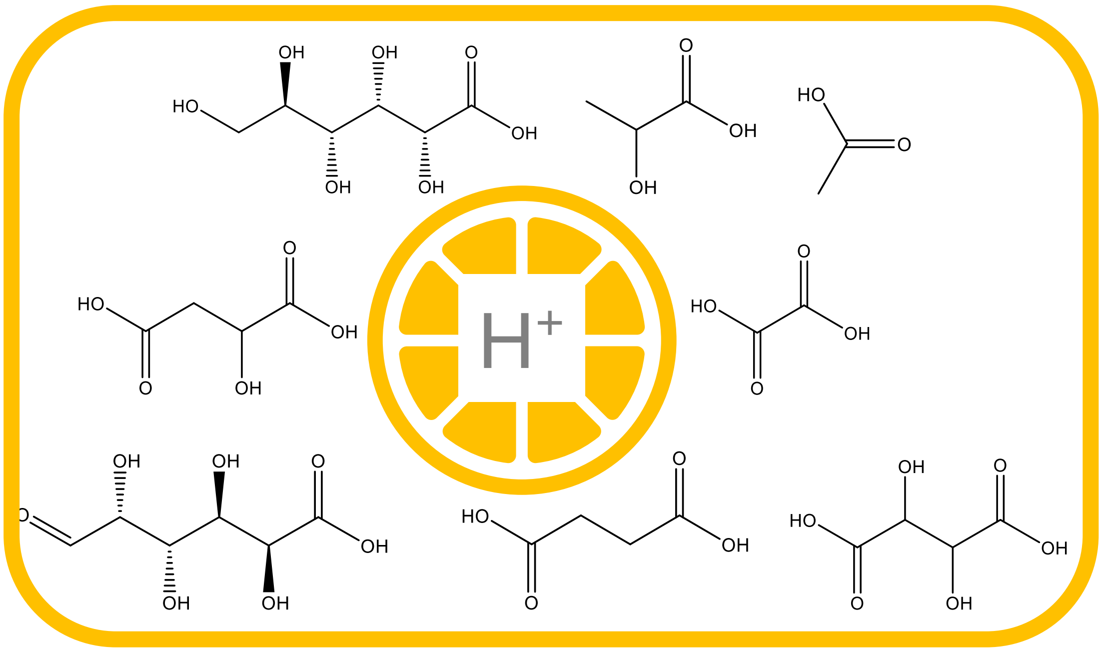
The Review on Aliphatic Organic Acids (AOA) of Honey and Pot-honey for Bee Science



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Mérida, Venezuela

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To honey consumers
delighted by the sour-sweet
honey flavor

To bee scientists intrigued
to decipher the AOA origin in honey,
their transformations
and functions in the nest

To our families
for the first imprinting
of healing honey
at home

Sweet sour-sweet honey

Foreword

By Dr. Natasha Hungerford and Professor Mary Fletcher, who identified the unusual low GI disaccharide trehalulose as a predominant sugar and distinctive marker of stingless bee honey.

Organic acid content of honey is an important determinant of honey characteristics including flavour and texture. Whilst minor in quantities, the organic acids in honey can be used as markers for distinguishing between honey types, and indeed be used as authenticity markers. In particular, aliphatic organic acids (AOA) are mono-, di- and tri-carboxylic acids of non-aromatic hydrocarbons with varied levels of oxygenation and in some cases unsaturation.

This e-book review collates AOA content of honey in the scientific literature from 1994–2023. This includes both honeybee (*Apis*) honey and stingless bee (Meliponini) or pot-honey. Systematically, the presence of twenty-four AOA (acetic, cis-aconitic, adipic, butyric, citric, citramalic, formic, fumaric, galacturonic, gluconic, glutaric, isocitric, lactic, maleic, malic, malonic, oxalic, propionic, pyruvic, quinic, shikimic, sorbic, succinic, and tartaric acids) were reviewed in *Apis mellifera* honeys and pot-honeys produced by stingless bee genera *Cephalotrigona*, *Nannotrigona*, *Partamona*, *Oxytrigona*, *Scaptotrigona*, *Tetragonisca* from Ecuador, *Geniotrigona* and *Heterotrigona* from Malaysia, *Melipona* from Brazil, Ecuador and Venezuela, *Axestotrigona* from Tanzania, and *Austroplebeia* and *Tetragonula* from Australia. The origins of these AOA in these honeys are discussed including the contributions from nectar sources, bee enzymes, microbes and the cerumen storage pots of the nest, and their association with the free acidity of honey.

In writing this book (together with bio-organic chemist Professor Svetlana Simova), Professor Patricia Vit continues her focus on stingless bees and as a powerful proponent of their pot-honey. With more than 600 stingless bee species worldwide, there is great diversity for honey production and pollination by stingless bees. The conclusion from the data is that the organic acid content of honeybee honey as < 0.5% has generally been underestimated and that typically *Apis mellifera* honeybee honey has up to 2.5% AOA content whereas stingless bee pot-honey contains up to 5.8% AOA. With honeybee honey regarded as a sweet product and stingless bee honey typically a delightfully sweet-sour honey, the relative sour flavours derive from the combination and quantities of AOA present. The relationship between AOA quantities in honey and the bees that make honey (*Apis* or Meliponini), their microbes, and the AOA plant sources is still to be fully understood.

This comprehensive review of AOA in honey provides a global view of honey and the contribution of AOA to honey's texture, flavour, and properties, and will undoubtedly prompt scientists to further decipher the AOA origins in honey, and to further understand their transformations and functions.

Dr. Natasha Hungerford
and
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Preface

The Review on Aliphatic Organic Acids (AOA) of Honey and Pot-honey for Bee Science covers bibliographic data in the period 1994 to 2023. The purpose of this e-book is to present a comprehensive and contemporary survey on the characterization of *Apis mellifera* honey and Meliponini pot-honey AOA profiles to readers involved in routine quality control and challenging research for true innovation.

The origin of AOA was introduced in the section *Natural honey AOA are secreted by plants, produced by action of bee enzymes, or by microbial fermentation.* In the section *Free acidity of honey and sourness is mostly based on AOA types and quantities*, free acidity was revisited in diverse European unifloral honey types. An important teaching effort was illustrating the useful *Conversion of free acidity into major AOA and AOA spectra into free acidity*, and highlighting that all *AOA are carboxylic acids*. A section was dedicated to recent advances on *Microbiota involved in aliphatic organic acids (AOA) from honey*, of utmost interest for pot-honey and the fermentation used as a conservation method of honey in the stingless bee nest. The core of this volume is the scoping review in the section on *Quantities of aliphatic organic acids (AOA) in honey* with systematic tables, carefuly tabulated from the literature for analytical comparisons, scrutinized to support consistent contribution, and to detect discrepancies in published units of AOA contents in literature. A general presentation of each AOA selected by authors of reviewed references was synthesized in *The aliphatic organic acids (AOA) of honey* section.

The molecular formulas of 24 AOA were compared besides their biochemical functions, and uses in the food industry. The chemical separation, and identification needed for quantitations were summarized in the section named *Briefing on techniques used for organic acid quantitation*, just before the *Integrative remarks* addressed to young generations –and the authors themselves– for a compact aliphatic organic acid database needed to think and build knowledge to understand the roles of microbiota associated with honey making bees.

The authors harmonized the AOA units available in the literature, useful to compare a set of AOA in different honey types, based on the 100 g compositional standard of food science adopted as the best way to compare nutrients in similar products.

Additionally, an updated reference for honey AOA contents was evident after the visualization of AOA < 2.5% for *Apis mellifera* honey replacing the current < 0.5%, as well as AOA < 5.8% for pot-honey, represented by the stingless bee *Axestotrigona* genus from Tanzania in this dataset.

The 2023 references included in this review were on Australian pot-honey with the largest sampling of Meliponini in current database, provided by the authors of the Foreword, revealing the advantage of HPIC-SCD to estimate the content of gluconic acid, not possible with targeted ^{1}H-NMR used for the Ecuadorian pot-honey.

We eagerly anticipate a positive response from our esteemed readers networking between pot-honey experts and integrative multidisciplinary interactions with ecologists, entomologists, health scientists, palynologists, sensory scientists, and stingless bee keepers of the world.

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Summary

An updated reference for Aliphatic Organic Acids AOA < 2.5% for *Apis mellifera* honey, as well as evidenced AOA < 5.8% for pot-honey are proposed after this review. The organic acids < 0.5% of *Apis mellifera* honey's constituents (2003) persisting along the years, was underestimated. Chronological data (1994–2023) for maximum concentrations of total AOA of *Apis mellifera* honeys were: 1.2% in strawberry tree honey Italy, 1.0% floral *Erica* Spain, 1.3% chestnut Turkey, 1.6% chestnut France, 1.8% polyfloral and chestnut Germany + 0.7% estimated gluconic acid, 1.6% bracatinga honeydew Brazil. The scope of this review was refining bibliometric support of 919 samples, 710 *Apis mellifera* honey and 209 Meliponini pot-honey, and harmonizing units for aliphatic organic acids (g/100 g honey). AOA are also named non-aromatic organic acids, and they contribute to the free acidity of honey standards, being gluconic acid the most abundant in honey, but not always assessed. However, in the pot-honey of Meliponini, acetic and lactic acids are distinctive and could be very high in the AOA spectra. The associated microbiota plays a role here. Twenty four AOA were reviewed (acetic, *cis*-aconitic, adipic, butyric, citric, citramalic, formic, fumaric, galacturonic, gluconic, glutaric, isocitric, lactic, maleic, malic, malonic, oxalic, propionic, pyruvic, quinic, shikimic, sorbic, succinic, and tartaric) in *Apis mellifera* honeys and pot-honeys produced by *Cephalotrigona*, *Nannotrigona*, *Partamona*, *Oxytrigona*, *Scaptotrigona*, *Tetragonisca* from Ecuador, *Geniotrigona* and *Heterotrigona* from Malaysia, *Melipona* from Brazil, Ecuador and Venezuela, *Axestotrigona* from Tanzania, and *Austroplebeia* and *Tetragonula* from Australia. AOA were tabulated from the literature for fast view and analytical comparisons. The botanical origin associated to organic acids in honey was introduced with the free acidity of major European unifloral and honeydew honey. AOA data was retrieved for multifloral, unifloral (*Acacia*, *Arbutus*, *Averrhoa*, *Brassica*, *Calluna*, *Castanea*, *Ceratonia*, *Citrus*, *Eucalyptus*, *Erica*, *Euphorbia*, *Helianthus*, *Lavandula*, *Leptospermum*, *Melaleuca*, *Onobrychis*, *Quercus*, *Paganum*, *Reseda*, *Rhododendron*, *Robinia*, *Rosmarinus*, *Thymus*, *Trifolium*, *Ziziphus*) and honeydew (bracating Brazil, fir France, metcalfa Germany, and pine Turkey) honeys from 15 countries (Australia, Brazil, Bulgaria, Chile, China, Ecuador, France, Germany, Italy, Latvia, Malaysia, Spain, Tanzania, Turkey, Venezuela).

Contents

1. Introduction

Previous reviews on nonaromatic organic acids in honey were concerned on their significance (Mato et al., 2003) and the analytical methods (Mato et al., 2006). To my knowledge this is the third review, with organic acids and honey in the title search of a Scopus database retrieval. And the first review on aliphatic organic acids (AOA) including recent data on honey produced by stingless bees (Meliponini). We will use here AOA for singular and plural, like DNA is used. The AOA present in honey are responsible for sourness in its flavor, added nutritional value, and stability against contamination due to undesirable microorganisms. Honey is a sour-sweet medicinal food, mostly known as a "sweet" bee product for the high concentration of sugars. However, low quantities of organic acids – compared to sugars and water – confer a sensory identity to the story of honey, and suggest a new integrated meaning. In some stingless bee taxa organic acids surpass a sensory threshold limit, producing a "sour" honey. That is the case for the Neotropical *Geotrigona* (Vit et al., 2017), the Indian *Trigona* (sic) *Tetragonula* (Thomas and Kharnaior, 2021), and diverse. Organic acids do not occur alone in nature; thus, a natural sour flavor derives from a combination of natural organic acids. Two organic acids that are frequently blended together for flavor modifications in food formulations are acetic and lactic (Dziezak, 2016), similar to the honey prepared in stingless bee nests containing 3.05 acetic and 2.30 lactic g/100 g *Axestotrigona* honey from Tanzania (cited as *Meliponula ferruginea* by Popova et al., 2021), and the following combinations of 2.43 and 1.96 *Geotrigona*, 0.16 and 1.33 *Melipona*, 0.16 and 1.21 *Scaptotrigona* honeys from Ecuador (Vit et al, 2023). The honey content of acetic acid being greater than lactic acid or viceversa, seems to be of entomological origin, or related to the microbial associations with the bee. Presumably acetic acid bacteria AAB are dominant in the colonies of Tanzanian *Axestotrigona*, and Ecuadorian *Geotrigona*, coordinated with lactic acid bacteria (LAB), producing high concentrations of lactic acid. For the Ecuadorian *Melipona* and *Scaptotrigona*, LAB seem to lead the fermentation processes in honeys with lactic acid being greater than acetic acid, which is also entomological origin oriented.

Aliphatic organic acids (AOA) have been studied by High Performance Liquid Chromatography HPLC in *Apis mellifera* unifloral and multifloral honeys from Italy

(Cherchi et al., 1994), from Spain (del Nozal et al., 1998; Suárez-Luque et al., 2002), and from Turkey (Tezcan, 2011), by Ion Chromatography IC in France (Daniele et al., 2012), by Capillary Zone Electrophoresis CZE in Spain (Mato et al., 2006b), Capillary Electrophoresis (CE) in Brazil (Seraglio et al., 2021a,b), by untargeted Nuclear Magnetic Resonance NMR in Germany (Ohmenhaeuser et al., 2013), by targeted NMR in Chilean (Fuentes Molina et al., 2020), and Iranian (Khansaritoreh et al., 2021) honeys. Largest numbers of *Apis mellifera* honeys were analyzed during the French study with 140 honeys (Daniele et al., 2012), and the German study with 328 honeys (Ohmenhaeuser et al., 2013).

Pot-honey is a recent term, coined for stingless bee honey produced in cerumen pots where nutritional, moist and warm anaerobic conditions facilitate fermentation (Vit et al., 2013). AOA from pot-honey were assessed by enzymatic methods in *Tetragonula carbonaria* from Australia (Persano-Oddo et al., 1998), and *Melipona favosa* from Venezuela (Sancho-Ortiz et al., 2013). Chromatographic methods were used to quantify honey AOAs in *Geniotrigona thoracica* and *Heterotrigona itama* from Malaysia (Shamsudin et al., 2019), *Melipona fasciculata* and *Melipona subnitida* from Brazil (Sant'Ana et al., 2020), 12 species of stingless bees from Ecuador (Villacrés-Granda et al., 2021), and five species from Australian *Austroplebeia* and *Tetragonula* genera (Hungerford et al., 2023). AOAs were identified and quantified by untargeted ^{1}H-NMR of *Axestotrigona ferruginea* honey from Tanzania (cited as *Meliponula ferruginea* by Popova et al., 2021), and targeted ^{1}H-NMR in *Geotrigona*, *Melipona* and *Scaptotrigona* honeys from Ecuador (Vit et al., 2023). For the pot-honeys, the largest sampling was 111 honeys produced by Australian *Austroplebeia australis*, *Austroplebeia cassiae*, *Tetragonula carbonaria*, *Tetragonula davenporti*, and *Tetragonula hockingsi* (Hungerford et al., 2023). These authors assessed variation by species and habitat using chemometrics.

AOA in honey originated from the plants visited by the bees for nectar or honeydew collection, by the action of bee enzymes, and even by microbial enzymes. Microbiota associated to the bees and substrates they visit, participate in fermentation processes inside the nest (bubbles are visible when opening some honey pots), which might be extended after pot-honey harvest. Also, the organic acids in the soil (Adeleke et al., 2017) may play a role in different habitats where some *Melipona* spp. collect mud for the production of geopropolis. In *A. mellifera* honey a post-harvest fermentation is considered spoilage caused by immature honey with higher water content than that suggested in the honey standards (CODEX STAN, 1987). For stingless bees, post-harvest programmed fermentation is a stabilizing process to extent shelf-life of their honey (Drummond, 2013). Bee pollen also contains organic acids and microbiota associated to pot-pollen (Rosa et al., 2003). Traces of pollen in honey may enrich its microbial spectra, contributing to its chemical profile. The honey bee harbors a simple microbiota producing AOA as fermentation products of dietary nectar and pollen (Ricigliano et al., 2020).

It is worth mentioning the use of organic acid and corresponding esters or salts in nutrition science. For example, the terms “oxalic acid” and “oxalate” are used interchangeably because this acid usually bounds to minerals in plants, forming oxalate salts, similarly acetic acid and acetate, lactic acid and lactate, and all the AOA. Numerous aliphatic organic acids, like acetic, citric, gluconic, lactic, malic, tartaric, and inorganic acids such as hydrochloric acid [H^+ Cl^- or H_3O^+ Cl^-], are responsible for sourness. Other organic acids together with inorganic anions also contribute to acidity (White, 1975; Cherchi et al., 1994). Aliphatic acids such as citric and malic, have an ability to chelate metals and this fact contributes to the antioxidant capacity of honey, which may facilitate the action of other antioxidants like polyphenols (Cavia et al., 2007). Acetic, ascorbic, citric, lactic, and malic acids, can increase the bioavailability of dietary copper and iron (Teucher, et al., 2004; Wapnir, 1998), a derived intake practice is to consume iron supplements with citrus juices. Organic acids regulate the pH and the microbial communities able to live in the honey substrate. They have a nutritional function for the bees, and the transformations of sugars is a nutritional benefit for the colony. The presence of diverse types and concentrations (transient or not) of organic acids in honey is of scientific interest to explain their biotic origin from plants, bees, microorganisms, but also related to environmental abiotic factors like temperature, altitude, soil minerals, and rain. As natural components of honey, organic acids are useful as authenticity markers of botanical origin by HPLC (Cherchi et al., 1994; del Nozal et al., 1998), IC (Daniele et al., 2012), NMR (Ohmenhaeuser et al., 2013), and CE (Seraglio et al., 2021a). They confer unique chemical spectra to fermentations, odor-aroma sensory perceptions, botanical and geographic origins.

Aliphatic and aromatic organic acids are present in honey. Aliphatic acids are also named non-aromatic organic acids in the literature, and they are more associated with the free acidity dictated by the honey standards. Gluconic acid is often the most abundant AOA in *Apis mellifera* honey. However, in the honey pots of Meliponini, other AOA such as acetic acid and lactic acid, may take over in particular species of stingless bees (Vit et al., 2023). On the other hand, aromatic acids belong to a category with aromatic structures like phenolic acids, they are more associated to biochemical parameters and bioactivity. Aromatic acids are phytochemicals but microbiota could also transform them not only in the bee gut or processing pots from the stingless bee nest, but also in the human gut. For example, urolithins are gut microbial metabolites better absorbed than their parent substrates ellagic acid and ellagitannin from berry fruits and nuts – also from honey – that are poorly absorbed in the intestine (Tomás-Barberán et al., 2017). The new genus *Ellagibacter* gen. nov. was proposed for the new bacteria species isolated from human gut (Beltrán et al., 2018). Thus microbial metabolites drive diet-based influence of microbiota on the host, by interactions between diet, microbiota, and host physiology. Returning to honey making bees and their gut microbial communities or microflora or microbiota or microbioma, all these terms are used on studies revolving two major related scopes: 1. Bees' health, and 2. Bees's efficacy as pollinators (Good et al., 2014).

The scope of this review is harmonizing literature on aliphatic organic acid data in honey, providing an insight on their biological (bee, botanical, microbial, geographic) and chemo diverse origin, and summarizing used analytical techniques. An important consideration will focus on the units to express their concentration, from bibliometric data on organic acids in honey towards better understanding of their qualitative and quantitative implications. Phenolic acids from honey will be the scope of a forthcoming review.

2. Natural honey AOA are secreted by plants, produced by action of bee enzymes, or by microbial fermentation

2.1 Plant secreted AOA

The plant and animal origin of honey encapsulate enormous chemical possibilities of this product of the bees' nest, with its derived physical and biological properties being continuously evaluated in science. Honey is a concentrated aqueous solution of inverted sugars hosting a complex natural product combination of components. These include minor saccharides, amino acids, enzymes, vitamins, metals, flavonoids, phenolic and aliphatic organic acids AOA, traces of pollen, beeswax and cerumen, as well as other residues. Visually, honey is liquid or crystallized, and ranges in the amber color scale almost colorless to dark brown. Tactually thin to thick or granulated honeys may have small to large, smooth to sharp textures of crystals. Gustatory sweet-sour-bitter-savory, and olfactorilly a plethora of volatiles conferring odor-aromas descriptors of diverse sensory families. These include floral-fruity, vegetable, fermentative, woody, bee nest, mellow, primitive, and industrial chemicals, and can be traced back to a story of origin and nature of the materials harvested and processed by the bees. Biological transformations by bees' secretions and associated microbiota integrate physicochemical properties of mature honey, which will continue post-harvest variations. This is the honey investigated along the years, focusing on aliphatic organic acids studied by bee scientists for this review. This includes honey collected by *Apis mellifera* and stingless bees (Meliponini). Their entomological richness consists on over 600 species of stingless bees, in contrast to 12 *Apis* spp. (Roubik and Vergara, 2021).

Floral nectar can be a source of natural AOA. Nectar has an acid neutral pH 2.6-6.4, rarely alkaline up to 9.1. Studies have shown that sugar composition can be governed by a number of internal and external factors, even by the position of the flower on a plant, such as the *Tilia* tree whose nectar has lower sugar concentrations in flowers of higher branches compared to flowers of lower branches (Maurizio, 1975). Sugar values are quantities of nectar (mg) produced by one flower in 24 h, and vary between plant species. Other factors influencing sugar concentration within nectar include floral size, maturity and age, as well as nectary surface, soil type and fertility, and the time of day. Nectar secreted at night is more diluted than nectar secreted during the day. Similarly, honeydew is produced after several plant-sucking insects secrete sugars with diverse spectra, the simplest being sucrose, glucose, and fructose, with wider possibilities being maltose, trehalose, turanose, kojibiose, nigerose, occasionaly melezitose, 1-kestose, raffinose, melibiose, mannose, rhamnose, and other oligosaccharides. Sugars can even be replaced by sugar alcohols such as dulcitol, sorbitol, inositol, ribitol. Few nectars contain substances harmful to bees, humans or both.

The sap of plants has a high acidity mostly caused by citric, malic and oxalic acids, which are generally present in their salt forms (Bennet-Clark, 1933). Organic acids are mainly non-carbohydrate organic compounds, for example gluconic and glucuronic acids are carbohydrates. Malic, citric and other AOA are intermediates of the Krebs cycle. Lipids and many amino acids are first converted to Krebs cycle organic acids, and then to sugars by gluconeogenesis (Walker et al., 2021). Organic acids play important roles in regulating osmotic pressure, pH homeostasis, stress resistance, and fruit quality. The transport of organic acids from the cytosol to the vacuole and their storage are complex processes. A large number of transporters carry organic acids from the cytosol to the vacuole with the assistance of various proton pumps and enzymes. The vacuolar storage and release of Krebs cycle acids/nitrogenous compounds has a role on gluconeogenesis and malic enzyme in this process; there is a fine cytosolic pH control of enzyme activity of gluconeogenesis and malate metabolism (Walker et al., 2021). However, the vacuolar transport mechanism of organic acids has complex regulatory networks involved in fruit acid formation. Organic acids synthesized in plants are recycled in soil. Citrate and malate are the major excreted organic acids, and some plants excrete large quantities of oxalate too, which crystals are visible with light microscope. Organic acids are the main source of fruit acidity. Edaphic sources of soil organic acids are plants, microbes and plant litter (Adeleke, 2017). Therefore, stingless bees collecting mud for geopropolis and nest structures, may also have a diverse source of organic acid. Medicinal plants such as chamomile (*Matricaria recutita*, Asteraceae), linden (*Tilia platyphyllos*, Tiliaceae) and mint (*Mentha piperita*, Lamiaceae) are sources of succinic, malic, citric, tartaric, and lactic acid (Truică et al., 2013).

2.2 The bee honey crop

The honey crop, or stomach, enables bees to collect and hold sugary liquids within a vessel, in order to transfer nectar from plants to the nest. In honey making bees the honey crop holds 50-60μL and weighs 40-70 mg. Studies have shown that a full honey crop can reach up to 90% of the weight of the bee (Maurizio, 1975). The honey crop has a proventriculus valve which aids in the filtration of impurities such as pollen, thus reducing solid sediments of the crop content.

2.3 Role of honey bee enzyme activity in producing AOA

If needed, the reader is advised to cite the literature source of honey enzymes, not the authors of this AOA review, who are condensing the classification of honey enzymes by White (1975). His work was instrumental in the differentiation of natural honey from artificial or adulterated honey, and has been pivotal in ongoing honey research. White refers back to Gothe's dissertation in Leipzig, 1913, more than a century ago, where he noted honey does not contain lactase, proteases, or lipases. Catalase, invertase and glucose oxidase enzymes were already known, as proteolytic and alcohol fermenting too. His scope was differentiating natural from artificial honey, which is a timeless scope in honey research – as one of the 10 most faked foods in the world – besides others important in human diets such as olive oil, milk, coffee, eggs, maple syrup, fruit juices, red chili, turmeric and saffron powders, rice, tea, and wine. Honey enzymes were classified in: 1. Diastase, 2. Invertase, 3. Glucose oxidase, and other enzymes such as catalase, peroxidase, and phosphatase (White, 1975).

1. Diastase is a starch digesting enzyme, classified into α-amylase, which splits the starch chain randomly, and β-amylase, which splits maltose end of the starch polymer). Diastase is used in *Apis mellifera* honey standards as a heating and aging indicator, but is not related with AOA.

2. Invertase was thought to be the enzyme responsible for honey making process. Honeybees add invertase to nectar, which remains active in harvested honey. Most biochemical changes that take place when nectar or honeydew are ripened into honey are invertase derived. The invertase substrate is sucrose. Two invertases are classified into fructoinvertase, also known as yeast invertase, and glucoinvertase producing several oligosaccharides originated from sucrose. The trisaccharide erlose is one that is related to honeydew honey. Melezitose is a product of transglucosylation by an aphid enzyme hydrolyzing sucrose, also present in honeydew honey. The α-glucosidase is a carbohydrase secreted from the hypopharingeal glands of the honey making bees (Gontarski, 1954 cited in Maurizio, 1975). Invertase is not related with AOA in honey.

3. Glucose oxidase origin of this enzyme present in honey was demonstrated in the hypopharingeal glands of the honey bees by Gauhe (1941), and was purified by Shepartz and Subers (1964). A glucose-oxidizing enzyme forming organic acids, basically gluconic acid, its gluconolactone and also peroxides (White et al., 1963). These authors demonstrated that the 'inhibine' antibacterial factor was hydrogen peroxide. Therefore, **glucose oxidase is the honey making bee enzyme originating AOA in honey**.

Some secondary metabolites may interact with the honey enzymes, such as the suspected inhibition of glucose oxidase by the manuka marker methylglyoxal affecting hydrogen peroxide accumulation in honey (Majtan et al., 2014). Enzymes secreted by bee glands such as α- and β-amylase, α-glucosidase, and glucose oxidase are the major enzymes found in most honey types (Erban et al., 2019), as indicated in the White's enzyme frame above. Glucose dehydrogenase (GDH) enzyme activity was studied in honey produced by four Indonesian 'kelulut' stingless bee species *Geniotrigona thoracica*, *Heterotrigona itama*, *Tetragonula biroi*, and *Tetragonula leaviceps*. Samples were harvested from West Java, Lampung, and West Kalimantan, looking for an authenticity marker, and GDH was detected only in *T. biroi* and *T. laeviceps* (Sahlan, 2019).

2.4 Microbial fermentation and other interactions

Acetic acid and lactic acid products of microbial fermentation are generally abundant in most pot-honey, and distinctive for some stingless bee species. Phenyl lactic acid is a lactic acid molecule in which one of the methyl hydrogens is substituted by a phenyl group; 3-phenyllactic acid was 3.7–12.7 mg gallic acid equivalents/100 g Australian *Tetragonula carbonaria* honey (Massaro et al., 2014), and it was more concentrated than lactic acid in Chinese pickles (Xu et al., 2021).

Enzymes produced by yeasts may be involved in the honey and pollen processing inside the nest, improving their nutritional quality for bees (Gilliam, 1997). *Starmerella meliponinorum* has been described as a novel ascomycetous yeast species associated with stingless bees (Teixeira et al., 2003), and is abundant in *Tetragonisca angustula* nests (Rosa et al., 2003; Teixeira et al., 2003). Biosurfactants visualized in a test of *Scaptotrigona vitorum* honey (Vit, 2022a) are defense molecules. These secondary metabolites are known as antimicrobial compounds paradoxically produced by ecological interactions of microbes in the honey microenvironment (Brudzynski, 2021). The Codex Stan (1987) honey definition lacks water, the second major chemical constituent of honey after sugars, driving nest fermentation of high moisture pot-honey. Micro-life honey cell factories in the culture media of sealed honey pots produce yeast-ethanol, AAB-acetic acid, and LAB-lactic acid (Vit, 2023), processing and stabilizing nectar transformations with bubbling fermentation. See more in Section 4. Microbiota involved in aliphatic organic acids (AOA) of honey.

A synthesis of this section on the origin of AOA in honey is provided by a chemist informing in his abstract the organic acids are produced in the TCA cycle and by worker bee enzymes such as glucose oxidase (Suto et al., 2020), comprising both the plant and the bee origin of AOA. The microbial scenario is a more recent asset in the scientific honey world.

3. Free acidity of honey and sourness is mostly based on AOA types and quantities

All the above-mentioned organic acids in honey dissociate in aqueous solutions, to generate protons or hydrogen ions (White, 1975). The molecular basis of sourness is mainly caused by the detection of these protons by sensory receptors. Sourness perception is therefore related to the acidity of honey due to the presence of organic acids, mainly the gluconic acid, and their lactones or internal esters and inorganic ions such as phosphate, sulphate and chloride (Silva et al., 2009). This tendency to dissociate into their ionic constituents is influenced by the anionic (negatively charged) component of the molecule and the pH. Both factors significantly affect H^+ ion liberation, and perception of sourness. Undissociated acid molecules do not stimulate receptor neurons, but their presence contributes to the overall perception of other organic acids (Ganzevles and Kroeze, 1987).

3.1 Free acidity in diverse honey types

The free acidity of honey standards is measured by titrimetric methods. It is explained by the types and concentrations of organic acids in honey, their modifications during processing in the bee nest, and post-harvest. The botanical origin of honey is one of those issues that needs to be understood. A unifloral origin is a convention, not an absolute label. It refers to the pollen counts on a microscopic slide prepared with the sediment of 10 g honey after washing away the sugars. If over 45% of them are from the same pollen type, it is considered a unifloral honey for that plant (e.g. *Brassica*, *Castanea*, *Helianthus*, *Lavandula*, etc.) tracking back the nectar sources visited by the honey making bee (Louveaux et al., 1978). It needs to consider under-represented and over-represented taxa, the presence of pollen from nectarless plants, honeydew elements, and other key-factors of melissopalynology.

Persano Oddo and Piro (2004) selected 17 unifloral and two honeydews European *Apis mellifera* honey types for their descriptive sheets with more than 35,000 entries. Their free acidity is given in Table 1a. *Arbutus*, *Calluna*, *Erica*, *Thymus* and *Metcafa* honeydew have the highest free acidities with more than 30 mEq/kg honey. Whereas *Brassica*, *Robinia*, *Rosmarinus* and *Taraxacum* have the lowest free acity near to 10 mEq/kg honey. Theoretically, these values should correspond with the concentrations of their AOA.

Table 1a. Free acidity of *Apis mellifera* European unifloral and honeydew honeys

Botanical source			**n**	**Free acidity** (mEq/kg honey)
Species	**Family**	**Common name**		
Arbutus unedo	Ericaceae	Strawberry tree	-	**35.1** ± 8.1
Brassica napus	Brassicaceae	Rape	652	10.3 ± 2.1
Calluna vulgaris	Ericaceae	Heather	79	**32.1** ± 5.6
Castanea sativa	Fagaceae	Chestnut	4,302	13.0 ± 3.5
Citrus spp.	Rutaceae	Orange, lemon	2,221	14.3 ± 3.2
Erica arborea	Ericaceae	Tree heather	-	**34.7** ± 5.0
Eucalyptus spp.	Myrtaceae	Eucalyptus	1,464	19.4 ± 5.3
Hedysarum	Hedysareae	Honeysuckle	-	27.2 ± 8.0
Helianthus	Asteraceae	Sunflower	2,991	23.1 ± 6.3
Lavandula	Lamiaceae	Lavender	1,957	17.3 ± 4.0
Phacelia	Boraginaceae	Phacelia	-	19.8 ± 7.5
Rhododendron	Ericaceae	Rhododendron	1,345	13.3 ± 3.3
Robinia	Fabaceae	Black locust	5,319	11.2 ± 3.4
Rosmarinus	Lamiaceae	Rosemary	3,508	11.5 ± 4.7
Taraxacum	Asteraceae	Dandelium	1,002	10.9 ± 2.0
Thymus	Lamiaceae	Thyme	1,850	**37.2** ± 6.3
Tilia	Tiliaceae	Lime	2,274	20.8 ± 7.7
Honeydew	Aphid	Honeydew	5,285	26.0 ± 5.6
Metcalfa pruinosa (aphid insect)	Rinchota Homoptera, Flatidae	*Metcalfa* honeydew	1,536	**37.2** ± 6.6
		Total	35,785	

Values are averages ± SD
In bold free acidity > 30 mEq/kg honey. These honey types should be richer in aliphatic organic acids.

After: Persano Oddo and Piro (2004)

Having about 600 stingless bee species worldwide does not facilitate building a database on tropical meliponine unifloral honey. Therefore, literature is focused on the entomological origin of the honey instead of the botanical origin, which is also important as well as the biome. The free acidity of pot-honey produced in 18 countries was reviewed last year (Vit, 2022b). In Table 1b the free acidity honey produced by 46 species of stingless bees worldwide (n=246) is given in ascending order from 30 to 592 meq/kg. These conspicuous variations of pot-honey free acidity according to the entomological origin, was investigated in three botanical origins of two Malysian stingless bees (Shamsudin et al., 2019). No variation for *Melaleuca cajaputi* unifloral honey of *Geniotrigona thoracica* (101.8) and *Heterotrigona itama* (103), medium entomological variation for unifloral *Averrhoa carambola* honey of *Geniotrigona thoracica* (170.5), and *Heterotrigona itama* (207.7), and maximum variation between the unifloral *Acacia mangium* honey of *Geniotrigona thoracica* (136.5) and *Heterotrigona itama* (64.5). Different nectars were processed differently by the bee enzymes of *G. thoracica* and *H. itama*, and also the microbial reservoir in the nectars may explain some of the observed bee-plant interactions. *H. itama* was more susceptible to the botanical origin, with free acidities from 64.5 to 207.7 meq/kg. Comparing botanical origins of 600 meliponine species has started, but progress will be very slow. Focus on the entomological origin of pot-honey still has great contributions ahead.

Table 1b. Ascending order of free acidity in pot-honey of 46 stingless bee species

No. species	Stingless bee species	Country	N 246	Free acidity (meq/kg)
1	*Meliponula togoensis*	Kenya	24	30 ± 14
2	*Hypotrigona* sp.	Nigeria	3	35.57 ± 0.42
3	*Axestotrigona ferruginea*	Kenya	17	38 ± 26
4	*Melipona cramptoni*	Ecuador	3	40.46 ± 3.03
5	*Nannotrigona chapadana*	Ecuador	1	42.07 ± 0.03
6	*Melipona indecisa*	Ecuador	6	44.21 ± 4.42
7	*Tetragona* sp.	Colombia	4	44.3 ± 21.8
8	*Paratrigona* sp.	Ecuador	1	46.53 ± 0.01
9	*Meliponula bocandei*	Kenya	4	48 ± 24
10	*Lisotrigona furva*	Thailand	1	48
11	*Scaptotrigona polysticta*	Bolivia	1	49.1
12	*Scaptotrigona depilis*	Bolivia	1	49.4
13	*Meliponula lendliana*	Kenya	1	52
14	*Melipona mondury*	Brazil	11	52.77 ± 31.93
15	*Tetragonula testaceitarsis*	Thailand	1	54
	Lisotrigona furva	Thailand	1	58
16	*Oxytrigona mellicolor*	Ecuador	1	58.84 ± 0.04
17	*Plebeia* sp1.	Venezuela	1	59.07

18	*Melipona grandis*	Ecuador	1	60.35 ± 0.03
19	*Tetragonisca silvestriana*	Ecuador	1	61.06 ± 0.04
20	*Scaptotrigona polysticta*	Ecuador	7	63.36 ± 4.17
21	*Heterotrigona itama A. mangium*	Malaysia	1	64.50 ± 4.00
22	*Melipona capixaba*	Brazil	9	67.94 ± 33.20
23	*Tetragonula fuscobalteata*	Thailand	1	69
24	*Tetragonisca angustula*	Venezuela	1	69.08
	Tetragonisca angustula	Ecuador	3	70.55 ± 1.01
25	*Tetragonisca fiebrigi*	Argentina	12	71.90 ± 2.25
26	*Tetragonula laeviceps-pagdeni complex*	Thailand	10	76 ± 30
	Tetragonisca angustula	Costa Rica	32	79.4 ± 43.9
27	*Scaura* aff. *latitarsis*	Venezuela	1	84.21
28	*Geotrigona acapulconis*	Guatemala	1	85.53
	Tetragonula testaceitarsis	Thailand	1	87
29	*Scaptotrigona* sp.	Venezuela	1	91.45
30	*Plebeia* sp2.	Venezuela	1	95.00
31	*Geniotrigona thoracica M. cajaputi*	Malaysia	1	101.83 ± 9.19
	Heterotrigona itama M. cajaputi	Malaysia	1	103.00 ± 2.83
32	*Scaptotrigona pectoralis*	Mexico	1	116.0
33	*Cephalotrigona* sp.	Ecuador	1	116.47 ± 0.20
34	*Plebeia wittmanni*	Argentina	10	117.5 ± 1.25
	Tetragonula fuscobalteata	Thailand	1	124
35	*Tetragonula carbonaria*	Australia	8	124.2 ± 22.9
36	unknown species	Venezuela	1	128.28
	Geniotrigona thoracica A. mangium	Malaysia	1	136.50 ± 6.36
37	*Plebeina armata*	Kenya	4	141 ± 63
	Tetragonula carbonaria	Australia	11	167.8 ± 32.8
38	*Lepidotrigona flavibasis*	Thailand	4	168 ± 47
	Geniotrigona thoracica A. carambola	Malaysia	1	170.50 ± 4.00
39	*Lepidotrigona doipaensis*	Thailand	1	188
40	*Lepidotrigona terminata*	Thailand	1	194
	Lepidotrigona doipaensis	Thailand	1	207
	Heterotrigona itama A. carambola	Malaysia	1	207.67 ± 3.30
	Heterotrigona itama	Malaysia	10	211.5 ± 93.0
	Geniotrigona thoracica	Malaysia	5	235.6 ± 95.8
41	*Frieseomelitta paupera*	Venezuela	1	248.52
42	*Liotrigona* sp.	Kenya	3	270 ± 38
43	*Tetrigona apicalis*	Thailand	1	440
44	*Homotrigona fimbriata*	Thailand	1	528
	Tetrigona apicalis	Thailand	1	550
45	*Geotrigona leucoblasta*	Ecuador	9	581.86 ± 54.43
46	*Tetrigona melanoleuca*	Thailand	1	592

Averages ± SD for all except for Ecuador ± SEM

3.2 Conversion of free acidity into major AOA and AOA spectra into free acidity

Estimating the major AOA with the 0.1 N NaOH volume (mL) used in the free acidity titration times a factor calculated with the Equivalent weight of the major acid of that food, (See blue panel below) is not that simple for honey. For honey that major AOA is not always gluconic acid, acetic, lactic, oxalic, succinic, and tartaric were major acids in some honey types, as will be elaborated upon throughout this review. Molecular weight (MW) and equivalent weight (EW) are the same in monocarboxylic acids with one carboxylic group (COOH), but EW is MW/2 in dicarboxylic acids and MW/3 in tricarboxylic acids. Gram Equivalent Weight (EW) is the mass of one equivalent in g (g/Eq), which corresponds to the mass of one milliequivalent in mg (mg/mEq).

AOA	**Chemical Formula**	**Major AOA Food**	**MW (g/mol) mg/mol[1]**	**No. COOH**	**EW (g/Eqmol) mg/mEqmol[1]**	**Factor**
Acetic acid	$C_2H_4O_2$	vinegar, pickles	60.05	1	60.05	0.0060
Citric acid	$C_6H_8O_7$	citrus fruits, pineapple, tomato	192.12	3	64.04	0.0064
Gluconic acid	$C_6H_{12}O_7$	honey	196.16	1	196.16	0.0196
Lactic acid	$C_3H_6O_3$	dairy products, fermented vegetables	90.08	1	90.08	0.0090
Malic acid	$C_4H_6O_5$	apple, pear, lettuce	134.09	2	67.05	0.0067
Oxalic acid	$C_2H_2O_4$	spinach, beets, nuts	90.04	2	45.02	0.0045
Succinic acid	$C_4H_6O_4$	blackberry, strawberry	118.09	2	59.05	0.0059
Tartaric acid	$C_4H_6O_6$	grapes, tamarind	150.08	2	75.04	0.0075

[1](F. Mora, Senior Researcher, School of Pharmacy, Universidad de Los Andes, personal communication, March 9, 2022)

After: Joslyn, 1970

3.2.1 Conversion of free acidity into major AOA

Honey is not one food product, it is a million foods in disguise, homogenized nutritious materials along honey making processes by bee colonies. Honey has a dual nature of a plant-animal natural product, but a third nature is uncovering: Microbial processes of associated microbiota with honey bees and stingless bees (SB). Considering the 12 *Apis* species and the over 600 stingless bee species, combinatory numbers speak on the variability of pot-honey. Possible undetermined Upper Cretaceous initiations of SB-phylo-microbes close connections originated microbial cell factories in SB nests (Vit, 2023). Bacteria, fungus and yeasts have particular functions in the nests, and may operate in successions as in other fermented foods like kombucha and pulque. What metabolites of honey have microbial origin? Metabolites of associated microbiota. In a seminal study of sympatric Australian stingless bee species, Acetobacteriaceae was present in the microbiome of two *Tetragonula* species and absent in the *Austroplebeia australis*, just to illustrate the dimension of ecological and phylogeographical implications (Leonhardt and Kaltenpoth, 2014), and the transformations of honey, pollen, propolis by nest micro-life.

Looking at this complex natural product narrowed to a viscous amber scale or crystallized amber pastels, the AOA types and dominances are not predictable as in grapes or milk. A honey jar is a bouquet of diverse flowers or honeydews foraged by bees, and their further transformations. Gluconic acid is often but not always the most abundant honey AOA, other acids such as acetic, lactic, oxalic, succinic, and tartaric can dominate the AOA composition too. Therefore, transforming free acidity into a major AOA of honey seems not adequate as it is done for other foods packed in their distinctive containers such as apples, blueberries, broccoli, carrots, fennels, lemons, parsley. Free acidity of honey can be transformed into corresponding major honey AOA, but what is the scope if to know that AOA must be measured in advance?

3.2.2 Conversion of AOA spectra into free acidity

Generally, papers on honey quality control do not have AOA data, and vice versa. Also, different laboratories and facilities are needed to analyze free acidity and content of AOA. The gowing literature in honey AOA, and the established free acidity for honey quality control need to meet and harmonize for comprehensive matters. Both scientists and technicians will benefit knowing how to convert them into each other, and students will learn that from early stages of honey research.

For this conversion exercise, we have chosen a study with both approaches and the combination of 2 stingless bees + 3 floral origins. An advantage is that they measured gluconic acid, with large variations that are suspected of microbial origin. Gluconic acid is difficult to assess with NMR due to the 16 isomers. Measurements of five AOA by HPLC and potentiometric free acidity let us do the calculations wanted for this review. However, these authors have a discrepancy between their reported AOA units (g/kg), and possibly correct AOA units (g/100 g), a X10 underestimated factor. These units need to be corrected by Shamsudin et al., who have to support scholar use of their important contributions. Our need was to write a note of amended units in this AOA review to anticipate editorial corrections that are not rare.

The correspondence between free acidity and total honey AOA needs to consider that: 1. All honey AOA are not measured, the total refers to the selected AOA measured in each paper, 2. Each AOA has its own Molecular Weight (MW), 3. Equivalent Weights (EW) vary according to the number of COOH in the AOA molecule, being equal to the MW in monocarboxylic acids, MW/2 in dicarboxylic acids, and MW/3 in tricarboxylic acids.

The right question to solve the problem is how many mEq/kg honey (free acidity) are explained by each concentration of AOA g/100 g honey? Simplified by calculating the mEq of each AOA in honey, as follows.

For example, let us consider the *Geniotrigona thoracica* **Gt** "Kelulut" gelam **Ge** *Melaleuca cajuputi* MYRTACEAE from Malaysia, **GtGe** honey (Samshudin et al., 2019).

The content (g) of five AOA quantified in the **GtGe** honey were transformed into meq AOA, for each acid after converting them to mg (X1000) and dividing by the corresponding Milligram Equivalent Weight (mg/mEq), to estimate the mEq of AcA acetic acid, CitA citric acid, GcA gluconic acid, LaA lactic acid, and MaA malic acid. See the calculations in the green panel below.

The total **GtGe** honey **AOA** (1.11 g) resulted from 0.06 g AcA, 0.03 g CiA, 0.55 g GcA, 0.17 g LaA, and 0.30 g MaA, that were transformed into **mg** first and corresponding **mEq**:

60 mg AcA/60.05 mg/mEq = 0.99 mEq AcA

30 mg CiA/64.04 mg/mEq = 0.47 mEq CiA

550 mg GcA/ 196.16 mg/mEq = 2.80 mEq GcA

170 mg LaA/90.08 mg/mEq = 1.89 mEq LaA

300 mg MaA/67.05 mg/mEq = 4.47 mEq MaA

Σ mEq AOA = mEq AcA + mEq CiA + mEq GcA + mEq LaA + mEq MaA = 10.62 **mEq**

Therefore the estimated **Total mEq AOA** contributing to free acidity of honey are 10.62 mEq for the gelam *Geniotrigona thoracica* honey. As it is reported g AOA/kg honey, the free acidity would be 10.62 mEq/kg honey, but their potentiometric free acidity was 101.83 mEq/kg honey (Shamsudin et al., 2019). If the authors may reach a consensus on their reported AOA units as g/100g, this value should be multiplied by ten, then the free acidity of this honey estimated by the mEq of five honey AOA, would be 106.2 mEq/kg honey, which is closer to their potentiometric free acidity 101.83 mEq/kg honey.

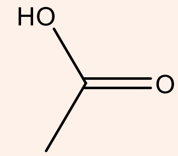
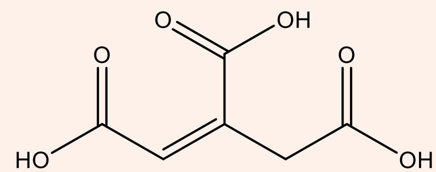
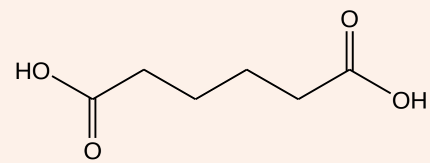
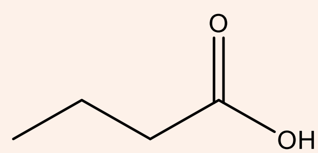
3.3 Aliphatic organic acids (AOA) are carboxylic acids

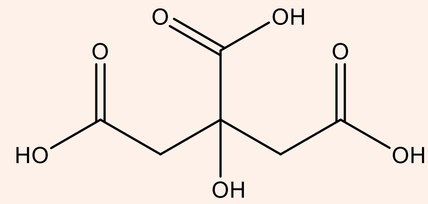
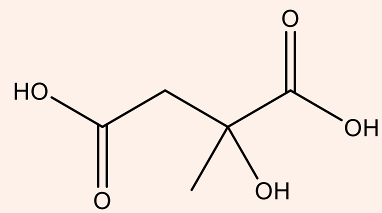
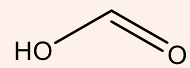
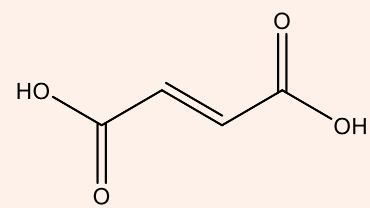
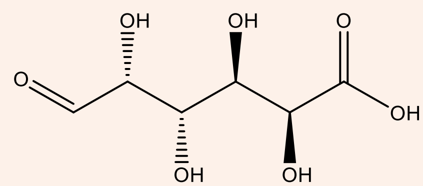
In the International Union of Pure and Applied Chemistry (IUPAC) system of nomenclature the functional carboxyl carbon (COOH or CO_2H) is designated #1 C. Other substituents are located and named accordingly as alkyl groups R. Thus, the general formula of a carboxylic acid is R–COOH or R–CO_2H. Their chemical formulas are short expressions considering the elemental numbers as for any other organic compound, for example the smallest carboxylic acid is methanoic or formic acid HCO_2H with a hydrogen as R, and chemical formula CH_2O_2.

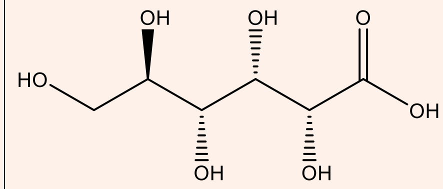
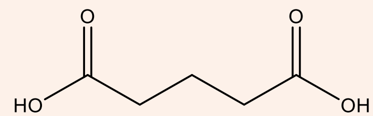
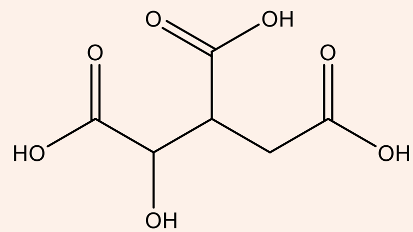
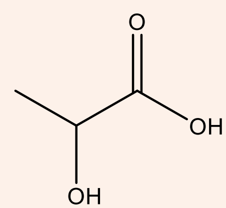
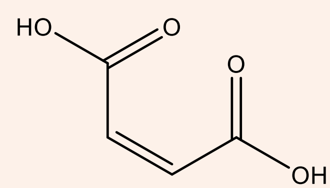
Monocarboxylic acids are molecules with one COOH functional group, simply named carboxylic acids. They are weak acids, which can deprotonate or lose a proton, to form carboxylate ions. They are soluble in water if they have fewer than 5 carbons. Dicarboxylic acids have two carboxyl groups, and the tricarboxylic acids are the strongest with three carboxylic groups.

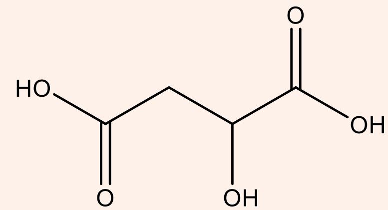
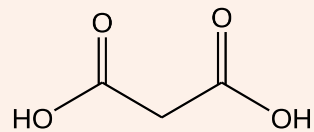
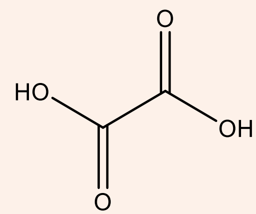
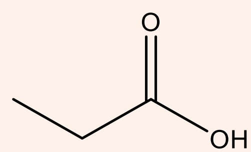
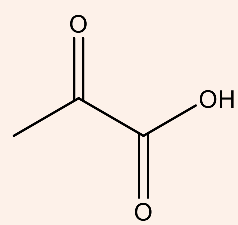
The following annotations from William Reusch (2021) summarize the chemical rules used in their nomenclature, and detailed explanations of their most distinctive physical property – acidity generated by carboxylic acids – is available in his page online. The International Union of Pure and Applied Chemistry (IUPAC) system of nomenclature assigns a characteristic suffix to carboxylic acids. The –e ending is removed from the name of the parent chain and is replaced -anoic acid. Since a carboxylic acid group must always lie at the end of the carbon chain, it is always given the #1 location position in numbering without the necessity to include it in the name. When a carboxyl group is added to a ring the suffix -carboxylic acid is added to the name of the cyclic compound. The ring carbon attached to the carboxyl group becomes the #1 C.

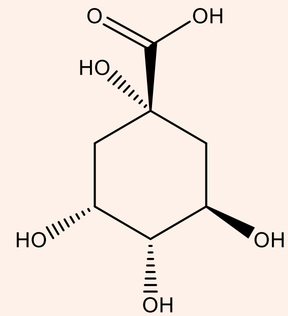
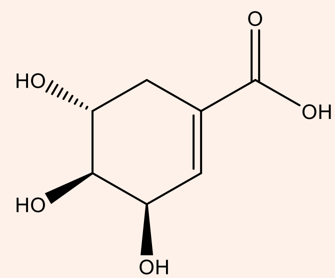
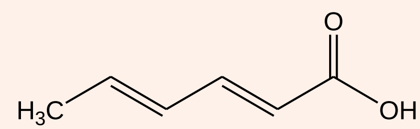
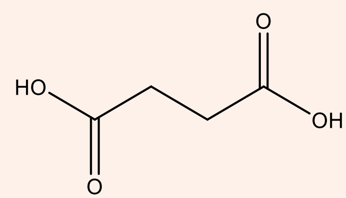
Each of the 21 AOA retrieved for this review on honey research, was framed in the pink panel to highlight their common name, source, etymology, molecular formula, Isomeric SMILES, chemical structure, molecular mass, and the IUPAC name for each honey AOA. SMILES is the acronym for Simplified Molecular Input Line – Entry System. SMILES notation visualizes in a linear manner the structure of chemical species with rings using short ASCII strings. Different configuration at rings, and/or double bond geometry could be distinguished by the isomeric SMILES encoding.

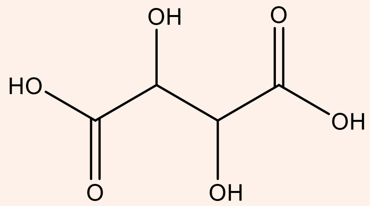
No.	**Common acid name**	**Source and/or Etymology**	**Molecular formula** (bold) **Isomeric SMILES**	**Chemical Structure**[1]	**Molar mass**[1] **(g/mol)**	**IUPAC acid name**
1	acetic	vinegar Latin *acetum*	$\mathbf{C_2H_4O_2}$ CC(=O)O		60.05	ethanoic
2	cis-aconitic	aconitum Greek ἀκόνιτον aconite Latin monkshood *Aconitum napellus*	$\mathbf{C_6H_6O_6}$ C(/C(=C/C(=O)O)/C(=O)O)C(=O)O		174.11	(Z)-prop-1-ene-1,2,3-tricarboxylic
3	adipic	animal fat Latin *adipis*	$\mathbf{C_6H_{10}O_4}$ C(=O)CCCCC(=O)O)O		146.14	1,6-hexanedioic
4	butyric	butter Latin *butyrum*	$\mathbf{C_4H_8O_2}$ CCCC(=O)O		88.11	butanoic

5	citric	lemmon Swedish *citron*	$\mathbf{C_6H_8O_7}$ C(C(=O)O)C(CC(=O)O)(C(=O)O)O		192.12	2-hydroxypropane-1,2,3-tricarboxylic
6	citramalic	citric + malic	$\mathbf{C_5H_8O_5}$ CC(CC(=O)O)(C(=O)O)O		148.11	(2R)-2-hydroxy-2-methylbutanedioic
7	formic	ants Latin *formica*	$\mathbf{CH_2O_2}$ C(=O)O		46.02	methanoic
8	fumaric	*Fumaria officinalis* fumitory PAPAVERACEAE Latin *fumaria*	$\mathbf{C_4H_4O_4}$ C(=C/C(=O)O)\C(=O)O		116.07	(E)-2-butenedioic
9	galacturonic	galac + uron galactose + Greek *ouron* urine	$\mathbf{C_6H_{10}O_7}$ [C@@H]1([C@H]([C@H](O[C@@H]([C@@H]1O)O)C(=O)O)O)O		194.14	(2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-oxohexanoic acid

10	gluconic	from glucose	**$C_6H_{12}O_7$** C([C@H]([C@H]([C@@H]([C@H](C(=O)O)O)O)O)O)O		196.16	(2R,3S,4R,5R)-2,3,4,5,6-pentahydroxy hexanoic
11	glutaric	glutamic + tartaric	**$C_5H_8O_4$** C(CC(=O)O)CC(=O)O		132.11	pentanedioic
12	isocitric	isomer of citric enzyme aconitase	**$C_6H_8O_7$** C(C(C(C(=O)O)O)C(=O)O)C(=O)O		192.12	1-hydroxypropane-1,2,3-tricarboxylic
13	lactic	milk Latin *lactātus*	**$C_3H_6O_3$** CC(C(=O)O)O		90.08	2-hydroxypropanoic
14	maleic	apples Latin *mālum*	**$C_4H_4O_4$** C(=C\C(=O)O)\C(=O)O		116.07	(Z)-but-2-enedioic

15	malic	apples Latin *mālum*	**$C_4H_6O_5$** C(C(C(=O)O)O)C(=O)O		134.09	2-hidroxy-1,4-butanedioic
16	malonic	apples Latin *mālum*	**$C_3H_4O_4$** C(=O)CC(=O)O)O		104.06	1,3-propanedioic
17	oxalic	wood-sorrels *Oxalys* spp. Greek *oxys* 'sharp'	**$C_2H_2O_4$** C(=O)(C(=O)O)O		90.03	ethanedioic
18	propionic	Greek πρῶτος + πίων *prōtos* + *piōn* 'first' + 'fat'	**$C_3H_6O_2$** CCC(=O)O		74.08	propanoic
19	pyruvic	pyro- + uva Greek pyro- 'fire' Latin *uva* 'grape'	**$C_3H_4O_3$** CC(=O)C(=O)O		88.06	2-oxopropanoic

20	quinic	Kichwa *kina* 'cinchona bark'	**$C_7H_{12}O_6$** C1[C@H](C([C@@H](CC1(C(=O)O)O)O)O)O		192.17	(1S,3R,4S,5R)-1,3,4,5-tetrahydroxy cyclohexane-1-carboxylic
21	shikimik	Japanese flower *shikimi Illicium verum* 'star anise'	**$C_7H_{10}O_5$** C1[C@H]([C@@H]([C@@H](C=C1C(=O)O)O)O)O		174.15	(3R,4S,5R)-3,4,5-trihydroxy cyclohex-1-ene-1-carboxylic
22	sorbic	Isolated from unripe berries *Sorbus aucuparia* ‘rowan tree’	**$C_6H_8O_2$** CC(=C)C(=C)C(=O)O		112.13	(E,E)-2,4-hexadienoic
23	succinic	Latin *succinum* 'amber'	**$C_4H_6O_4$** C(CC(=O)O)C(=O)O		118.09	butane-1,4-dioic

24	tartaric	Medieval, alchemical term *tartarus* Latin *tartarum*	**$C_4H_6O_6$** C(C(C(=O)O)O)(C(=O)O)O		150.09	(2R,3R)-2,3-dihydroxy-1,4-butanedioic

[1] Chemical structures and molecular mass data were retrieved from https://pubchem.ncbi.nlm.nih.gov/

4. Microbiota involved in aliphatic organic acids (AOA) of honey

Hugh and Leifson (1953) pointed out a taxonomic difference between fermentation and oxidative metabolism of carbohydrates. Functional services to the bee host by microbial associations are evidenced in the abstract on microbiology of ripening honey (Ruiz-Argueso and Rodriguez-Navarro, 1975), almost 50 years ago: "Two main groups of bacteria, identified as *Gluconobacter* and *Lactobacillus*, are present in ripening honey. A third bacterial group, classified as *Zymomonas*, and several types of yeast are occasionally isolated. Both in natural honey and in synthetic syrup the bacterial population decreases in the course of the ripening process. *Lactobacillus* and *Gluconobacter* disappear after minimum moisture (about 18%) is reached, but the former does so sooner than the latter. The presence of these bacteria in different parts of the bee has been also investigated". Despite numerous inhibiting factors, microorganisms such as yeasts and spore-forming bacteria can survive in honey. Distinct bee foraging behaviors, along with different floral resources in a particular habitat can shape stingless bee gut microbial communities (Vásquez et al., 2012). These authors suggested a long, more than 80-million-year history of association between LAB microbiota (*Lactobacillus* and *Bifidobacterium*) symbionts to modulate *A. mellifera* health. Symbionts are beneficial microbes, when associated with bees, they secrete antimicrobial compounds that prevent the spoilage of food stores (Shanahan and Spivak, 2021).

A dynamic microbial community was described for honey processing and ripening inside the honeycomb (Wen et al., 2017). Moreover, a recent idea attributed the origin of antibacterial compounds in honey to antagonistic microbial interactions in floral nectars, honey itself, and the bee (Brudzynski, 2021). Therefore, not only transient successions on microbiota, but also fingerprints of bioactive signals are propagated along microbiological processes in the honey matrix. The organic acids are a portion of this molecular universe. A fraction of them has microbial origin. Acetic acid bacteria (AAB) produce acetic acid, lactic acid bacteria (LAB) produce lactic acid, and yeasts produce alcohols among other metabolites. Honey bee gut microbes (8-10 core bacterial species are active *in vivo*) assist in host nutrition through fermentation, expressing key enzymatic genes critical for utilizing plant-derived molecules, breaking down saccharides and producing organic acids (Lee et al., 2018). An understanding of the metabolic contributions of symbionts was based on metagenomes, metatranscriptomes, and a combination of molecular and biochemical approaches. These authors found that core taxa may contribute to weight gain in honey bees, especially by the organic acid production. Therefore, the organic acids of honey are produced for the bees, not to please our human senses. Dietary pollen restriction and organic acid feeding treatments were applied for assessing roles of bacterial fermentation products on the diet-microbiota-host axis in honey bees. Organic acid feeding significantly impacted hindgut enteroendocrine signaling gene expression, demonstrating some effects of pollen restriction. Ricigliano et al. (2020), suggested bee health intervention are crucially affected by diet-based microbiota manipulations, and recommended further studies in this area. Consistent compartment-specific core bee-gut-microbiome components –1. Recurring bacteria, 2. Less abundant environmental bacteria, 3. Fungus or yeast– were mapped in the honey bee gut (Callegari et al., 2021). These authors hypothesized that ecological and physiological interactions of host-microbiome adapt to physicochemical and metabolic conditions, thus shaping the gut. When you find a title like this "Honey bees avoid nectar colonized by three bacterial species, but not by a yeast species, isolated from the bee gut" (Good et al., 2014), all the microbial associations with honey making bees convey the importance of yeast traits.

Recently, bacterial spectra in gut microbioma of eight Kenyan stingless bee species from sub-Saharan Africa were elucidated considering dominant genera from three bacterial families: 1. LACTOBACILLACEAE: *Acetilactobacillus* and *Apilactobacillus* dominated in *Hypotrigona* sp. 2, *Meliponula lendliana*, and *Axestotrigona ferruginea*; *Bifidobacterium* was dominant in *Hypotrigona* sp. 1; *Lactobacillus* dominated in *Hypotrigona* sp. 1 and *M. togoensis*, and *Bombilactobacillus* in *Dactylurina schmidti*. 2. ACETOBACTERACEAE: *Bombella* was dominant in *M. bocandei*, and *Saccharibacter* in *D. schmidti*, and 3. RICKETSIACEAE: the endosymbiont *Wolbachia* was the most abundant in *Liotrigona* sp. (Tola et al., 2021). Their bacterial-bee associations were demonstrated, and further clarification of their functions within the nest will take longer.

The following examples of studies on traditional fermented beverages, encourage us to ponder on the microbial processing of honey within the nest and during post-harvest: 1. In a whole-genome shotgun sequencing of pulque fermentation in Mexico, the abundance of genera and species varied with sucrose depletion and production of ethanol and lactic acid, suggesting that resource competition shapes microorganism diversity (Chacón-Vargas et al., 2020). 2. A 15-day snapshot on microbial-physicochemical integrated analysis comparing green and black tea kombucha fermentations, found different phenolic profiles with 16 fermentative volatiles, identified the most frequent microorganisms: *Komagataeibacter* and *Zygosaccharomyces* (Barbosa et al., 2021). They suggested *Komagataeibacter* and catechins (natural polyphenols) were preventing microbial contamination. A similar approach for integrating chemical-microbial-sensory indicators would be a contribution to identify post-harvest successions of microorganisms involved in pot-honey fermentation. A longer period of 30 days would be needed because LAB inactivated over a month storage in *Heterotrigona itama* Malaysian honey (Yaacob et al., 2021), and ethanol production stabilized in that time for *Tetragonisca angustula* honey from Venezuela (Pérez-Pérez et al., 2007). NMR or CE would be powerful techniques to measure the evolution of aliphatic organic acids (AOA) along the post-harvest honey fermentation process. Manuka honey was used as a fermentation substrate and revealed its prebiotic potential for a lactobacilli probiotic strain. A high biomass after 36 h, was partly explained by the presence of honey sugars and oligosaccharides (Mohan et al., 2021). With a pH below 4.0, lactic acid was the major metabolite among beneficial acetic, succinic, and propionic acids also known as short-chain fatty acids (SCFA).

Microbial parameters and antibacterial activities have recently merged to physicochemical parameters in databases for multivariate statistics useful to discriminate stingless bee entomological (Avila et al., 2019) and geographic (Rosiak et al., 2021) origins of honey. Rosiak et al. (2021) used the reference plate method (total viable count, yeast and molds, lactic acid bacteria, *Bacillus* spp.) to compare honey from tropical and temperate climates, and concluded that total viable count could be an alternative to estimate *Bacillus* spp. *Bacillus cereus* was the most frequently isolated species in a *Melipona fasciata* nest from Panama (Gilliam et al., 1990), and *Bacillus* species were the major bacteria found in a *Heterotrigona itama* nest from Malaysia (Ngalimat, 2019). However, the role of microorganisms associated with stingless bees, either food defense from pathogens, conservation or nutritional benefits for the colony, need further research to prove mutualism between stingless bees and microbiota harboured in their nests (Morais et al., 2013, de Paula et al., 2021).

5. Quantities of aliphatic organic acids (AOA) of honey

Different authors have studied diverse organic acids in honey. This is not necessarily for a biological scope but is more related to the availability of laboratory resources for capillary electrophoresis, enzymatic, chromatographic, spectrophotometric or spectroscopic techniques, for chemical scopes on methodology and honey authenticity. Precise measurements of previously reported and novel AOA challenge the complex honey matrix. Therefore, comparisons of data are not immediate in terms of organic acid types, their origins and functions for the bee colony. Additionally, the units used to quantify these AOA in honey are not harmonized (g/100 g, mg/100 g, g/kg, mg/kg), and may induce miscalculations in the literature. Recently, a targeted NMR for multiparametric analysis operating with a mixture tube of ten organic acids (acetic, citric, formic, fumaric, lactic, malic, pyruvic, quinic, shikimic and succinic) from the reference sample for quantification in food applications (Hl 44518 - QuantRefA-NMR-Tube 5mm, 600µL), became available to generate a successful dataset for chemists. But this challenges other professional fields, because it needs to be interpreted by biologists, ecologists, microbiologists and related areas from agronomy to health science. The analysis of a set of 12 organic acids from French honey (Daniele et al., 2012) by ion chromatography was also an important achievement besides all contributions reviewed here. This sudden access to new information is an impact for understanding different types and concentrations of AOA in honey. Also note that some of these 10-12-14 organic acids were not detected in some honeys e.g. fumaric and tartaric acids were absent in Brazilian honeys from Santa Catarina, but tartaric was a major acid for honeys of the German (Ohmenhaeuser et al., 2013) and Latvian (Keke and Cinkmanis, 2019) studies. In this vast universe of honey, shikimic acid from the targeted NMR reference was absent in Ecuadorian pot-honeys (Vit et al., 2023) but it was present in Tanzanian pot-honey (Popova et al., 2021), thus could be a potential entomological marker for that habitat where *Axestotrigona ferruginea* thrives. Therefore, exploring non reported organic acids in honey using multiple techniques and detectors is justified.

The AOA identified and quantified in diverse honey types (bee, floral or honeydew botanical and geographical origins), were tabulated in this section for comparative analysis, providing a fast view of AOA in honey retrieved from literature search. For practical reasons, different tables were produced and data harmonized to g/100g. Some articles merited individual tables either for the variety of tested honey types or organic acids types. Separate tables were also produced when the published concentrations (too low) did not correspond to reference values validated along the years across diverse habitats for particular botanical and entomological origins. Their relative values should be good but the absolute values need refining of the reported concentrations. Authors were contacted and a personal communication note was adequate for corrections in this review. The reader is advised to use the original references with detailed sampling, analytical techniques, statistics and discussions. The columns with total contents of quantified organic acids in 100 g of honey were mostly calculated for this review, because generally authors do not report that addition, facilitated now by adopting the harmonized g/100 g concentration.

Influential lactic acid bacteria were known for the honey making process (Ruiz-Argueso and Rodriguez-Navarro, 1975), but instead of measuring lactic acid, galacturonic, citramalic and pyruvic acids were chosen. The authors did not explain the reason for their choices. More than a decade later, galacturonic acid was the major acid in honeydew honey (Daniele et al., 2012), so it seemed it was a good choice for these authors to predict it might be a useful acid to measure in honey. Concentrations of organic acids were expressed in g/kg honey (del Nozal et al., 1998), and here they were harmonized to g/100 g in Table 2, corresponding to standard values found in later studies. Fumaric and pyruvic acid concentrations are visible in mg but become zero in g., similarly for galacturonic acid except for a multifloral honey with 0.01 g/100 g. Since gluconic acid is often the major AOA of honey, its concentration affects the total content of AOA. Such is the case for honey produced from flowers in the *Erica* genus, which has the highest content of gluconic acid (0.49) and quinic acid (0.39), which resulted in the highest total AOA content of 1.00 g/100 g honey. This could have been a valid reference, but instead, Mato et al. (2003) decided that a 50% of that will do, and made it as organic acids < 0.5% honey. This reference is still successfully cited after more than 20 years, despite evidences confirming it was underestimated since 1998, which was the leading contribution on AOA in honey. Two chromatographic procedures were needed for the measurements: 1. The first one used two reversed-phase columns (Spherisorb ODS-1 S5) connected in series and only three organic acids (galacturonic, pyruvic and citric) were quantified, 2. The second one needed one ion-exchange. The eluent was filtered with membrane filter column for the quantification of four acids (malic, citramalic, quinic and formic). Other two organic acids (succinic and fumaric), were quantified using both chromatographic methods (del Nozal et al., 1998).

Table 2. Average contents of AOA (g/100 g) in 57 Spanish *Apis mellifera* honeys originally (mg/kg) of nine botanical origins (Cav *Calluna vulgaris* n=11, Eri *Erica* spp. n=2, Lae *Lavandula esteoechas* n=3, Lal *Lavandula latifolia* n=12, Mfl Multifloral n=9, Ons *Onobrychis sativa* n=4, Que *Quercus* spp. n=10, Roo *Rosmarinus officinalis* n=2, Thy *Thymus* spp. n=4) from Spain by Solid-phase extraction **SPE** High Performance Liquid Chromatography **HPLC** (del Nozal et al., 1998), originally reported in g/kg.

Bee	**Aliphatic Organic Acids AOA (g/100g)**										**Total**
	CiA	**CmA**	**FoA**	**FuA**	**GaA**	**GcA**	**MaA**	**PvA**	**Qia**	**SuA**	
Cav	0.03	0.01	0.01	0.00	0.00	0.38	0.02	0.00	0.01	0.14	0.60
Eri	0.01	0.01	0.02	0.00	0.00	0.49	0.00	0.00	0.35	0.12	**1.00**
Lae	0.02	0.00	0.02	0.00	0.00	0.41	0.00	0.00	0.00	0.05	0.50
Lal	0.01	0.01	0.01	0.00	0.00	0.37	0.00	0.00	0.01	0.08	0.49
Mfl	0.04	0.02	0.01	0.00	0.01	0.27	0.03	0.00	0.02	0.11	0.51
Ons	0.01	0.00	0.01	0.00	0.00	0.16	0.02	0.00	0.00	0.01	**0.21**
Que	0.02	0.02	0.00	0.00	0.00	0.22	0.10	0.00	0.00	0.18	0.54
Roo	0.01	0.01	0.01	0.00	0.00	0.24	0.00	0.00	0.01	0.01	0.29
Thy	0.07	0.01	0.01	0.00	0.00	0.18	0.03	0.00	0.00	0.14	0.44

In bold minimum and maximum total contents of AOA.

CiA citric acid, **CmA** citramalic acid, **FoA** formic acid, **FuA** fumaric acid, **GaA** galacturonic acid, **GcA** gluconic acid, **MaA** malic acid, **PvA** pyruvic acid, **QiA** quinic acid, **SuA** succinic acid.

After: del Nozal et al. (1998)

In Table 3, the content of organic acids in honey in the last two decades since 2002 is presented with correct values. Besides the Cherchi et al. (1994) recoveries of 29 AOA, limits of detection of 31 AOA and six of them assessed in honey of four botanical origins. The Turkish study assessed six AOA in six botanical origins (Tezcan et al., 2011), the French study with 12 AOA assessed in seven botanical origins (Daniele et al., 2012), and the German study with seven AOA assessed in 19 botanical origins (Ohmenhaeuser et al., 2013) deserved their own tables within this group of *A. mellifera* honey from Brazil, Bulgaria, Chile, China, Latvia, Italy, Spain, and stingless bee honey from Australia, Brazil, Tanzania and Venezuela. The highest total AOA contents corresponded to the Venezuelan *Melipona favosa* honey with 6.36 g/100 g and the Tanzanian *Axestotrigona ferruginea* honey with 5.77 g/100 g. A higher content than the maximum 5% sucrose permitted in the honey standards. Evidence for tropical stingless bee sour honey from Africa and Venezuela. Moreover, the gluconic acid was not measured by NMR. More studies are needed, but a new reference for organic acids in stingless bee honey is proposed here. The aforementioned stingless bee honeys were more than ten-fold higher than the reported <0.5% AOA in *A. mellifera* honey (Mato et al., 2003). Using HPLC, del Nozal et al. (1998) reported *Erica* honey with 1% organic acids, mostly explained by the high gluconic and quinic acid contents, and lower for the other seven botanical origins (0.21-0.60 g/100 g honey). Daniele et al. (2012) observed 1.27 % AOA in chestnut and 1.15% in fir honeys, lower contents for the five other botanical sources (0.24 to 0.89 g/100 g). In 2013, an NMR screening of 19 diverse botanical origins of *Apis mellifera* honey only had one polyfloral honey with <0.5% AOA, and the rest varied in the range from 0.52 to 1.78 g/100 g (Ohmenhaeuser et al., 2013), see Table 6. Suárez-Luque et al. (2002) focused on quantifying five minor AOA (citric, fumaric, maleic, malic, succinic), and their reports of maleic acid were the first in honey, although 0.00 g/100 g. There is a study with 225 Iranian honeys where 10 AOA were quantified by targeted NMR, but the data are not usable in our Table 3 since they were reported only in boxplots and a biplot of Principal Component Analysis (PCA) (Khansaritoreh et al., 2021).

Table 3. Average contents of AOA (g/100 g) in ***Apis mellifera* honeys** (AmIf multifloral, AmIau strawberry tree *Arbutus unedo*, AmIs *Asphodel*, AmIy red gum *Eucalyptus*) from Italy **SPE-HPLC** (Cherchi et al., 1994); (AmSc *Castanea* n=3, AmSe *Eucalyptus* n=21, AmSt *Trifolium* n=1, AmSu multifloral n=25) from Spain **HPLC-UV** (Suárez-Luque et al., 2002); (AmS) n=10 from Spain **CZE** (Mato et al., 2006); (AmH) n=1 from China **EME-IC** (Tan et al., 2018); (AmLa apiary n=5, AmLc n=4 commercial) from Latvia **HPLC** (Keke and Cinkmanis, 2019); (AmC) from Chile n=12 targeted **^{1}H-NMR** (Fuentes Molina et al.,

2020); (AmBf floral n=32, AmBh honeydew n=60) bracatinga *Mimosa scabrella* from Brazil **CE** (Seraglio et al., 2021a); (AmBq) n=1 at 0 months storage, bracatinga *Mimosa scabrella* from Brazil (Seraglio et al., 2021b); and (AmU) n=10 from Bulgaria untargeted **1H-NMR** (Popova et al., 2021); and **Stingless bee honeys** produced by *Tetragonula carbonaria* (Tcar1A) n=8 from Australia **ENZ** (Persano Oddo et al., 2008); *Melipona favosa* (MfavV) n=7 from Venezuela **ENZ** (Sancho et al., 2013); *Melipona fasciculata* (MfasB) n=18 and *Melipona subnitida* (MsubB) n=11 from Brazil **HPLC** (Sant'ana et al., 2020); *Axestotrigona ferruginea* (AferT) n=1 from Tanzania untargeted **^{1}H-NMR** (cited as *Meliponula ferruginea* by Popova et al., 2021); *Austroplebeia australis* (AausA) n=19, *Austroplebeia cassia*e (AcasA) n=3, *Tetragonula carbonaria* (Tcar2A) n=61, *Tetragonula davenporti* (TdavA) n=3, and *Tetragonula hockingsi* (ThocA) n=25 from natural forest, suburban garden, and farm habitats Australia **HPIC-SCD** (Hungerford et al., 2023); and *Geotrigona* (GeoE) n=8 , *Melipona* (MelE) n=4, and *Scaptotrigona vitorum* (SvitE) n=8 from Ecuador targeted **^{1}H-NMR**.

Honey Types	Aliphatic Organic Acids (AOA) g/100g																Total	Year
	AcA	CiA	FoA	FuA	GcA	GtA	GyA	LaA	MaA	MeA	OxA	PyA	QiA	ShA	SuA	TaA		
***Apis mellifera* Honeys**																		
AmIau	-	0.02	-	0.00	1.16	-	-	-	0.01	-	-	0.01	-	-	0.01	-	1.21	1994
AmIfl	-	0.01	-	0.00	0.69	-	-	-	0.01	-	-	0.01	-	-	0.00	-	0.72	
AmIs	-	0.01	-	0.00	0.20	-	-	-	0.00	-	-	0.00	-	-	0.00	-	0.21	
AmIy	-	0.01	-	0.00	0.61	-	-	-	0.02	-	-	0.01	-	-	0.00	-	0.63	
AmSc	-	0.03	0.00	0.00	-	-	-	-	0.03	0.00	-	-	-	-	0.05	-	0.11	2002
AmSe	-	0.00	0.00	0.00	-	-	-	-	0.01	0.00	-	-	-	-	0.01	-	0.02	
AmSt	-	0.01	0.00	0.00	-	-	-	-	nd	0.00	-	-	-	-	nd	-	**0.01**	
AmSu	-	0.01	0.00	0.00	-	-	-	-	0.01	0.00	-	-	-	-	0.01	-	0.03	
AmS	0.02	0.01	0.03	-	0.00	-	-	0.02	0.02	-	0.00	0.00	-	-	0.00	-	0.10	2006
AmH	-	-	0.02	0.00	-	-	-	-	0.00	0.00	0.00	-	-	-	0.00	-	0.02	2018
AmLa[1]	-	0.04	-	0.00	-	-	-	-	0.01	-	0.00	-	0.24	-	0.06	0.61	0.96	2019
AmLc[1]	-	0.01	-	0.00	-	-	-	-	0.02	-	0.00	-	0.00	-	0.01	0.62	0.66	
AmC[2]	0.00	0.03	0.01	0.00	-	-	-	0.01	0.04	-	-	-	0.01	0.00	0.01	-	0.11	2020
AmBf	0.00	0.03	0.00	-	0.87	nd	0.00	0.00	0.00	-	-	-	-	-	0.00	-	0.93	2021
AmBh	0.02	0.05	0.02	-	0.96	0.03	0.03	0.17	0.14	-	-	-	-	-	0.13	-	1.53	
AmBq	0.02	0.05	0.05	-	1.00	0.02	0.00	0.26	0.10	-	-	-	-	-	0.13	-	**1.62**	2021
AmU	0.02	0.00	0.02	-	-	-	-	0.01	0.00	-	-	0.00	-	0.00	0.01	-	0.06	2021
Stingless Bee Honeys																		
Tcar1A	-	0.01	-	-	0.99	-	-	-	0.02	-	-	-	-	-	-	-	1.02	2008
MfavV[3]	-	0.00	-	-	0.64	-	-	-	0.00	-	-	-	-	-	-	-	0.64	2013
MfasB[4]	0.16	0.01	-	0.00	-	-	-	-	0.00	-	-	-	-	-	0.00	0.56	0.73	2020
Msub[4]	0.39	0.04	-	0.00	-	-	-	-	0.00	-	-	-	-	-	0.00	0.94	1.37	
AferT	3.05	0.18	0.01	-	-	-	-	2.38	0.12	-	-	0.01	-	0.02	0.00	-	**5.76**	2021

AausA[5]	0.10	0.01	-	nd	2.50	-	-	0.01	0.04	0.01	0.00	-	0.03	0.01	0.00	nd	2.72[6]	
AcasA[5]	0.03	0.00	-	nd	1.64	-	-	0.00	0.00	0.03	0.00	-	0.02	0.00	0.00	nd	1.72	
Tcar2A[5]	0.43	0.03	-	nd	1.65	-	-	0.58	0.01	0.02	0.01	-	0.05	0.00	0.01	nd	2.81[6]	2023
TdavA[5]	0.87	0.02	-	nd	1.90	-	-	1.04	0.01	0.01	0.00	-	0.08	0.01	0.01	nd	3.96	
ThocA[5]	0.33	0.02	-	nd	1.29	-	-	0.43	0.01	0.03	0.00	-	0.03	0.00	0.01	nd	2.15[6]	
GeoE	1.96	0.02	0.01	0.00	-	-	-	2.43	0.01	-	-	0.00	nd	-	0.01	-	4.44	
MelE	0.13	0.00	0.00	0.00	-	-	-	0.16	0.00	-	-	0.00	nd	-	0.00	-	**0.29**	2023
SvitE	0.12	0.01	0.00	0.00	-	-	-	0.16	0.00	-	-	0.00	nd	-	0.00	-	**0.29**	
Honey Types	**AcA**	**CiA**	**FoA**	**FuA**	**GcA**	**GtA**	**GyA**	**LaA**	**MaA**	**MeA**	**OxA**	**PyA**	**QiA**	**ShA**	**SuA**	**TaA**	**Total**	**Year**
	Aliphatic Organic Acids (AOA) g/100g																	

nd *not detected or below LOD.* Latvian and Chinese honeys were analyzed for oxalic acid, but it was 0.00 g/100 g, as well as fumaric and tartaric acid from Australia.
[1] Averages were calculated for this review.
[2] Units were updated to mg/kg Chilean honey instead of reported g/kg (P. Vásquez Quitral, personal communication).
[3] Errata sheet (M.T. Sancho, personal communication).
[4] Honey AOA averages were provided for this review (C.A.L. Carvalho, personal communication) because medians were published.
[5] Honey AOA averages for the Australian stingless bee species (N.L. Hungerford, personal communication).
[6] Total was calculated by adding the sub-total of three additional AOA for **AdA** adipic acid (0.01g/100g Tcar2A honey), **MoA** malonic acid ND, and **SoA** sorbic acid (0.01g/100g AausA, Tcar2A, and TdavA honeys) (N.L. Hungerford, personal communication).

In bold, the lowest and highest AOA total contents within each group of honey, *Apis mellifera* and stingless bee (Meliponini)

EME-IC Electro Membrane Extraction - Ion Chromatography, **ENZ** Enzymatic, **HPLC** High Performance Liquid Chromatography, **NMR** Nuclear Magnetic Resonance, **HPIC-SCD** High Performance Ion Chromatography combined with Suppressed Conductivity Detection, **AcA** acetic acid, **CiA** citric acid, **FoA** formic acid, **FuA** fumaric acid, **GcA** gluconic acid, **GtA** glutaric acid, **GyA** glycolic acid, **LaA** lactic acid, **MaA** malic acid, **MeA** maleic acid, **OxA** oxalic acid, **PyA** pyruvic acid, **QiA** quinic acid, **ShA** shikimic acid, **SuA** succinic acid, **TaA** tartaric acid.

After: Cherchi et al., 1994, Suárez-Luque et al., 2002, Mato et al., 2006, Persano Oddo et al., 2008, Sancho et al., 2013, Tan et al., 2018, Keke and Cinkmanis, 2019, Fuentes Molina et al., 2020, Sant'ana et al., 2020, Popova et al., 2021, Seraglio et al., 2021a, Hungerford et al., 2023, Vit et al., 2023.

Six organic acids were identified and quantified with Capillary Electrophoresis (CE) for six botanical origins of Turkish *A. mellifera* honey, as given in Table 4. Turkish acacia and floral honey had a simple organic acid spectrum consisting in low quantities of gluconic acid (0.15-0.20), intermediate for floral from the Anzac plateau, pine and *Rhododendron* (0.35-0.61), and chestnut was top with 1.12 g/100 g (Tezcan et al., 2011)

Table 4. Average contents of six AOA (g/100 g) in *Apis mellifera* Turkish honey from six botanical origins by Capillary Electrophoresis **CE** (Tezcan et al., 2011) originally reported as mg/kg and g/kg for gluconic acid, Acacia n=1, Chestnut n=3, Floral n=1, Floral Anzer plateau n=2, Pine n=1, Rhododendron n=2. Acronyms were not needed, because the common name of the honey type fits in the column.

Botanical origin	**Aliphatic organic acids AOA (g/100 g)**						
	CiA	**FoA**	**GcA**	**MaA**	**OxA**	**SuA**	**Total**
Acacia	nd	nd	0.15	nd	nd	nd	**0.15**
Chestnut	0.03	0.11	1.12	0.05	nd	0.01	**1.32**
Floral	nd	nd	0.20	nd	nd	nd	0.20
Floral Anzer plateau	nd	0.00	0.49	nd	nd	nd	0.49
Pine (honeydew)	0.01	0.01	0.35	0.03	nd	nd	0.40
Rhododendron	nd	0.00	0.61	0.02	nd	0.00	0.63

nd *not detected.* In bold minimum and maximum total contents of AOA.

CiA citric acid, **FoA** formic acid, **GcA** gluconic acid, **MaA** malic acid, **OxA** oxalic acid, **SuA** succinic acid.

After: Tezcan et al., 2011

In 2012, Daniele et al. analyzed twelve organic acids using ion chromatography (IC) with an electrochemical detector (ED) in 140 French honeys from seven unifloral types. The $^{13}C/^{12}C$ isotopic ratios of the honeys, and of the organic acids extracted from them with an anion exchange resin, were additionally determined by isotope ratio mass spectrometry. The isotopic ratios of honeys and of their acids were strongly linked. Table 5 presents the contents of AOA in this study. Glutamic acid and pyroglutamic acid were not included in this review because they are amino acids. Citramalic and isocitric acid were not present in the French honeys, like the *cis*-Aconitic and butyric acids that were not detected or 0.00 g/100 g. Total organic acids >1 % for chestnut and fir honeys, and lowest for acacia varying from 0.24 to 1.63 g/100 g honey. These honey types were separated according to their botanical origins in distinctive clusters by Principal Component Analysis. The best PCA for organic acids of honey in the reviewed literature. Fir honeys higher contents of pyruvic and quinic acid were distinctive, with the major AOA being galacturonic acid. Lavender honey's acidity was mostly explained by 0.50 g/100 g gluconic acid.

Table 5. Average contents of 12 organic acids (g/100 g) in n=140 *Apis mellifera* French honey from seven botanical origins by **IC-ED** (n=20 each) ion chromatography and electrochemical detector (Daniele et al., 2012), originally reported as mg/kg.

Botanical origin	Aliphatic organic acids AOA (g/100 g)												
	AoA	**BuA**	**CiA**	**CmA**	**FoA**	**GaA**	**GcA**	**IsA**	**LaA**	**PrA**	**PyA**	**QiA**	**Total**
Acacia	nd	nd	0.01	0.00	0.00	0.01	0.19	0.00	0.02	0.00	0.00	0.01	**0.24**
Chestnut	0.00	nd	0.01	0.00	0.19	0.01	1.27	0.00	0.08	0.00	0.00	0.07	**1.63**
Fir (honeydew)	0.00	nd	0.04	0.00	0.05	0.46	0.24	0.00	0.02	0.03	0.13	0.18	1.15
Lavender	nd	0.00	0.00	0.00	0.03	0.00	0.50	0.00	0.03	0.00	0.00	0.03	0.59
Linden	nd	0.00	0.01	0.00	0.09	0.01	0.71	0.00	0.05	0.00	0.00	0.02	0.89
Rapeseed	nd	0.00	0.01	0.00	0.01	0.01	0.23	0.00	0.01	0.00	0.00	0.03	0.30
Sunflower	0.00	nd	0.02	0.00	0.02	0.00	0.51	0.00	0.03	0.00	0.00	0.02	0.60

nd *not detected*. In bold minimum and maximum total contents of AOA.

CE capillary electrophoresis, **AoA** *cis*-Aconitic, **BuA** butyric acid, **CiA** citric acid, **CmA** citramalic acid, **FoA** formic acid, **GaA** galacturonic acid, **GcA** gluconic acid, **IsA** isocitric acid, **LaA** lactic acid, **PrA** propionic acid, **PyA** pyruvic acid, **QiA** quinic acid.

After: Daniele et al., 2012

Seven organic acids were identified and quantified with untargeted NMR, spectroscopic ^{1}H NMR and ^{13}C NMR in 328 samples from 19 unifloral, polyfloral and honeydew types of honey purchased in Germany. These samples had the following chemical shifts in ppm for citric (2.68-2.69), formic (8.44-8.47), fumaric (6.53-6.55), malic (2.73-2.70), pyruvic (6.42-6.45), and succinic (2.50-2.52) acids (Ohmenhaeuser et al., 2013). It was the most extensive evaluation of honey types by NMR. Table 6 presents the contents of AOA in the German study. A mixture of floral honey and royal jelly was removed from the original data, and also the phtalic acid because it is an aromatic dicarboxylic acid, which was not detected anyway. These authors are unique at informing concentrations for not detectable organic acids in the legend of the table. Gluconic acid was not included in this German NMR study with the highest AOA content of 1.8% honey, thus a quantity of 2.5% was set as reference value, considering a conservative average gluconic acid content of 0.7% honey. See values of gluconic acid in *A. melliffera* honeys up to 1.16 in Italian honey (1994) and 0.87-1.00 g/100 g Brazilian honey (2021) in Table 3.

Table 6. Average contents of seven organic acids (g/100 g) in n=328 *Apis mellifera* honey from 19 botanical origins by untargeted **NMR** (Ohmenhaeuser et al., 2013) originally reported as mg/kg.

Botanical origin	**Aliphatic organic acids AOA (g/100 g)**							
	CiA	**FoA**	**FuA**	**MaA**	**PyA**	**SuA**	**TaA**	**Total**
Manuka	0.09	0.15	nd	nd	nd	0.03	0.31	0.58
Floral	nd	0.05	nd	0.20	nd	0.01	0.31	0.57
Sunflower	0.49	0.06	0.03	0.40	nd	0.01	0.38	1.37
Honeydew	0.25	0.08	0.02	nd	nd	0.17	0.58	1.10
Chestnut	nd	0.71	0.05	nd	nd	0.08	0.64	1.48
Polyfloral	nd	0.15	0.04	0.90	nd	0.10	0.59	**1.78**
Robinia pseudoacacia	0.17	0.04	nd	nd	nd	0.01	0.48	0.70
Orange	0.26	0.06	nd	0.25	nd	0.02	0.61	1.20
Polyfloral	nd	0.07	nd	a	nd	0.02	0.35	**0.44**
Floral mountains	0.10	0.04	nd|	nd	nd	nd	0.38	0.52
Rape	nd	0.07	nd	nd	nd	0.03	0.39	0.49
Fruit tree blossom	nd	0.12	0.03	nd	nd	0.04	0.44	0.63
Polyfloral	nd	0.08	0.03	nd	nd	0.03	0.46	0.60
Polyfloral	nd	0.09	0.03	nd	nd	0.07	0.47	0.66
Chestnut	0.19	0.80	0.04	nd	nd	0.18	0.74	1.75
Honeydew	0.28	0.11	0.04	nd	nd	0.28	0.66	1.36

Polyfloral	nd	0.11	nd	nd	nd	0.03	0.54	0.68
Eucalyptus	nd	0.10	nd	0.28	nd	0.06	0.45	0.89
Polyfloral	nd	0.63	nd	nd	nd	0.05	0.59	1.27

nd *not detectable* 0.04 (citric acid), 0.02g/kg (fumaric acid), 0.03g/kg (malic acid), 0.18g/kg (pyruvic acid), and 0.01g/kg (succinic acid).
a Overlapped signal, direct quantification is not possible.
In bold minimum and maximum total contents of AOA.

CiA citric acid, **FoA** formic acid, **FuA** fumaric acid, **MaA** malic acid, **PyA** pyruvic acid, **SuA** succinic acid, **TaA** tartaric acid.

After: Ohmenhaeuser et al., 2013.

From the eight organic acids studied by HPLC in *Geniotrigona thoracica* (Gt) and *Heterotrigna itama* (Hi) honey from Malaysia (Table 7), formic acid was not detected (Shamsudin et al., 2019). These authors analyzed three monofloral types (Ac) acacia *Acacia mangium* FABACEAE, (Ge) gelam *Melaleuca cajaputi* MYRTACEAE, and (St) starfruit *Averrhoa carambola* OXALIDACEAE, to observe further variations in AOA contents (g/kg) caused by these stingless bee species and the nectar origin interactions. These units should be divided by 10 to become (g/100g) as in Table 7, but by doing that, calculated values will become too low, and total values of organic acids will range from 0.02 to 0.20 which seems too low compared with the reviewed database. Possibly these authors reported their values in g/100 g and not in g/kg.

Table 7. Average contents of organic acids (g/kg) in honey from two species of Malaysian stingless bees foraging three nectar types (Ac Acacia, Ge Gelam, Sf Starfruit) by **HPLC** (Shamsudin et al., 2019). *Geniotrigona thoracica* (Gt) GtAc, GtGe and GtSf, and *Heterotrigna itama* (Hi) HiAc, HiGe, and HiSf.

Bee and flower	**Aliphatic organic acids AOA (g/kg)**								
	AcA	**CiA**	**FoA**	**GcA**	**LaA**	**MaA**	**SuA**	**TaA**	**Total**
GtAc	0.09	0.04	nd	0.48	0.20	nd	0.52	0.04	1.37
GtGe	0.06	0.03	nd	0.55	0.17	0.30	nd	nd	1.11
GtSf	0.08	0.05	nd	nd	0.03	nd	0.07	nd	**0.23**

HiAc	0.30	0.04	nd	0.90	0.15	0.03	0.32	0.06	1.80
HiGe	0.01	0.09	nd	1.48	0.18	0.03	0.34	nd	**2.13**
HiSf	0.06	0.06	nd	0.39	0.18	nd	0.38	0.03	1.10

nd *not detected*. In bold minimum and maximum total contents of AOA.

AcA acetic acid, **CitA** citric acid, **FoA** formic acid, **GcA** gluconic acid, **LaA** lactic acid, **MaA** malic acid, **SuA** succinic acid, **TaA** tartaric acid.

After: Shamsudin et al., 2019

In some documents, units are reported with a misrepresentation of AOA concentrations (mg/100 g instead of g/100 g perhaps) like in Villacrés-Granda et al. (2021), see Table 8. Oxalic acid was most abundant in this set of honeys, except for *Cephalotrigona* sp. with top citric acid (0.68) and *Tetragonisca angustula*, with major concentrations of acetic acid (1.15) and lactic acid (0.72) followed by *Scaptotrigona polysticta* (0.69) in this group of 26 honey samples. *Melipona grandis* (0.73) and *Oxytrigona mellaria* (0.72) are leading the oxalic acid concentration, followed by *Nannotrigona chapadana* (0.69), *Tetragonisca angustula* (0.65), and *Nannotrigona chapadana* (0.63). Their AOA total contents varied from 0.44 to 3.00 for the four evaluated organic acids, but without correct units, they are not valid as Table 3 data are. This is the first study of this type for stingless bee species in Ecuador.

Positive interaction aimed to pave a harmonized expression g/100 g, adapting for this review and a further editorial action if needed. With the aim of increasing knowledge in this area, for a wider diversity of honey producing bee species, this review has adapted the units of measurement to harmonize all results for the reviewed literature confirm the necessity of harmonizing the AOA units to the familiar g/100 g proposed here instead of a variety of g/kg, mg/kg, mg/100 g found in literature. However, each laboratory follows internal norms and will continue accordingly.

Table 8. Average contents of organic acids (mg/100 g) in honey from 12 species of Ecuadorian stingless bees by **HPLC** (Villacres-Granda et al., 2021). *Cephalotrigona* sp. (Cep) n=1, *Melipona* sp. (Mel) n=1, *Melipona cramptoni* (Mcra) n=3, *Melipona grandis* (Mgra) n=1, *Melipona indecisa* (Mind) n=6, *Melipona mimetica* (Mmi) n=1, *Nannotrigona chapadana* (Ncha) n=1, *Oxytrigona mellaria* (Omel) n=1, *Paratrigona* sp. (Par) n=1,

Scaptotrigona polysticta (Spol) n=7, *Tetragonisca angustula* (Tang) n=3, *Tetragonisca silvestriana* (Tsil) n=1. These units are not comparable with the g/100 g honeys; thus, not possible to include a discussion. The relative values of AOA in Table 8 are good.

Stingless Bee	**Aliphatic organic acids AOA(mg/100 g)**				
	AcA	**CiA**	**LaA**	**OxA**	**Total**
Cep	0.06	**0.68**	0.04	0.44	1.22
Mel	0.06	nd	nd	0.50	0.56
Mcra	0.09	0.12	0.09	0.24	0.54
Mgra	0.15	0.03	0.17	**0.73**	1.08
Mind	0.18	0.10	0.13	0.30	0.71
Mmi	nd	0.07	nd	0.37	**0.44**
Ncha	0.19	0.12	0.33	0.63	1.27
Omel	0.06	0.12	0.28	0.72	1.18
Par	0.06	0.13	nd	0.40	0.59
Spol	0.41	0.40	0.69	0.31	1.81
Tang	**1.19**	0.44	**0.72**	0.65	**3.00**
Tsil	0.24	0.13	0.16	0.69	1.22

nd *not detected*
In bold major organic acids other than oxalic acid. Also minimum and maximum total contents of AOA.

AcA acetic acid, **CiA** citric acid, **LaA** lactic acid, **OxA** Oxalic acid

After: Villacres-Granda et al., 2021

6. Aliphatic organic acids (AOA) of honey

Aliphatic organic acids (AOA) have an organic backbone of one to six carbons, are also known as short-chain fatty acids (SCFA) starting by the smallest with a 2C chain, acetic acid. Formic acid is the simplest of all with only one carbon. They are known as carboxylic acids, with one, two or three carboxylic groups, thus monocarboxylic, dicarboxylic and tricarboxylic. They are cell signaling molecules in plant metabolism. Natural AOA in honey are intermediates or final products of plant and microbiota Krebs cycle (citric, succinic, glutaric, fumaric, oxalic), derived from bee enzymes (gluconic), aerobic or anaerobic processes (acetic, lactic). Many of them turnover into other related molecules (e.g. succinic acid to fumaric acid, and further fumarate to malate, or pyruvate to lactate). It seems confusing so many intermediates of the citric acid cycle, but should be an evolutionary molecular versatility for organic biosynthesis.

Some AOA of honey are volatile (acetic, formic, propionic, pyruvic), they contribute to odor-aroma descriptors. Interestingly, some of these AOA may have specialized functions within the bee colony (communication, nutrition, prolonged storage), but their exact origin is complex, as they are important in a mixture of convergent synthesis within the honey matrix. More than 600 volatile organic compounds (VOCs) have been identified in honey (Montenegro et al., 2009). They are classified in functional groups or chemical families, originating from various metabolic pathways in plant sources (nectar, honeydew, microbiota), individual bees vectoring products to the nest, within the nest (nectar or honeydew, bee, honey making process, microbiota) and also post-harvest, processing, storage, and environmental factors.

In this section each organic acid studied in honey is presented with information on its origin, uses in food science (chemical and molecular formulas are available on-line, including metabocards) and some examples of their concentrations in different botanical and entomological types tabulated in the previous section of the review. Metabocard synthesis from the Metaboloma Database were used for the biochemical descriptions of AOA, mostly related to the Krebs cycle or TCA cycle. The menu string gives access to Identification, Taxonomy, Ontology, Physical properties, Spectra, Biological Properties, Concentrations, Links, and References. It has entries for metabolites, diseases, pathways, proteins and reactions (See Supplementary Table S1 in Supplementary data after References). These metabocards in Human Metabolome Database (HMDB) were used for the descriptions of the 24 selected AOA studied in honey and pot-honey.

All of the AOA detected in honey actively contribute to its free acidity. They prepare the substrate for further microbiota developments. Their pattern was suggested as authenticity markers of *A. mellifera* unifloral French honeys (Daniele, 2012) and for bracatinga honeydew honey, which had been adulterated with *A. mellifera* floral honey from Brazil (Seraglio et al., 2021). Acetic acid "exists in all living species, ranging from bacteria to plants to humans", is a frequent expression for the diverse organic acids quantified in honey. It means their significance is wider than a marker, since they are protagonists of life processes. The acetyl group, derived from acetic acid, is fundamental to the biochemistry of virtually all forms of life. When bound to coenzyme A (to form acetylCoA) it is central to the metabolism of carbohydrates and fats. Citrate is an intermediate in the Tricarboxylic Acid Cycle (TCA) (also known as the Citric Acid cycle or Krebs cycle). The TCA cycle is a central metabolic pathway for all animals, plants, and microbes. As a result, citrate is found in all living organisms, from bacteria to plants to animals. Fumaric acid is a dicarboxylic acid. It is a precursor to L-malate in the TCA cycle. It is formed by the oxidation of succinic acid by succinate dehydrogenase. Fumarate is converted by the enzyme fumarase to malate.

Few authors included ascorbic acid in their AOA profile for honey from Latvian *A. mellifera* (Keke and Cinkmanis, 2019), and *Melipona* from Brazil (Sant'ana et al., 2020), but it was removed from the tabulated AOA because ascorbic acid is not a carboxylic acid, chemically it is (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one, IUPAC name (https://pubchem.ncbi.nlm.nih.gov/#query=ascorbic%20acid).

6.1 Acetic acid

Acetic acid $C_2H_4O_2$ is the smallest short-chain fatty acid (SCFA), with molecular formula CH_3COOH. It has a sour taste and pungent smell. Used in plastic soft drink bottles, photographic film, and polyvinyl acetate for wood glue, as a cleaning agent. Acetic acid bacteria (AAB) produce acetic acid. The family Acetobacteriaceae breaks down carbohydrates under acidic media. Acetic acid bacteria (AAB) are symbionts of the Australian stingless bee *Tetragonula carbonaria* but not of the stingless bee *Austroplebeia australis* (Leonhardt and Kaltenpoth, 2014), indicating a possible endemism and segregation in the forage sites. Acetobacter-like operational taxonomic unit (OTU) were associated with some stingless bee gut microbiota (Kwong et al., 2017). *Acetobacter* and *Gluconobacter* are the two main genera of AAB for obligate aerobic acetic acid fermentations. However, the honey making process in the bee nest is anaerobic. Therefore, another anaerobic microbiota may be involved. Acetic acid is volatile and was identified in *A. mellifera* honey (Siegmund et al., 2017). This volatile quality may have a functional role for bee behavior and communication. The highest concentration of acetic acid in this review was 3.05 g/100 g Tanzanian *Axestotrigona ferruginea* honey (Popova et al., 2021) may suggest this species process pot-honey with AAB. Similarly, *Geotrigona* honeys from Ecuador had an average of 1.96 g/100 g of acetic acid in honey, but lower 0.13 and 0.12 g/100 g were observed in *Melipona* and *Scaptotrigona* honey respectively (Vit et al., 2023). In Australia, acetic acid was higher in *Tetragonula* than *Austroplebeia*, and varied with species, 0.10 g/100 g *Austroplebeia australis* honey, 0.03 g/100 g *Austroplebeia cassiae* honey, 0.43 g/100 g *Tetragonula carbonaria honey*, 0.87 g/100 g *Tetragonula davenporti* honey, and 0.33 g/100 g *Tetragonula hockingsi* honey (Hungerford et al., 2023). This AOA is an important marker for pot-honey, besides the lactic acid, both originated from microbial fermentations in the stingless bee nest.

6.2 cis-Aconitic acid

Aconitic acid $C_6H_6O_6$ belongs to a class of tricarboxylic acids with three carboxyl groups, with molecular formula $HOOCCH_2C(COOH){=}CHCOOH$, *cis*-aconitate is an intermediate in the isomerization of citrate to isocitrate in the TCA cycle. The metabocard information shows a unique human exposome nature of this AOA in the set of retrieved organic acids of honey for this review. Aconitic acid is a primary metabolite, metabolically or physiologically essential, directly involved in reproduction, growth or development. Exposome is the collection of all exposures of an individual in a lifetime and how those exposures relate to health. An individual's exposure begins before birth and includes environmental and occupational sources. The *cis*-aconitic acid was included in the French study but it was not detected (Daniele et al., 2012).

6.3 Adipic acid

Adipic acid $C_6H_{10}O_4$ or hexanedioic acid molecular formula $(CH_2)_4(COOH)_2$, is a white crystalline solid that melts at 152 ºC, rare in nature. It is an important monomer in the polymer industry, especially nylon. It is a human xenobiotic metabolite, used as food acidity regulator in evaporated milk, puddings, desserts, and flavourings. Also used as additive and gelling agent in gelatins. In the pharmaceutical industry it is known for its pH-independent release for both weakly basic and weakly acidic drugs. In bioanalysis, is a good biomarker of jello consumption. A microbial metabolite from *Escherichia*. The adipic acid was investigated in the Australian stingless bee honeys, characterized by low concentrations of 0.01g/100g *Tetragonula carbonaria* honey (Hungerford et al., 2023).

6.4 Butyric acid

Butyric acid $C_4H_8O_2$ molecular formula $CH_3CH_2CH_2COOH$ is a short-chain fatty acid (SCFA) formed by bacterial fermentation of carbohydrates (including dietary fiber). It is a straight-chain alkyl carboxylic acid with an unpleasant (rancid butter) odor. The name butyric acid comes from the Greek word for butter, the substance in which it was first found. Triglycerides of butyric acid constitute 3-4% of butter. When butter goes rancid, butyric acid is liberated from the short-chain triglycerides via hydrolysis. It is present in animal fat and plant oils, bovine and human milk, butter, parmesan cheese. A paradox is the butyric acid unpleasant odor, but pleasant buttery aroma by retronasal perception in the mouth. Low-molecular-weight esters of butyric acid, such as methyl butyrate, also known under the systematic name methyl butanoate, have very pleasant aromas. Thus, several butyrate esters are used as food flavoring agents and perfume additives. Butyrate is naturally produced by fermentation processes performed by obligate anaerobic bacteria found in the mammalian gut. Butyric acid was measured in French honeys but it was either not detected or 0.00 g/100 g (Daniele et al., 2012).

6.5 Citramalic acid

Citramalic acid $C_5H_8O_5$ molecular formula $HO_2CCH_2C(CH_3)(OH)COOH$ is related structurally to malic acid, in which the hydrogen at position 2 has been replaced by a methyl group, thus also known as 2-methylmalic acid or hydroxysuccinic acid. Citramalate has mostly a bacterial origin, and is present in almost all living organisms from microbes to humans. This acid is used as a urinary marker for gut dysbiosis (Paprotny et al., 2019). It reduces facial skin wrinkle, and is the dominant acid in pitaya *Hylocereus* spp. CACTACEAE fruit pulp and peel (Wu et al., 2020). As an example of foodomics approaches, citramalate synthase (IPMS), particularly pitaya HuIPMS2 was a chloroplast-localized protein positively correlated with the synthesized quantities of citramalic acid in four Chinese pitaya cultivars (Chen et al., 2022). This AOA was 0.00-0.02 g/100 g *A. mellifera* Spanish honey (del Nozal et al., 1998).

6.6 Citric acid

Citric acid $C_6H_8O_7$ is a tricarboxylic acid, with molecular formula $HOC(COOH)(CH_2COOH)_2$ found in citrus fruits (grapefruit, lemon, orange, tangerine) and pineapple. Citric acid was a frequently measured AOA in this review. In Table 3, 0.01–0.04 g/100 g floral honey were found in Brazil, Chile, Italy, Latvia, Spain, and 0.05 in honeydew honey from Brazil. In Table 4, it was 0.01 g/100 g honeydeey and 0.03 chestnut Turkish honey (Tezcan, 2011). In Table 5, citric acid varied from 0.01 g/100 g acacia, chestnut, linden, rapeseed, 0.02 sunflower, and 0.04 g/100 g honeydew honey in the French study (Daniele et al., 2012). In the German study, see Table 6, it varied from 0.09 g for manuka, to 0.49 g/100g for sunflower honey (Ohmenhaeuser et al., 2013). See Table 7 for variations in the Malaysian *Geniotrigona thoracica* (0.03–0.05) and *Heterotrigona itama* (0.04–0.09) acacia, gelam, and starfruit honey (Shamsudin et al., 2019). In Table 3 citric acid was 0.01–0.03 for Chilean, Latvian, Italian, and Spanish floral honey; 0.01 g/100 g *Melipona favosa* honey from Venezuela (Sancho et al., 2013); 0.18 g/100 g *Axestotrigona ferruginea* honey from Tanzania (Popova et al. 2021); in the Australian honey was 0.02 g/100 g *Tetragonula carbonaria* (Persano Oddo et al., 2008), and 0.03 g/100 g *Tetragonula carbonaria honey*, 0.02 *Tetragonulaa davenporti* and *Tetragonula hockingsi* honey, and 0.01 g/100 g *Austroplebeia Australia* honey (Hungerford et al., 2023); and in the Ecuadorian study 0.02 g/100 g *Geotrigona* honey, and 0.01 *Scaptotrigona* honey (Vit et al., 2023).

6.7 Formic acid

Formic acid CH_2O_2 molecular formula HCOOH is the simplest carboxylic acid also called methanoic acid. It is a strong acid, used in textile and leather processing. Inhibition of cytochrome oxidase by formate causes cell death by increased production of cytotoxic reactive oxygen species (ROS). Used as preservative and antibacterial agent to extend nutritive value of livestock feed. It has a pungent odor. Formic acid has signaling properties and its presence in honey has a background in the social chemistry of bee communication and defense. *Geotrigona* and *Scaptotrigona* chemical alarm signals near to their nests, do not contain formic acid, which is used by *Oxytrigona* in characteristic caustic defensive secretion (Roubik, 1989). Formic acid, is the alarm pheromone in ant species (Blum and

Brand, 1972). In Table 5 it varied from 0.00 for acacia to 0.19 g/100 g chestnut honey from France (Daniele et al., 2012). In Table 6, it ranged from 0.04 in *Robinia* and floral honey, to 0.71 g/100 g in chestnut honey from Germany (Ohmenhaeuser et al., 2013). Like acetic acid, formic acid is volatile and was identified in Austrian honey (Siegmund et al., 2017).

6.8 Fumaric acid

Fumaric acid $C_4H_4O_4$ molecular formula $HO_2CCH=CHCO_2H$ is a key intermediate metabolite in the TAC used to produce cellular energy as adenosine triphosphate (ATP). Many microorganisms produce fumaric acid in small amounts. Its salt fumarate has a fruit-like flavor, it is used as acidity regulator, and to add tartness to food. It is frequently measured in honey by HPLC and NMR, but concentrations are so low and mostly zero or not detected. It varied between 0.02 and 0.05 g/100 g in chestnut, honeydew, polyfloral and sunflower honey by untargeted NMR (Ohmenhaeuser et al., 2013).

6.9 Galacturonic acid

The galacturonic acid $C_6H_{10}O_7$ is an aldehyde acid, in its open molecular form $HOOC(CHOH)_4CHO$ the main component of the polysaccharide pectin, as monomer of polygalacturonic acid polymer. This is a sugar acid, a derived form of D-galactose by oxidation of the terminal CH_2OH to a COOH (at C6). Beet, flaxseeds, guava contain this uronic acid. It was present (0.01) of floral honey in the study conducted by del Nozal et al. (1988), and was the major organic acid for honeydew honey 0.46 g/100 g (Daniele et al., 2012).

6.10 Gluconic acid

Gluconic acid $C_6H_{12}O_7$ is a carboxylic acid with linear structural formula $HOCH_2(CHOH)_4COOH$ as one of the 16 isomeric 2,3,4,5,6-pentahydroxyhexanoic acids. In aqueous solution at neutral pH, gluconic acid forms the gluconate ion. Citric acid was previously proposed as the principal acid of honey, but Stinson et al. (1960) demonstrated that gluconic acid is the most abundant acid in honey, produced after the oxidation of the aldehyde group to a carboxyl group from D-glucose, by the enzyme glucose oxidase of hypopharyngeal glands of the honey making bee *A. mellifera* (Gauhe, 1941). However, gluconic-acid producing bacteria, like the obligate aerobic *Gluconobacter* spp. also contribute to this process. Ruiz-Argueso and Rodriguez-Navarro (1973) used high glucose media to isolate them from healthy honey bees and from ripening honey, and suggested a relationship with acetic-acid bacteria and pseudomonads. Non-pathogenic bacteria are involved in the biochemical transformations of materials collected by bees inside their nests (Pain and Maugenet, 1966; Rodriguez-Navarro and Ruiz-Argueso, 1970; Gilliam, 1997). Gluconic acid is in equilibrium with its lactone, gluconolactone. For this reason, besides the free acidity of honey, also the lactonic acity is measured to estimate the total acidity. Gluconic acid occurs naturally in fruit, honey, kombucha tea and wine. Variations of 0.02 to 1.16 g gluconic acid/100 g Italian floral honey (Cherchi et al., 1994), and a D-gluconic acid 0.99 g/100 g Australian *Tetragonula carbonaria* honey (Persano Oddo et al., 2008) was comprised in that *Apis mellifera* honey range. Gluconic acid varied from 0.15 to 1.12 g/100 g Turkish honey (Tezcan et al., 2011), and from 0.19 to 1.27 g/100 g French honey (Daniele et al., 2012); 0.64 g/100 g in *Melipona favosa* honey from Venezuela (Sancho et al., 2013), errata sheet. Gluconic acid was 0.87–1.00 g/100 g floral and honeydew Brazilian honey (Seraglio, 2021a,b). In the Australian pot-honeys, gluconic acid was 2.50 g/100 g *Austroplebeia australis* honey –the highest concentration in this review– 1.64 g/100 g *Austroplebeia cassiae* honey, 1.65 g/100 g *Tetragonula carbonaria honey*, 1.90 g/100 g *Tetragonula davenporti* honey, and 1.29 g/100 g *Tetragonula hockingsi* honey (Hungerford et al., 2023).

6.11 Glutaric acid

Glutaric acid $C_5H_8O_4$ is a medium-strong 5C linear dicarboxylic acid with molecular formula $HOOC(C_3H_6)COOH$. It is used as building blocks for polymers (polyesters and polyamides), and into organometallics, leading to antimicrobial agents. It is synthesized with some amino acids like lysine and tryptophan. The water solubility of glutaric acid is over 50% at room temperature, compared to the low solubility of succinic acid, a related form. In this review glutaric acid was evaluated in Brazilan *A. mellifera* honey. It was not detected in floral honey but was detected in Brazilian honeydew honey at 0.03 g/100 g (Seraglio et al., 2021a), and 0.02 g/100 g (Seraglio et al., 2021b).

6.12 Isocitric acid

Isocitric acid $C_6H_8O_7$, also available in ionic forms as isocitrate belongs to the class of organic compounds known as tricarboxylic acids and derivatives, containing exactly three carboxyl groups, it is a TCA (tricarboxylic acid) cycle intermediate, a structural isomer of citric acid formed from citrate by the enzyme aconitase. More specifically, isocitric acid is synthesized from citric acid via the intermediate *cis*-aconitic acid by the enzyme aconitase hydratase. Isocitrate is acted upon by isocitrate dehydrogenase (IDH) to form alpha-ketoglutarate. This is a two-step process, which involves oxidation of isocitrate to oxalosuccinate (a ketone), followed by the decarboxylation of the carboxyl group beta to the ketone, forming alpha-ketoglutarate. In humans, IDH exists in three isoforms: IDH3 catalyzes the third step of the citric acid cycle while converting NAD^+ to NADH in the mitochondria. Isocitric acid is found in most fruit juices, especially in blackberries and vegetables like carrots. A high ratio of citric to isocitric acid can indicate the addition of citric acid as an adulterant. In authentic orange juice, for example, the ratio of citric acid to D-isocitric acid is < 130. It is also used as an acidulant in the food industry. This AOA was measured only in the French study, besides *cis*-aconitic acid.

6.13 Lactic acid

Lactic acid bacteria (LAB) produce lactic acid $C_3H_6O_3$ molecular formula $CH_3CH(OH)COOH$. It is found in yogurt, buttermilk, and fermented kombucha, kimchee, and sauerkraut. The acid becomes more undissociated at lower pH, and imparts more of a sour taste. The intense sourness of lactic acid at pH 3.5 is caused by 70% of undissociated acid compared to 30% for citric acid. At pH 3.5–4.5 lactic acid is the most astringent AOA (Dziezak, 2016). In animals, L-lactate is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH) in a process of anaerobic fermentation during normal metabolism and exercise, produced during contraction of muscle tissue as a by-product of anaerobic glycolysis. The LAB genera *Lactobacillus* and *Bifidobacterium* were isolated and identified in the *Apis mellifera* stomach (gut) by Olofsson and Vásquez (2008). The LAB microbiota are symbionts of *A. mellifera* (Olofsson et al., 2016), and they were also identified in Mexican stingless bees. Torres Moreno et al. (2021) isolated and characterized lactic acid bacteria (LAB) from the gastrointestinal tract of *Melipona beecheii*, *Scaptotrigona pectoralis*, *Plebeia llorentei* and *Plebeia jatiformis* from Veracruz, southeast Mexico. These included Gram-positive and catalase-negative rods and cocci: *Lactiplantibacillus plantarum*, *Weissella paramesenteroides*, *Leuconostoc citreum*, and *Apilactobacillus* spp. Lower concentrations of lactic acid (0.01-0.02) in honey from Spain (Mato et al., 2006), Bulgaria (Popova et al., 2021), Chile (Fuentes Molina et al., 2020), and (0.17) in bracatinga honeydew honey from Brazil (Seraglio et al., 2021). Lactic acid was 2.38 g/100 g honey of *Axestotrigona ferruginea* from Tanzania (Popova et al., 2021). Lactic acid in the Australian honey was 0.01 g/100 g *Austroplebeia australis* honey, 0.00 g/100 g *Austroplebeia cassiae* honey, 0.58 *Tetragonula carbonaria honey*, 1.04 *Tetragonula davenporti* honey, and 0.43 g/100 g *Tetragonula hockingsi* honey (Hungerford et al., 2023). Ecuadorian honeys varied from 2.43 g/100 g for *Geotrigona* –the highest in this review– 1.33 g/100 g *Melipona* and 1.21 g/100 g *Scaptotrigona* honeys (Vit et al., 2023).

6.14 Maleic acid

Maleic acid $C_4H_4O_4$ is a dicarboxylic acid molecular formula HOOCCH=CHCOOH, the Z-isomer of butenedioic acid, and the isomer of E-configured fumaric acid. It is a weak diprotic acid and has a faint odor. Maleic acid is converted into malic acid by hydration, and to succinic acid by hydrogenation. It is used as acidulant for tartness flavoring, adjusting acidity in cosmetics, lubricant additive manufacture, quick setting inks, furniture lacquers. Suárez-Luque et al. (2002) reported 0.00 g/100 g Spanish honey. Maleic acid in the Australian honey was 0.01 g/100 g *Austroplebeia australis* honey, 0.03 g/100 g *Austroplebeia cassiae* honey, 0.02 *Tetragonula carbonaria* honey, 0.01 *Tetragonula davenporti* honey, and 0.03 g/100 g *Tetragonula hockingsi* honey (Hungerford et al., 2023).

6.15 Malic acid

Malic acid $C_4H_6O_5$ is a tart-tasting organic dicarboxylic acid with molecular formula $HOOCCH_2CH(OH)COOH$ that plays a role in sour or tart foods. Apples and pears contain malic acid, which contributes to the sourness of a green apple. Malic acid can make a wine taste tart, and a honey too. Malate is an intermediate of the TCA cycle along with fumarate. It can also be formed from pyruvate. Malic acid is both derived from food and synthesized by the TCA in the mitochondria. "Under aerobic conditions, the oxidation of malate to oxaloacetate provides reducing equivalents to the mitochondria through the malate-aspartate redox shuttle. During anaerobic conditions, where a buildup of excess reducing equivalents inhibit glycolysis, malic acid's simultaneous reduction to succinate and oxidation to oxaloacetate is capable of removing the accumulating reducing equivalents" from metabocard (See S1). It might be of interest for aerobic-anaerobic transformations in the nest. Malic acid was present in less than 30% of the honeys 0.02–0.10 g/100 g (del Nozal et al., 1998) Spanish study, 0.20–0.90 g/100 g (Ohmenhaeuser et al., 2013) German study, 0.00-0.05 g/100 g (Tezcan et al., 2011) Turkish study, and varied from 0.00-0.14 g/100 g *Apis mellifera* honey in Table 3. For stingless bees, 0.02 g/100 g *Tetragonula carbonaria* honey from Australia (Persano Oddo et al., 2008), 0.12 g/100 g *Axestotrigona ferruginosa* from Tanzania (Popova et al., 2021), 0.00 g/100 g *Melipona* species *favosa* from Venezuela (Sancho et al., 2013), *fasciculata* and *subnitida* from Brazil (Sant'ana et al., 2020). Malic acid in the Australian honey was 0.04 g/100 g *Austroplebeia australis honey*, and 0.01g/100 g *Tetragonula carbonaria*, *Tetragonula davenporti*, and *Tetragonula hockingsi* honey (Hungerford et al., 2023), and 0.01 g/100 g *Geotrigona* honey from Ecuador (Vit et al. 2023).

6.16 Malonic acid

Malonic acid $C_3H_4O_4$ is a dicarboxylic acid with structure $CH_2(COOH)_2$. It is a precursor in polymers and polyester. A highly hydrophobic molecule, practically water insoluble, and relatively neutral. It has applications in the flavors, fragrance and pharmaceutical industry. It is used to control acidity, and is involved in fatty acid biosynthesis. Malonic acid is the precursor substrate of mitochondrial fatty acid synthesis. The coenzyme A derived from malonate, malonyl-CoA, and the acetyl-CoA are precursora in cytosolic fatty acid biosynthesis. Malonic acid was not detected in the Australian study of pot-honey produced by five species of stingless bees (Hungerford et al., 2023).

6.17 Oxalic acid

Oxalic acid $C_2H_2O_4$ molecular formula $HO_2C{-}CO_2H$ is the simplest dicarboxylic acid. Having two joint carboxyl groups, this is one of the strongest organic acids. It is also a reducing agent. Oxalic acid is a marker for yeast overgrowth from *Aspergillus*, *Penicillum* and/or *Candida*. Veterinary treatments of honey bees with oxalic acid did not increase its content in honey (Bogdanov et al., 2002). Thus, a putative fungal origin could be suspected for the Ecuadorian pot-honeys, if they may be associated with those stingless bee species and would be detected in their nests. Oxalic acid was the most abundant acid in the Ecuadorian stingless bee honeys (Table 8) –except for *Cephalotrigona* sp., *Scaptotrigona polysticta* and *Tetragonisca angustula* honeys where citric acid, lactic acid and acetic acid were more concentrated respectively. *Melipona grandis* (0.73) and *Oxytrigona mellicolor* (0.72) primed the oxalic acid concentration in Ecuadorian pot-honey, followed by *Tetragonisca silvestriana* (0.69), *Tetragonisca angustula* (0.65), and *Nannotrigona chapadana* (0.63) units to be updated by authors (Villacres-Granda et al., 2021). This is in contrast with other groups from China and Latvia where oxalic acid was measured but not found in *Apis mellifera* honey. Oxalic acid was present in one of the five Australian species, 0.01 g/100 *Tetragonisca angustula* honey (Hungerford et al., 2023).

6.18 Propionic acid

Propionic or propanoic acid $C_3H_6O_2$ is a carboxylic acid with molecular formula CH_3CH_2COOH commercially produced by fermentative, biosynthetic or amino acid catabolic pathways (Gonzalez-Garcia et al., 2017). Its pungent and unpleasant smell that reminds body odor. It is widely used as an antifungal agent in food, food preservative, with some immunosuppressive action, food intake, plasma and liver fatty acid reduction, and improved tissue insulin sensitivity. It is one of the main metabolic end products after fermentation of undigested food by human colon microbiota. Propionic acid was 0.03/100 g fir honeydew French honey (Daniele et al., 2012).

6.19 Pyruvic acid

Pyruvic acid $C_3H_4O_3$ is a carboxylic acid with molecular formula $CH_3COCOOH$. The decarboxylation of two pyruvic acids leads to the formation of one molecule of acetoin ($C_4H_8O_2$), which is a natural volatile that became a marker for the Australian *Eucalyptus* species *E. leucoxylon* and *E. melliodora* (D'Arcy et al., 1997). It is also detected in heather honey from France (Radovic, 2000), Germany and Norway (Guyot et al., 1999). This volatile organic acid was not detected in honey of any botanical origin by HPLC (del Nozal et al., 1998) see Table 3, and untargeted NMR (Ohmenhaeuser et al., 2013) in Table 6 German study, except for the Italian study 0.01 g/100 g (Cherchi et al., 1994) and the French study where it was 0.13 g/100 g fir honeydew honey (Daniele et al., 2012). Pyruvic acid content in pot-honey was 0.01g/100 g *Axestotrigona ferruginosa* from Tanzania (Popova et al., 2021).

6.20 Quinic acid

This is an astringent cyclic polyol $C_7H_{12}O_6$ from plant sources. Linear molecular formula is not available for cyclic acids. Quinic acid and shikimic acid are key intermediates in the biosynthesis of aromatic compounds in living systems. Therefore, this AOA has an important role in the formation of phenolic compounds. Citric, malic and quinic acids are the major portion of total AOA in coffee. Quinic acid is involved in sour perception of coffee. In some honey types quinic acid was detected at 0.35 g/100 g *Erica* spp. honey from Spain and 0.00–0.02 in others (del Nozal et al., 1998), 0.18 in fir honeydew and 0.07 g/100 g in chestnut from France, in other French floral honeys it varied from 0.01 to 0.03 (Daniele et al., 2012), and 0.24 g/100 g in Latvian honey from apiary (Keke and Cinkmanis, 2019). It was very low in few other Spanish and 0.01 g/100 g Chilean honey (Fuentes Molina et al., 2020) and not detected in the rest of *A. mellifera* honeys. Quinic acid was quantitated in the five Australian species 0.03 g/100 g *Austroplebeia australis* honey, 0.02 g/100 g *Austroplebeia cassia*e honey, 0.05 g/100 g *Tetragonula carbonaria* honey, 0.08 g/100 g *Tetragonula davenporti* honey, and 0.03 g/100 g *Tetragonula hockingsi* honey (Hungerford et al., 2023).

6.21 Shikimic acid

Shikimic acid $C_7H_{10}O_5$ is a cyclohexene, cyclitol and cyclohexanecarboxylic acid. Its name originated from the Japanese flower shikimi from which it was first isolated. Linear molecular formula is not available for cyclic acids. The enzymes responsible for the regulation of phenolic metabolism are known, and shikimic acid is a central metabolite. Shikimic acid is considered a marker in honey. It was present in the Tanzanian stingless bee *Axestotrigona ferruginea* 0.02 g/100 g honey (Popova et al., 2021), and the Australian stingless bees *Austroplebeia australis* and *Tetragonisca davenporti* 0.01 g/100 g honey, see Table 3. In this review, these pot-honeys are the unique values for shikimic acid.

6.22 Sorbic acid

Sorbic acid $C_6H_8O_2$ with molecular formula $CH_3(CH)_4CO_2H$ is a natural preservative used in meats, cheeses, baked goods, and fresh foodstuffs to prevent yeast and mold growth, and some pharmaceutical drugs and cosmetics. More effective in acidic food. Generally used as K salt, and less frequently as Ca salt. Sorbic acid concentration was very low 0.01 g/100 g honey of *Austroplebeia australis*, *Tetragonula carbonaria*, and *Tetragonula davenporti* from Australia (Hungerford et al., 2023).

6.23 Succinic acid

Succinic acid $C_4H_6O_4$ is a dicarboxylic acid with molecular formula $(CH_2)_2(COOH)_2$. In Latin *succinum* means amber. It is an important component of the citric acid or TCA cycle and is capable of donating electrons to the electron transfer chain. Succinate has multiple biological roles as a metabolic intermediate, cell signaling molecule, altering gene expression patterns, thus modulating epigenetics by hormone-like signaling functions, with flavor-enhancement properties (Dziezak, 2016). Its concentration varied between unifloral honeys 0.01–0.28 g/100 g (Ohmenhaeuser et al., 2013). It was a distinctive acid for the Malaysian bees *G. thoracica* and *H. itama*, similar in the three floral honeys for *H. itama* 0.32–0.38, but for *G. thoracica* 0.52 in acacia, not detected for gelam and 0.07 starfruit (Shamsudin et al., 2019). A pattern impossible to explain without further experiments. Succinic acid 0.13 g/100 g in bracatinga honeydew honey, from Brazil (Seraglio et al., 2021), was higher than 0.06 in Latvian honey (Keke and Cinkmanis, 2019), and 0.05 in Spanish chestnut honey (Mato et al., 2006). Succinic acid values were 0.01 g/100 g *Tetragonula carbonaria*, *Tetragonula davenporti*, and *Tetragonula hockingsi* Australian honey (Hungerford et al., 2023), and *Geotrigona* Ecuadorian honey (Vit et al., 2023).

6.24 Tartaric acid

Tartaric acid $C_4H_6O_6$ is a dicarboxylic acid with molecular formula HOOCCH(OH)CH(OH)COOH for the major organic acid in grapes, and also abundant in tamarind, citrus fruit and bananas. It is used as an acidulant additive and antioxidant in the food industry. In the organic acid spectra for 19 botanical origins of honeys purchased in Germany, tartaric acid was the most abundant AOA (0.31–0.66) by untargeted NMR (Ohmenhaeuser et al., 2013) –except for sunflower with top citric acid– see Table 6, and similarly for Latvian honeys (0.61–0.62) (Keke and Cinkmanis, 2019) in Table 3. This organic acid is not frequently included in the organic acids evaluated for honey, but it would deserve a routine analysis. The Brazilian pot-honeys had conspicuous tartaric acid in their AOA spectra *Melipona fasciculata* (0.56) and *Melipona subnitida* 0.94 g/100 g honey by HPLC (Sant'ana et al., 2020).

7. Briefing on techniques used for organic acid quantitation

Organic acids in honeys have been quantified by enzymatic, capillary electrophoresis, chromatographic, enzymatic, spectrometric and spectroscopic methods mostly used for *Apis mellifera* honey and recently for stingless bee products. Ion-exchange adsorption, silicic acid partition chromatography and ion-exchange chromatography were used to separate organic acids from clover honey; butyric, acetic, formic, lactic, succinic, pyroglutamic, malic, citric, and gluconic acids were identified by paper chromatography with six solvent systems (Stinson et al., 1960). Solid-phase extraction (SPE) with anion exchange cartridges and with High Performance Liquid Chromatography (HPLC) were used to quantify pyruvic, citric, citramalic, formic, fumaric, galacturonic, gluconic, glycolic, malic, pyruvic, quinic and succinic acids in honey of nine botanical origins (del Nozal et al., 1998). Separation techniques are coupled to diverse detectors like Diode Array Detector (DAD). Suárez-Luque et al., (2002) continued with HPLC, further series-coupled ion-exclusion columns to assess low molecular weight organic acids in honey (Nozal et al., 2003), and later with Capillary Zone Electrophoresis (CZE) to separate inorganic anions (chloride, nitrate, phosphate, sulphate) and formic acid from three botanical honey types (Mato et al., 2006). Capillary Electrophoresis-Diode Array Detection (CE-DAD) was used to measure citric, formic, gluconic, malic, oxalic and succinic acids (Tezcan et al., 2011). Untargeted Nuclear Magnetic Resonance (NMR) based spectroscopic ^{1}H-NMR and ^{13}C-NMR methods quantified citric, formic, fumaric, malic, phtalic, pyruvic, and succinic acids in 24 unifloral, polyflora and honeydew types of honey (Ohmenhaeuser et al., 2013). The

^{1}H-NMR was used to compare Bulgarian honey with a Tanzanian pot/honey (Popova et al., 2021). A recent method from Singapore and Saudi Arabia proposed an electro membrane extraction (EME) of organic acids from honey and further ion chromatographic (IC) analysis (Tan et al., 2018). Stable carbon isotope ratios (δ^{13}C) adapted from elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) to liquid chromatography (LC/IRMS) to correlate δ^{13}C between organic acids and sugars in honey (relatively weak values with disaccharide, and trisaccharide) were proposed by (Kawashima et al., 2019). Later, determination of gluconic acid, tartaric acid, malic acid, citric acid, and succinic acid in honey was obtained by liquid chromatography with tandem mass spectrometry (LC-MS/MS)–based from Japan (Suto et al., 2020). A targeted ^{1}H-NMR based system for multiparametric analysis included ten AOA in honey from Chile (Fuentes Molina et al., 2020) and pot-honey of three stingless bee genera from Ecuador (Vit et al., 2023). High-performance ion chromatography HPIC combined with suppressed conductivity detection (SCD) was used to detect formic acid in honey from Poland (Matysiak et al., 2018), and 15 AOA in pot-honey produced by five species of stingless bees from Australia, but fumaric acid and tartaric acid were not detected, and malonic acid was <LOQ (Hungerford et al., 2023).

8. Integrative remarks

This review aims to integrate information from the literature available on honey AOA. It leaves open questions on further relationships between honey making bees and their microbiota, to explain the pathways from plant sources to their presence and quantities in honey. Identified LAB fill the gap for lactic acid, and AAB for acetic acid. However, for yeasts it is not only alcohol, but also AOA and other metabolites that may represent an adaptive value for their metabolic ability serving their host. It has given us an opportunity to view honey as an ecological reservoir of microorganisms producing organic acids with the dual function of improving the nutritional quality of the sugary sources collected by the honey making bees. These microorganisms also protect honey from antagonistic microbial interactions, thus exploiting their ability of living in that hyperosmotic substrate.

The situation on the absolute and relative concentrations is hopefully addressed here, despite the diverse techniques available for AOA analysis, and the different organic acids chosen by authors of honey research. It is important that the most frequently studied AOA in honey have agreed upon reference values with the available literature. Some comments on those reported with units not corresponding to the accurate concentration are a positive way to harmonize perhaps to the familiar g/100 g proximate analysis expression used in food science and technology. This expression is favorable for expressing major organic acids such as gluconic, citric and lactic, secondary succinic, malic and malonic in honeydew honey, and especially acetic acid in some stingless bee sour honeys, instead of having unnecessary elevated values in mg. For the minor organic acids, unless it is a targeted study to pinpoint a particular acid, values in the range 0.01-0.10 g/100 g cover most of the quantities reviewed here. Corresponding authors of AOA contributions in honey research were contacted, and sometimes positive feedback was received. Editorial adjustments were advised when needed. We hope that in its simplicity, the reader will find useful information on AOA not compacted before as it is here. Our expectation is nurturing a motivation to formulate new questions in this topic, and also completing what was missing here by future improved versions. The creation of new AOA reviews may focus on distant aspects such as the evolution of these metabolites in what this sugary-watery interactive honey media is for them.

Evidence was retrieved from literature to update the reference on AOA <0.5% of *Apis mellifera* honey's constituents (2003), which is underestimated for *Apis mellifera* honeys. This could be adjusted to a conservative <2.5% for *Apis mellifera* and to <4.4% for stingless bee (Meliponini) honeys. More research is needed and active groups are progressing on applications of AOA in honey authentication, quality, and methodology.

Using aliphatic or non-aromatic descriptors for organic acids in database searches does not provide further focus. To be practical, organic acids in honey refer to the aliphatic and non-aromatic, since the aromatic group of organic acids are simply named phenolic acids and there is no reason to confuse both types. A note on distraction by regulatory standards, causing delay for understanding by leaders and reviewers distant from the biological significance of AOA in honey –and procrastinating contributions on sour honey– overcoming this contradiction will benefit both: 1. The standards, and 2. Grasping new knowledge from nature, the endless source.

Besides harmonized units, also listing the organic acids in alphabetical order is advantageous to facilitate reading. Sometimes they are listed at random or less frequently following an order of concentration or distant elution patterns perhaps. Using fancy graphics is fine, but not a reason for removing tables with data. Knowing the editorial limitations, and the choice of statistical plots needed for visualization, authors are of great support providing their databases upon request for further analysis. Growing scientific interest on phytochemical and geographic origin is visible, entomological predictors have just started. Young generations needed a solid aliphatic organic acid reference platform for thinking and understanding the microbiota associated with honey making bees, and we too. A library on yeast diets and transformations would possibly be more equipped to provide some answers, than the analytical efforts in chemistry sometimes exhausted by Limits of Detection (LODs), Limits of Quantification (LOQs), and Relative Standard Deviations (% RSD), needed for validations of the methods and sound quantifications, the description of the findings and the multivariate statistics applied to them. A brief approach to explain the presence of that organic acid spectra of such a specialized acquisition is recurrently missing. Indeed, in a Chilean contribution that received targeted NMR service, details were given to expand information on the organic molecules. Due to modern multiparametric developments, a melittologist may suddenly have access to more AOA components than his ability to explain their origin, roles and fate in honey.

Twenty four AOA were reviewed (acetic, aconitic, adipic, butyric, citric, citramalic, formic, fumaric, galacturonic, gluconic, glutaric, isocitric, lactic, maleic, malic, malonic, oxalic, propionic, pyruvic, quinic, shikimic, sorbic, succinic, and tartaric) in *Apis mellifera* honeys as well as pot-honeys produced by stingless bee genera *Cephalotrigona*, *Nannotrigona*, *Partamona*, *Oxytrigona*, *Scaptotrigona*, *Tetragonisca* from Ecuador, *Geniotrigona* and *Heterotrigona* from Malaysia, *Melipona* from Brazil, Ecuador and Venezuela, *Axestotrigona* from Tanzania, *Austroplebeia* and *Tetragonula* from Australia. These AOA were tabulated from the literature for fast view and analytical comparisons. The botanical origin associated to organic acids in honey was introduced with the free acidity of major European unifloral and honeydew honey types (database > 35000 entries, 2004). The AOA data were retrieved for multifloral, unifloral (*Acacia*, *Arbutus*, *Averrhoa*, *Brassica*, *Calluna*, *Castanea*, *Ceratonia*, *Citrus*, *Eucalyptus*, *Erica*, *Euphorbia*, *Helianthus*, *Lavandula*, *Leptospermum*, *Melaleuca*, *Onobrychis*, *Quercus*, *Paganum*, *Reseda*, *Rhododendron*, *Robinia*, *Rosmarinus*, *Thymus*, *Trifolium*, *Ziziphus*) and honeydew (bracatinga Brazil, fir France, metcalfa Germany, and pine Turkey) honeys from 15 countries (Australia, Brazil, Bulgaria, Chile, China, Ecuador, France, Germany, Italy, Latvia, Malaysia, Spain, Tanzania, Turkey, Venezuela).

A literature review on one metabolite family such as the honey AOA, permitted a global view of honey from diverse countries, botanical and entomological origins, although with edaphic components never recorded before. Finding some discrepancies on concentration units was not expected. Thus, solid data and covered mistakes became evident solely when comparing values in a database with harmonized units to g AOA/100 g honey.

This review was also an attempt to expand on knowledge from our ABC in the two seminal pages and nine lines of acids in honey from Chapter 5 by Jonathan W. White Jr., in the Eva Crane's legendary book: Honey. A comprehensive survey (Crane, 1975).

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Table S1, Supplementary data

Table S1. Metabocards of Aliphatic Organic Acids (AOA) in Human Metabolome Database (HMDB)

No.	Aliphatic Organic Acid	Salt or Ester	Link
1	Acetic acid (HMDB0000042)	Acetate	//hmdb.ca/metabolites/HMDB0000042
2	Adipic acid (HMDB0000448)	Adipate	//hmdb.ca/metabolites/HMDB0000448
3	Aconitic acid (HMDB0247961)	Aconitate	//hmdb.ca/metabolites/HMDB0247961
4	Butyric acid (HMDB0000039)	Butyrate	//hmdb.ca/metabolites/HMDB0000039
5	Citric acid (HMDB0000094)	Citrate	//hmdb.ca/metabolites/HMDB0000094
6	Citramalic acid (HMDB0000426)	Citramalate	//hmdb.ca/metabolites/HMDB0000426
7	Formic acid (HMDB0000142)	Formate	//hmdb.ca/metabolites/HMDB0000142
8	Fumaric acid (HMDB0000134)	Fumarate	//hmdb.ca/metabolites/HMDB0000134
9	Galacturonic acid (HMDB0002545)	Galacturonate	//hmdb.ca/metabolites/HMDB0002545
10	Gluconic acid (HMDB0000625)	Gluconate	//hmdb.ca/metabolites/HMDB0000625
11	Glutaric acid (HMDB0000661)	Glutarate	//hmdb.ca/metabolites/HMDB0000661
12	Isocitric acid (HMDB0000193)	Isocitrate	//hmdb.ca/metabolites/HMDB0000193
13	Lactic acid (HMDB0000190)	Lactate	//hmdb.ca/metabolites/HMDB0000190
14	Maleic acid (HMDB0000176)	Maleate	//hmdb.ca/metabolites/HMDB0000176
15	Malic acid (HMDB0000156)	Malate	//hmdb.ca/metabolites/HMDB0000156
16	Malonic acid (HMDB0000691)	Malonate	//hmdb.ca/metabolites/HMDB0000691
17	Oxalic acid (HMDB0002329)	Oxalate	//hmdb.ca/metabolites/HMDB0002329
18	Propionic acid (HMDB0000237)	Propionate	//hmdb.ca/metabolites/HMDB0000237
19	Pyruvic acid (HMDB0000243)	Pyruvate	//hmdb.ca/metabolites/HMDB0000243
20	Quinic acid (HMDB0003072)	Quinate	//hmdb.ca/metabolites/HMDB0003072
21	Shikimic acid (HMDB0003070)	Shikimate	//hmdb.ca/metabolites/HMDB0003070
22	Sorbic acid (HMDB0029581)	Sorbate	//hmdb.ca/metabolites/ HMDB0029581
23	Succinic acid (HMDB0000254)	Succinate	//hmdb.ca/metabolites/HMDB0000254
24	Tartaric acid (HMDB0000956)	Tartrate	//hmdb.ca/metabolites/HMDB0000956