltulosyl-glycine in the presence of cysteine. *Journal of Agricultural of Food Chemistry, 54*, 5127–5131.

Pohl, P., Stecka, H., Greda, K., & Jamroz, P. (2012). Bioaccessibility of Ca, Cu, Fe, Mg, Mn and Zn from commercial bee honeys. *Food Chemistry, 134*, 392–396.

Pontes, M., Marques, J. C., & Câmara, J. S. (2007). Screening of volatile composition from Portuguese multifloral honeys using headspace solid-phase microextraction-gas chromatography–quadrupole mass spectrometry. *Talanta, 74*, 91–103.

Puscas, A., Hosu, A., & Cimpoiu, C. (2013). Application of a newly developed and validated high-performance thin-layer chromatographic method to control honey adulteration. *Journal of Chromatography A, 1272*, 132–135.

Pyrzynska, K., & Biesaga, M. (2009). Analysis of phenolic acids and flavonoids in honey. *Trends in Analytical Chemistry, 28*, 893–902.

Radovic, B. S., Careri, M., Mangia, A., Musci, M., Gerboles, M., & Anklam, E. (2001). Contribution of dynamic headspace GC–MS analysis of aroma compounds to authenticity testing of honey. *Food Chemistry, 72*, 511–520.

Rebane, R., & Herodes, K. (2010). A sensitive method for free amino acids analysis by liquid chromatography with ultraviolet and mass spectrometric detection using precolumn derivatization with diethyl ethoxymethylenemalonate: Application to the honey analysis. *Analytica Chimica Acta, 672*, 79–84.

Ribeiro, R. O. R., Márcico, E. T., Carneiro, C. S., Monteiro, M. L. G., Conte Júnior, C., & Oliveira de Jesus, E. F. (2014). Detection of honey adulteration of high fructose corn syrup by Low Field Nuclear Magnetic Resonance (LH ^{1}H NMR). *Journal of Food Engineering, 135*, 39–43.

Rice-Evans, C. A., & Packer, L. (2003). Flavonoids in health and disease. In A. AnnieFleuriet & J.-J. Macheix (Eds.), *Phenolic acids in fruits and vegetables*. New York: Marcel Dekker.

Rizelio, V. M., Gonzaga, L. V., Borges, G. S. C., Micke, G. A., Fett, R., & Costa, A. C. O. (2012). Development of a fast MECK method for determination of 5-HMF in honey samples. *Food Chemistry, 133*, 1640–1645.

Rybak-Chmielewska, H. (2007). Changes in the carbohydrate composition of honey undergoing during storage. *Journal of Apicultural Science, 51*, 39–48.

Sak-Bosnar, M., & Sakac, N. (2012). Direct potentiometric determination of diastase activity in honey. *Food Chemistry, 135*, 827–831.

Saxena, S., Gautam, S., & Sharma, A. (2010). Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry, 118*, 391–397.

Sghaier, M. B., Skandrani, I., Nasr, N., Franca, M. D., Chekir-Ghedira, L., & Ghedira, K. (2011). Flavonoids and sesquiterpenes from *Tecurium ramosissimum* promote antiproliferation of human cancer cells and enhance antioxidant activity: A structure–activity relationship study. *Environmental Toxicology and Pharmacology, 32*, 336–348.

Silici, S., Sagdic, O., & Ekici, L. (2010). Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chemistry, 121*, 238–243.

Soldatkin, O. O., Peshkova, V. M., Saiapina, O. Y., Kucherenko, I. S., Dudchenko, O. Y., Melnyk, V. G., et al. (2013). Development of conductometric biosensor array for simultaneous determination of maltose, lactose, sucrose and glucose. *Talanta, 115*, 200–207.

Suárez-Luque, S., Mato, I., Huidobro, J. F., & Simal-Lozano, J. (2005). Capillary zone electrophoresis method for the simultaneous determination of cations in honey. *Journal of Chromatography A, 1083*, 193–198.

Suárez-Luque, S., Mato, I., Huidobro, J. F., Simal-Lozano, J., & Sancho, M. T. (2002). Rapid determination of minority organic acids in honey by high-performance liquid chromatography. *Journal of Chromatography A, 955*, 207–214.

Tornuk, F., Karaman, S., Ozturk, I., Toker, O. S., Tastemur, B., Sagdic, O., et al. (2013). Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products, 46*, 124–131.

Tosun, M. (2013). Detection of adulteration in honey samples added various sugar syrups with $^{13}C/^{12}C$ isotope ratio analysis method. *Food Chemistry, 138*, 1629–1632.

Trautvetter, S., Koelling-Speer, I., & Speer, K. (2009). Confirmation of phenolic acids and flavonoids in honeys by UPLC–MS. *Apidologie, 40*, 140–150.

Truchado, P., Ferreres, F., Bortolotti, L., Sabatini, A. G., & Tomás-Barberán, F. A. (2008). Nectar flavonol rhamnosides are floral markers of acacia (*Robinia pseudoacacia*) honey. *Journal of Agricultural and Food Chemistry, 56*, 8815–8824.

Truzzi, C., Annibaldi, A., Illuminati, S., Finale, C., & Scarponi, Giuseppe. (2014). Determination of proline in honey: Comparison between official methods, optimization and validation of the analytical methodology. *Food Chemistry, 150*, 477–481.

Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. Review. *Nutrients, 2*, 1231–1246.

Tuberoso, C. I. G., Jerkovic, I., Sarais, G., Congiu, F., Marijanovic, Z., & Kus, P. M. (2014). Color evaluation of seventeen European unifloral honey types by means of spectrophotometrically determined CIE $L^*C^*_{ab}H^\circ_{ab}$ chromaticity coordinates. *Food Chemistry, 145*, 284–291.

Vaikousi, H., Koutsoumanis, K., & Biliaderis, C. G. (2009). Kinetic modelling of non-enzymatic browning in honey and diluted honey systems subjected to isothermal and dynamic heating protocols. *Journal of Food Engineering, 95*, 541–550.

Vorlova, L., & Pridal, A. (2002). Invertase and diastase activity in honeys of Czech provenience. *Acta Univarsitatis Agriculturae et Silviculturae Mendelianae Brunensis, 5*, 57–66.

Wang, S., Guo, Q., Wang, L., Lin, L., Shi, H., Cao, H., & Cao, B. (2015). Detection of honey adulteration with starch syrup by high performance liquid chromatography. *Food Chemistry, 172*, 669–674.

Wang, Y., Juliani, R., Simon, J. E., & Ho, C. (2009). Amino acid-dependent formation pathways of 2-acetylfuran and 2,5-dimethyl-4-hydroxy-3[2H]-furanone in the *Maillard* reaction. *Food Chemistry, 115*, 233–237.

Won, S., Lee, D., Ko, S. H., Kim, J., & Rhee, H. (2008). Honey major protein characterization and its application to adulteration detection. *Food Research International, 41*, 952–956.

Won, S. A., Li, C., Kim, J., & Rhee, H. (2009). Immunological characterization of honey major protein and its application. *Food Chemistry, 113*, 1334–1338.

Yücel, Y., & Sultanoglu, P. (2013). Characterization of honeys from Hatay region by their physicochemical properties combined with chemometrics. *Food Bioscience, 1*, 16–25.