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Review article

Fatty acids profile in the honeybee: metabolic pathways, stressor interactions, and analytical approaches

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Abstract – Fatty acids play a crucial role in the metabolic activities, health, cognitive development, and behaviors of honeybees. The primary source of fatty acids for honeybees is the pollen consumed, though they can also produce specific fatty acids through *de novo* synthesis. Among the saturated fatty acids in honeybees, palmitic and stearic acids are the most abundant, while common unsaturated fatty acids include oleic, eicosenoic, palmitoleic, linoleic, and linolenic acids, which are present in various body parts and tissues. The composition and concentration of these fatty acids can be influenced by multiple biotic and abiotic factors such as developmental stage, nutrition, pathogens, season, temperature, sanitation conditions, industrial pollution, pesticides, and radiation. Therefore, monitoring the fatty acid profile of honeybees can be used as a bioindicator for monitoring the environmental conditions and the health status, enabling management actions that could improve honeybee sustainability. This study aims to provide foundational knowledge on the fatty acids identified in honeybees, examining their physiological roles, the impact of environmental stressors, and the analytical techniques used to determine their composition.

***Apis mellifera* / saturated and unsaturated fatty acids / climatic changes / pesticides / gas chromatography**

1. INTRODUCTION

Honeybees are vital to the sustainability of life on Earth due to their contribution to pollination. At the same time, they provide a diversity of products such as honey, bee pollen, bee bread, or propolis, consumed by humans as nutritious and health-promoting products. More than 100 million bee colonies worldwide support plant pollination, thereby playing a key role in maintaining ecosystem stability (Domínguez et al. 2024). Monitoring honeybee populations and addressing the pressure factors that threaten them are critical priorities for conservation. Various stressors—including habitat loss, malnutrition, climate change, pathogens, pesticide exposure, inadequate beekeeping practices, and industrial pollution—can negatively impact honeybee health, development, and the longevity of colonies (Mayack et al. 2022; Sarioğlu-Bozkurt et al. 2022). Fatty acids serve as bioindicators for understanding the effects of these stressors on honeybees (Domínguez et al. 2024). As they are essential components in development, energy metabolism, pheromone biosynthesis, cellular membrane structure, task differentiation, lifespan regulation, environmental adaptation, and immune defense (Girgis Sawires et al. 2024; Mackei et al. 2023; Wegener et al. 2018), changes in fatty acid composition directly impact honeybee metabolism. Therefore, several studies explored the fatty acid composition of honeybees and how it varies across developmental stages,

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dietary conditions, and exposure to environmental stressors.

Fatty acid evaluation in honeybees has been carried out with chromatographic methods, where gas chromatography systems equipped with flame ionization (GC-FID) or mass spectrometry (GC-MS) detectors are the mainly used techniques for the identification and quantification of methyl-esterified fatty acids (Arien et al. 2020; Bennett et al. 2022; Haber et al. 2019). Understanding the fatty acid composition of honeybees and the mechanisms behind their variability may help to design beekeeping management protocols that minimize the impact of stressor effects on honeybees, assuring their resilience to climatic changes. Towards this goal, in this literature review, we survey the fatty acid composition in honeybees, their physiological effects, stress factors, and fatty acid relations, as well as the methodologies used for determining fatty acid composition in honeybees.

2. FATTY ACIDS

Fatty acids are fundamental in biological systems because they serve as building blocks for triglycerides, energy storage and distribution, and phospholipids, which are essential for membranes and signaling molecules (Furse et al. 2023). Chemically, fatty acids are organic compounds with a methyl ($–CH_3$) and carboxyl (–COOH) group at their ends. Those naturally occurring typically have unbranched chains containing 4 to 28 carbon atoms (Chen and Liu 2020). Based on chain length, fatty acids are categorized into four groups: short chain (1-5 C atoms; acetic, propionic, and butyric acids), medium chain (6-12 C atoms; caproic, caprylic, capric, and lauric acids), long chain (13-21 C atoms; myristic, palmitic, and stearic acids), and very long chain (>22 C atoms; lignoceric, cerotic, montanic, and ghedoic acids) (Kaczmarek and Boguś, 2021). Fatty acids are also classified by the presence or absence of double bonds: saturated fatty acids lack double bonds, while monounsaturated and polyunsaturated fatty acids contain one or more double bonds, respectively (Chen and Liu 2020). Saturated fatty acids generally comprise 30–40% of the total fatty acids in animal tissues, with palmitic, stearic, myristic, and lauric acids being the most common types (Legrand and Rioux 2015).

Among monounsaturated fatty acids, oleic, palmitoleic, and vaccenic acids are generally reported, while common polyunsaturated fatty acids include α-linolenic, timnodonic (EPA), clupadonic (DHA), docosapentaenoic (DPA), linoleic, γ-linolenic, dihomo-γ-linolenic (DHGLA), arachidonic, adrenic, and docosapentaenoic acids (Tvrzicka et al. 2011).

3. FATTY ACID BIOSYNTHESIS MECHANISM IN HONEYBEES

The fatty acids found in honeybees are originated from dietary sources such as pollen and the *de-novo* fatty acid synthesis pathway (Seltzer et al. 2023), as presented in Figure 1. Fatty acid biosynthesis begins with the formation of malonyl coenzyme A and acetyl coenzyme A (Kaur et al. 2018), and the action of several enzymes such as acetyl-CoA carboxylase, fatty acid desaturase, fatty acid synthase, very long-chain fatty acid elongase, and long-chain acyl-CoA synthetase. Nevertheless, the complexity of the fatty acid synthesis mechanism and synthesis abilities may vary among living organisms (Kaczmarek and Boguś, 2021).

These biosynthetic pathways typically involve three main steps: (1) synthesis of precursor fatty acids from acetate, (2) elongation and adjustment of the carbon chain length, and (3) modification of the carboxyl group (Yang et al. 2017). Fatty acid synthase, elongase, and desaturase enzymes participate in these stages. Fatty acid synthases generate unbranched and methyl-branched fatty acids; elongases extend both saturated and unsaturated fatty acids; and desaturases introduce double bonds, converting saturated chains to unsaturated forms (Falcón et al. 2014). In *Apis mellifera*, a range of fatty acid synthase (GB52590, GB53412), elongase (GB40681, GB46038, GB54396, GB54397, GB51250, GB51247, GB54399, GB54401, GB54404, and

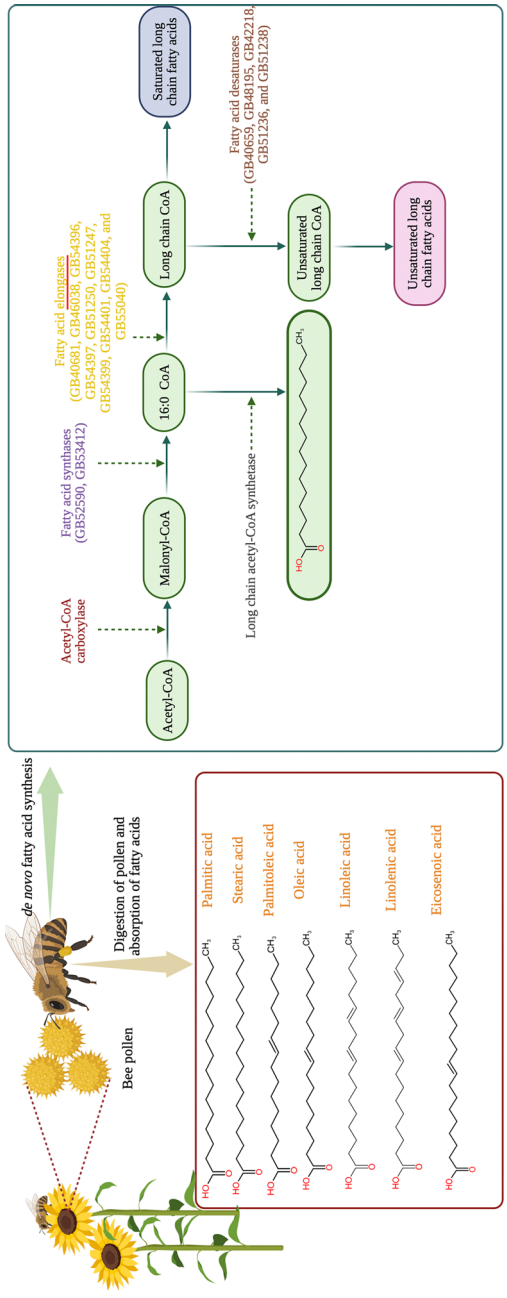

Figure 1. Biosynthesis mechanisms of fatty acids in honeybees.

GB55040), and desaturase (GB40659, GB48195, GB42218, GB51236, and GB51238) enzymes have been identified in the fat body of honeybees, a multifunctional organ essential for bee survival and health, indicating complex and specialized fatty acid metabolism (Moris et al. 2023).

One of the fatty acids whose synthesis mechanism is most studied in honeybees is 10-hydroxydecenoic acid (10-HDA), which is secreted from the mandibular glands of the honeybee and is the characteristic fatty acid of royal jelly (Furse et al. 2023). The biosynthesis of 10-HDA varies across honeybee castes but generally begins with the hydroxylation of stearic acid at the C17 or C18 position via cytochrome P450 enzymes. The resulting compound undergoes β-oxidation to yield a 10-carbon chain (decanoic and decenoic acids), regulated by carnitine O-palmitoyltransferase and peroxisomal acyl-CoA oxidase enzymes, leading to 10-HDA (Hu et al. 2023; Plettner et al. 1998; Zhang et al. 2022).

3.1. Fatty acid composition of honeybees and their physiological effects

Several studies have reported that honeybees contain a wide range of saturated, monounsaturated, and polyunsaturated fatty acids, as shown in Table I (Domínguez et al. 2024; Ghosh et al. 2020a, 2020b, 2020c; Girgis Sawires et al. 2024; Haber et al. 2019; Martin et al. 2022; Vishchur et al. 2019). Besides, functions of some identified fatty acids in honeybee physiology are given in Table II. The findings have indicated that saturated fatty acids account for over 50% of the total fatty acid content in honeybee larvae and pupae oils, with concentrations decreasing as honeybees mature (Yap et al. 2023). A study examining *Apis mellifera ligustica* drones from Denmark reported saturated fatty acid contents of 6.3, 6.6, and 0.7 g/100 g dm (dry matter) in larvae, pupae, and adult honeybees, respectively. These results highlight a remarkable decrease in saturated fatty acid levels as honeybees progress through maturation stages (Ghosh et al. 2020c). Similarly, research on worker honeybees from the same subspecies reported saturated fatty acid percentages of 52%, 51%, and 25% in larvae, pupae, and adult honeybees, respectively (Ghosh et al. 2016). This decreasing trend is also observed in *Apis mellifera carnica* and *Apis mellifera mellifera* drones, where the lower lipid content in adult honeybees has been attributed to its use, together with trehalose and proline, as the source of energy required for flight (Ghosh et al. 2021).

The main saturated fatty acids reported in honeybees are caprylic, capric, lauric, myristic, palmitic, and stearic acids. Additionally, arachidonic, behenic, and lignoceric acids have been identified in some honeybees (Girgis Sawires et al. 2024; Vishchur et al. 2019; Yu et al. 2022). Among these, palmitic and stearic acids are the most abundant in honeybees (Ghosh et al. 2016; Haber et al. 2019; Vishchur et al. 2019). In mature honeybees, palmitic and stearic acid levels were reported to range from 251 to 384 mg/100 g dm and 162 to 342 mg/100 g dm, respectively (Ghosh et al. 2016, 2020c). Myristic acid levels in honeybees were relatively low at 10 mg/100 g, representing about 0.6% of total fatty acids (Ghosh et al. 2016). In honeybees, palmitic acid serves as a precursor for stearic acid, synthesized through the activity of the ELOVL 6 fatty acid elongase enzyme (Chinarak et al. 2022; Hoc et al. 2020). This conversion mitigates the potential negative impact of palmitic acid on honeybee metabolism because stearic acid does not have a cholesterol-increasing effect due to its metabolization to oleic acid (Olaleye et al. 2023). Besides, stearic acid plays a role in the biosynthesis of the 10-HDA molecule found in royal jelly, the primary nutritional source for queen honeybees (Hu et al. 2022, 2023). Caprylic and capric acids have been reported to play a role in the defense mechanisms of insects (Sönmez et al. 2016) and may be synthesized for similar purposes in honeybees.

The unsaturated fatty acid content in honeybees also varies according to their developmental stages, similar to the changes observed in saturated fatty acid levels. However, the changes in total monounsaturated and polyunsaturated fatty acid contents follow distinct patterns. During

Table I Fatty acids detected in honeybees

Honeybee specie/stage	Isolated body part	Identified fatty acids	References
Apis mellifera			
Not specified	Honeybee head tissue	Caprylic Capric Lauric Myristic Pentadecanoic Palmitic Palmitoleic Stearic Oleic Linoleic Linolenic Arachidic Eicosanoic Eicosadienoic Eicosatrienoic Arachidonic Eicosapentaenoic Docosadienoic Docosatrienoic Docosatetraenoic Docosapentanoic Docosahexaenoic	(Vishchur et al. 2019)
Worker honeybee	Cellular membrane	Palmitic Stearic Palmitoleic Oleic Linoleic Linolenic	(Martin et al. 2022)
Worker honeybee	Whole body	Lauric Myristic Palmitic Stearic Behenic Lignoceric Oleic Linolic Calendic	(Girgis Sawires et al. 2024)
Worker honeybee	Honeybee without midgut	Caprylic Capric Lauric Tridecylic Myristic Pentadecylic Palmitic Estearic Heneicosylic Palmitoleic Oleic Eicosatrienoic	(Domínguez et al. 2024)

Table I (continued)

Honeybee specie/stage	Isolated body part	Identified fatty acids	References
Honeybee larvae and pupae	Whole body	Myristic Palmitic Stearic Arachidic Behenic Oleic Linoleic Linolenic	(Haber et al. 2019)
Worker honeybee larvae	Whole body	Lauric Myristic Palmitic Stearic Hexadecenoic Oleic acid	(Ghosh et al. 2016)
Worker honeybee pupae	Whole body	Lauric Myristic Palmitic Stearic Hexadecenoic Oleic acid Eicosenic	(Ghosh et al. 2016)
Worker honeybee adults	Whole body without wings	Capric Lauric Myristic Palmitic Margaric Stearic Hexadecenoic Oleic acid Eicosenic Linoleic	(Ghosh et al. 2016)
Honeybee drone larva	Whole body	Lauric acid Myristic acid Palmitic acid Stearic acid Palmitoleic acid Oleic acid Linoleic acid	(Ghosh et al. 2020c)
Honeybee drone pupae	Whole body	Lauric acid Myristic acid Palmitic acid Stearic acid Arachidic acid Palmitoleic acid Oleic acid Linoleic acid	(Ghosh et al. 2020c)

Table I (continued)

Honeybee specie/stage	Isolated body part	Identified fatty acids	References
Honeybee drone adult	Whole body without wings	Lauric Myristic Palmitic Stearic Arachidic Behenic Palmitoleic Oleic cis11-Eicosenic Linoleic	(Ghosh et al. 2020c)
Apis cerana adult worker honeybees	Whole body without wings	Lauric acid Tridecanoic Myristic acid Palmitic acid Stearic acid Arachidic acid Palmitoleic acid Oleic acid cis11-Eicosenic acid Linoleic acid Linolenic	(Ghosh et al. 2020a)
Apis dorsata adult worker honeybees	Whole body without wings	Lauric acid Tridecanoic Myristic acid Palmitic acid Stearic acid Arachidic acid Palmitoleic acid Oleic acid cis11-Eicosenic acid Linoleic acid	(Ghosh et al. 2020a)
Apis florea adult worker honeybees	Whole body without wings	Lauric acid Tridecanoic Myristic acid Palmitic acid Stearic acid Arachidic acid Palmitoleic acid Oleic acid cis11-Eicosenic acid Linoleic acid	(Ghosh et al. 2020b)

the transition of worker honeybees from the larval stage to maturity, monounsaturated fatty acid levels decrease significantly, from 2381 to 1164 mg/100 g dm, while linoleic acid level increases from nothing to 136 mg/100 g dm (Ghosh et al. 2016). Likewise, Martin et al. (2019) observed that polyunsaturated fatty acid content rises while monounsaturated fatty acid content declines from the late larval stage to emergent worker or drone honeybees. It was emphasized that this change in the composition and quantity of unsaturated fatty acids may be related to the beginning of pollen feeding by honeybees after emergence from the comb (Martin et al. 2019). Furthermore, the fatty acid composition of the cell membranes in worker

Table II Functions of identified fatty acids in honeybee physiology

Fatty Acids	Category	Functions in Honeybee Physiology	References
Caprylic acid (C8:0)	Medium-chain saturated fatty acid	· Energy source · Antimicrobial activity	(Kaczmarek and Boguś, 2021; Khyzhnyak et al., 2018)
Capric acid (C10:0)	Medium-chain saturated fatty acid	· Energy source · Antimicrobial activity · Influence the learning process	(Khyzhnyak et al., 2018; Schleifer et al., 2024)
Lauric acid (C12:0)	Medium-chain saturated fatty acid	· Antimicrobial activity	(Elhoseny et al., 2024)
Myristic acid (C14:0)	Long-chain saturated fatty acid	· Antimicrobial activity	(Manning, 2001)
Palmitic acid (C16:0)	Long-chain saturated fatty acid	· Precursor for stearic, linoleic and linolenic acids synthesis · Nestmate recognition clue	(Buchwald et al., 2009; Castaños et al., 2023; Chinarak et al., 2022)
Stearic acid (C18:0)	Long-chain saturated fatty acid	· 10-HDA synthesis	(Hu et al., 2023)
Arachidonic acid (C20:0)	Very long-chain saturated fatty acid	· Improve the growth, survival, and immune functions	(Yu et al., 2022)
Palmitoleic acid (C16:1, ω−7)	Monounsaturated fatty acid	· Nestmate recognition clue · Antimicrobial activity · Synthesis of pheromones · Synthesis of cuticular chemical signals	(Buchwald et al., 2009; Domínguez et al., 2024)
Oleic acid (C18:1, ω−9)	Monounsaturated fatty acid	· Cellular membrane material · 10-HDA synthesis · Pheromone synthesis · Nestmate recognition clue	(Buchwald et al., 2009; Hu et al., 2023; Qin et al., 2019; Wegener et al., 2022)
Linoleic acid (C18:2, ω−6) Linolenic acid (C18:3, ω−3)	Polyunsaturated fatty acid	· Nestmate recognition clue · Cognitive functions (learning, memory, etc.) · Metabolic activities	(Bennett et al., 2022; Buchwald et al., 2009; Furse et al., 2023)
Eicosadienoic acid (C20:2, ω−6) cis-11,14,17-eicosatrienoic acid (C20:3, ω−3) cis-5,8,11,14,17-eicosapentaenoic acid	Polyunsaturated fatty acid	· Modulate immune function, inflammatory response, and colony development	(Dmitryjuk et al., 2015)

honeybees differs from that in queens, with these differences becoming more noticeable as they develop, likely due to variations in their diets. Both workers and queens exhibit similar membrane compositions, characterized by a high level of monounsaturated fatty acids and a low

level of polyunsaturated fatty acids as emergent adults. However, while worker honeybee membranes progressively become more polyunsaturated, resulting in reduced monounsaturated fatty acids from the emergent to the nursing phase, queen honeybees maintain consistently high monounsaturated fatty acid levels in their cell membranes throughout adulthood (Martin et al. 2022).

The monounsaturated fatty acids identified in adult honeybees, based on quantity, can be ordered as oleic, eicosenoic, and palmitoleic acids (Ghosh et al. 2016; Teerawanichpan et al. 2010; Yap et al. 2023). Honeybees can synthesize oleic acid via the elongase and desaturase enzymes, and it is the main fatty acid of their cellular membrane material, also used for pheromone synthesis (Qin et al. 2019; Wegener et al. 2022). The imbalance of oleic acid together with palmitic acid may lead to learning deficiencies in honeybees and can also affect immune responses (Diaz et al. 2024). Oleic acid has been reported to increase in female insects during the egg-laying period, potentially stimulating its synthesis. In males, which have the potential to mate continuously, oleic acid is thought to serve as an energy source (Nurullahoğlu et al. 2004; Sönmez et al. 2016). A similar effect of oleic acid may also occur in honeybees. The eicosenoic acid was reported to be one of the common fatty acids in newly emerged and nurse honeybees (Naccarato et al. 2019), and its anionic forms have been reported in high amounts in the head tissues of honeybees located at high traffic intensity and industrial areas in Ukraine (Vishchur et al. 2019). Although no specific physiological role has been clearly established for eicosenoic acid in honeybees, certain eicosenoic compounds identified in bee venom—such as (Z)−11-eicosenol, methyl cis-11-eicosenoate, and cis-11-eicosenoic acid—have demonstrated immunostimulatory effects (Alqarni et al. 2019). The palmitoleic acid content in honeybees varies across subspecies and developmental stages. It was found in *Apis mellifera mellifera* pupae at 56 mg/100 g, but slightly lower, 48 mg/100 g in *Apis mellifera carnica* pupae (Ghosh et al. 2021). Palmitoleic acid content has also been reported as 167 mg/100 g in *Apis mellifera ligustica* drones and 93 mg/100 g in Buckfast *Apis mellifera* drones (Ghosh et al. 2020c). Another study evaluating the fatty acid composition of worker honeybees according to developmental stages reported that the palmitoleic acid contents in *Apis mellifera ligustica* at adult and pupal stages were 45 and 31 mg/100 g, respectively (Ghosh et al. 2016). Although the exact function of palmitoleic acid in honeybees remains unclear, several hypotheses have been proposed. It was demonstrated that palmitoleic acid exhibits antimicrobial activity against *Paenibacillus larvae*, suggesting a potential defensive role in honeybee immunity. Additionally, palmitoleic acid may contribute to synthesizing pheromones and cuticular chemical signals (Domínguez et al. 2024).

Many different polyunsaturated fatty acids have been reported in honeybees, including linoleic acid (Ghosh et al. 2021, 2016), linolelaidic acid (Ghosh et al. 2021), linolenic acid (Ghosh et al. 2020a), calendic acid (Girgis Sawires et al. 2024), eicosadienoic acid (Guiné et al. 2022), cis-11,14,17-eicosatrienoic acid, cis-13,16-docosadienoic acid, and cis-5,8,11,14,17-eicosapentaenoic acid (Ghosh et al. 2021).

Linoleic (ω−6) and linolenic (ω−3) acids play essential roles among the polyunsaturated fatty acids in honeybees. Honeybees can synthesize these fatty acids via elongase and desaturase enzymes from palmitic acid (Castaños et al. 2023; Qin et al. 2019) or directly supplemented from the pollens collected (Corby-Harris et al. 2022). Their levels generally increase as honeybees progress through developmental stages. While linoleic acid was not detected in *Apis mellifera ligustica* worker honeybees at larval and pupal stages (Ghosh et al. 2016; Guiné et al. 2022), its concentration reached 136 mg/100 g in adult honeybees (Ghosh et al. 2016). Beyond developmental differences, polyunsaturated fatty acid content varies between honeybee subspecies. Linolenic acid has been quantified at 154 mg/100 g and 119 mg/100 g in *Apis mellifera carnica* and *Apis mellifera mellifera* drones at the pupal stage, respectively (Ghosh et al. 2021). Besides, in a study on honeybee castes,

polyunsaturated fatty acid levels were found to decrease over time in drones, increase significantly in workers, and increase at a slower rate in queens throughout their lifespan (Martin et al. 2019). It was concluded that linoleic and linolenic acids affect the metabolic activities, health, cognitive development, and behaviors of honeybees (Bennett et al. 2022; Furse et al. 2023). Besides, linoleic acid can serve as a source of arachidonic acid, a key precursor of eicosanoid biosynthesis, playing a crucial role in mediating insect immune responses (Hasan et al. 2019). The linoleic/linolenic ratio is particularly important for honeybee health and functionality: a high ratio reduces hypopharyngeal gland size, brood-rearing capacity, learning ability, and olfactory sensitivity, while increasing mortality rates (Arien et al. 2020; Corby-Harris et al. 2022; Jorjani et al. 2023). In addition, Minahan et al. (2024) reported that an unbalanced dietary omega-6:3 ratio negatively impacted task allocation and nursing behaviors in honeybees. These effects are not immediately reversible, leading to delayed onset of nursing, a reduced frequency of nursing visits, and altered attention allocation between 3-day-old and 4-day-old larvae. According to Arien et al. (2018), the optimal linoleic/linolenic ratio of pollen for cognitive benefits in honeybees is between 0.3 and 0.9. Their findings indicate that honeybees fed with a diet containing 4% total lipid concentration and a linoleic/linolenic ratio of 1.0 achieved the highest learning scores compared to other ratios (0.3 and 5.0) and lipid concentrations (1, 2, and 8%) (Arien et al. 2018).

Although polyunsaturated fatty acids offer many health benefits, high levels of these fatty acids can induce lipid peroxidation, particularly in cell membranes, which can alter their composition. The difference in lifespan among different honeybee castes has been linked to variations in polyunsaturated fatty acid content (Arien et al., 2020; Martin et al. 2022; Vaudo et al. 2016). The increase in polyunsaturated fatty acid content in cellular membranes during the development of worker honeybees, unlike in queens, leads to oxidative damage, and this may contribute to the significantly shorter lifespan of workers compared to the long-lived queens (Martin et al. 2019). It has also been revealed that honeybees fed with linoleic acid-rich soybean oil had lower survival rates compared to those fed with oleic acid-rich palm oil (Wang et al. 2021).

In honeybees, linolelaidic, calendic, eicosadienoic, cis-11,14,17-eicosatrienoic, cis-13,16-docosadienoic, and cis-5,8,11,14,17-eicosapentaenoic acids are present in lower amounts than linoleic and linolenic acids (Ghosh et al. 2021; Girgis Sawires et al. 2024), and there is limited knowledge about their functions. The linolelaidic acid is a cis–trans isomer of linoleic acid, and its anti-inflammatory, hypocholesterolemic, hepatoprotective, antiarthritic, and cancer-protective properties have been noted in previous studies in human cell line models (Chaudhary and Tripathy 2015; Nazarparvar et al. 2020). Additionally, cis-13,16-docosadienoic and cis-5,8,11,14,17-eicosapentaenoic acid levels in honeybees have been associated with the synthesis of HSP 70 protein, which is produced in response to heat shock conditions (Sarioğlu-Bozkurt et al. 2022). The calendic acid was also identified in the whole body of *Apis mellifera carnica* workers (Girgis Sawires et al. 2024). It is recognized for its anticarcinogenic, antioxidative, anti-inflammatory, and hypotensive effects for humans. However, there is no report on its function in honeybees or other insects (Barut et al. 2022).

3.2. Factors affecting fatty acid composition in honeybees

The composition and concentration of fatty acids in honeybees can vary in response to factors like diet (Giri et al. 2018), seasonal temperature changes (Gooley and Gooley 2019), industrial pollution (Vishchur et al. 2019), hygiene, pathogens (Domínguez et al. 2024), pesticide exposure (Furse et al. 2023; He et al. 2020), and radiation (Girgis Sawires et al. 2024) (Figure 2).

The diet of honeybees is strongly dependent on pollen collected by the forager bees, providing essential lipids, proteins, vitamins, and minerals necessary to support the colony's vitality and development (Al-Kahtani et al. 2021). The

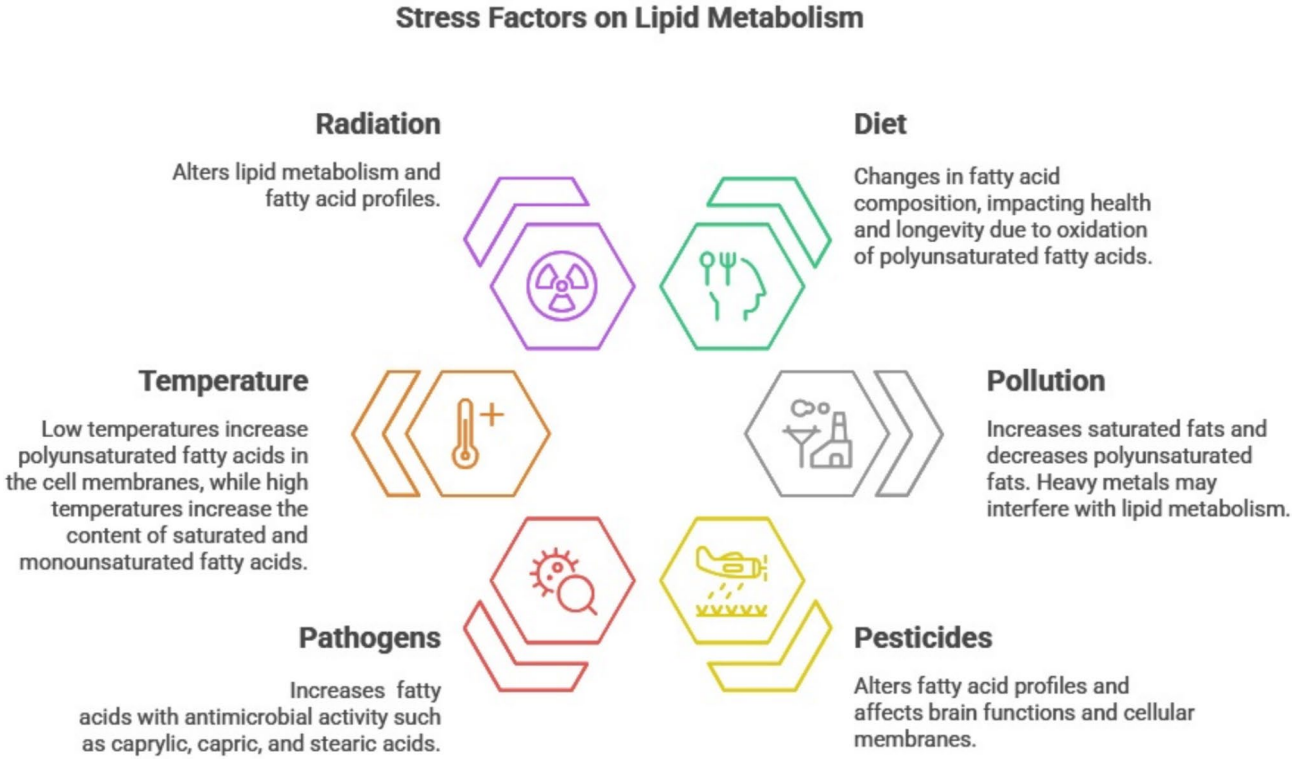

Figure 2. Stressors effects on fatty acid composition in honeybees.

bee pollen mainly contains palmitic, stearic, oleic, linoleic, and linolenic acids (Wang et al. 2021), and their contents can vary by botanical origin, seasonal changes, and storage conditions (Al-Kahtani et al. 2021). Honeybees' polyunsaturated fatty acid content largely reflects their diet, as pollen is the primary source of these essential fatty acids. The high polyunsaturated fatty acid-containing pollen diet increases their concentration in cellular membranes, which may cause short longevity of worker honeybees due to the oxidation susceptibility of these fatty acids (Wegener et al. 2018). Corby-Harris et al. (2022) recommend that bee diets maintain a balanced linoleic-to-linolenic acid ratio, as an elevated ratio has been shown to have detrimental effects on bee health. Arien et al. (2020) noted that varying dietary lipid concentrations alter the fatty acid composition of honeybee bodies, with total fatty acid content rising in response to increased dietary lipids. In another study, the incorporation of sunflower oil in the diet of honeybees caused an increase in caprylic, capric, lauric, myristic, pentadecanoic, palmitic, palmitoleic, linoleic, eicosadienoic, eicosatrienoic, arachidonic, docosadienoic, and docosatetraenoic acids, but decreased oleic acid in the tissue of honeybees (Saranchuk et al. 2021).

The seasonal cycle can lead to the differentiation between summer and winter honeybees. This adaptation to changing climatic conditions results in differences in the life span of honeybees, body size, and composition, including protein, hormone, and fat levels (Knoll et al. 2024). Studies have shown that honeybees reached the highest fat body content before cold weather conditions around late fall and winter seasons (Knoll et al. 2024; Koubová et al. 2021). According to the homeoviscous adaptation hypothesis, organisms in colder environments require a higher unsaturated-to-saturated fatty acid ratio to maintain membrane fluidity and function, whereas those in warmer climates benefit from increased saturated fatty acid content for greater energy efficiency (Giri et al. 2018). Similarly, studies have reported that alterations in the unsaturation level of membrane lipids represent an adaptive mechanism in honeybees under extreme temperature conditions. In addition, the concentration of unsaturated fatty acids in honeybee cell membranes was described to increase with decreasing air temperature. Consequently, drones and queen honeybees with high saturated fatty acid content in their membranes can live above 20 °C, while forager honeybees, rich in unsaturated fatty acids, are capable of flight at temperatures below 12 °C (Wegener et al. 2022). It was also reported that the amounts of stearic and linoleic acids in honeybees fluctuate with the season, peaking during spring and summer when honeybees are more active. In general, the amount of saturated and monounsaturated fatty acids increases during summer, reaching the highest level in late fall, then remains constant during winter and decreases as spring begins. On the other hand, the seasonal trend of polyunsaturated fatty acids has the opposite behavior, with maximum levels during spring and the lowest value when winter ends (Gooley and Gooley 2019).

Heavy metals are key indicators of industrial pollution in the environment, but they play, as well, a role in the synthesis, desaturation, and oxidation of long-chain fatty acids across various tissues in living organisms (Rivis et al. 2023). Findings reported that some heavy metals can bind to non-esterified fatty acids, while others, such as Cu and Zn, were found in the structure of enzymes involved in the elongation and desaturation of long-chain fatty acids (Vishchur et al. 2019). A study evaluating honeybees exposed to different levels of technological pollution (categorized as high, medium, and low based on heavy metal concentrations) found that honeybees from hives located in high-activity industrial areas had lower overall fatty acid content (633 mg/kg) compared to the other honeybees (643–674 mg/kg). Moreover, honeybees from areas having high technological burden exhibited higher levels of saturated fatty acids and lower levels of unsaturated fatty acids (Vishchur et al. 2016). Rivis et al. (2023) investigated the impact of heavy metals on honeybee fatty acid composition across various regions. They reported that the quantity of esterified fatty acids in worker honeybee tissues increased in areas with higher

concentrations of toxic heavy metals, such as foothills and forest-steppe regions. On the other hand, the conversion of esterified linolenic acid to longer-chain biologically active unsaturated fatty acids in honeybee head tissues was significantly reduced. In honeybees collected from mountainous areas, essential fatty acids in abdominal tissues—specifically linoleic and linolenic acids—were found at concentrations of 2.8 and 4.0 g/kg, respectively. However, in honeybees from the foothills and forest-steppe regions, these concentrations decreased approximately 18% for both acids.

Pesticides are chemicals used for crop protection from insects, fungi, and the growth of unwanted weeds. Its application during flowering may expose honeybees to these chemicals when collecting nectar or pollen (Mackei et al. 2023). Moreover, miticides such as coumaphos, fluvalinate-tau, or amitraz are applied directly to honeybees to control *Varroa destructor* infestations (Milone et al. 2021). It was revealed that intense tebuconazole contact with honeybees caused changes in fatty acid composition due to severe oxidative stress, which may result in possible damage to the brain of honeybees. The concentrations of lauric, myristic, and palmitic acids were notably higher at a medium dosage (4.2 µg/bee/day) of tebuconazole, compared to both lower (2.1 µg/bee/day) or higher (8.3 µg/bee/day) doses, as well as untreated honeybees (Mackei et al. 2023). In another study, honeybees fed with desmedipham, phenmedipham, or ethofumesate exhibited reduced saturated fatty acid content and increased unsaturated fatty acids in their thoracic and abdominal tissues compared to controls. It was also observed that this increase was more than 2 times in total ω−3 fatty acids content (α-linolenic acid, docosahexaenoic acid**,** eicosapentaenoic acid) (Khyzhnyak et al. 2018), which may influence cellular stability and metabolic functions, crucial for honeybee health.

Honeybees are susceptible to various pathogens, including *Paenibacillus larvae*, *Melissococcus plutonius*, *Spiroplasma apis*, *Serratia marcescens* (Fünfhaus et al. 2018), *Nosema ceranae* (Broadrup et al. 2019), and particularly to the parasitic mite *Varroa destructor,* which also transmits honeybee viruses (Posada-Florez et al. 2020). It was reported that, although no changes were observed in the total lipid concentration and fatty acid types of *Apis mellifera* worker honeybees infected with *P. larvae*, differences were detected in the ratios of some fatty acids. The levels of caprylic, capric, tridecylic, and oleic acid contents increase in *P. larvae* injected honeybees compared to control honeybees, and the regulation of fatty acids under the immunological threat is associated with the defense mechanism of honeybees (Domínguez et al. 2024). Broadrup et al. (2019) noted altered fatty acid metabolism in *Apis mellifera* honeybees infected with *Nosema ceranae*. Stearic acid and (9Z)-octadecenoic acid, both known for their antimicrobial properties, were found in relatively higher levels in infected honeybees. In contrast, hexadecanoic acid—a key link in fatty acid metabolism—was relatively reduced (Broadrup et al. 2019). In contrast, Aliferis et al. (2012) found no significant differences in palmitic, linoleic, oleic, and stearic acid levels between healthy and *N. ceranae*-infected *Apis mellifera*. The similarities of fatty acid composition between *Varroa destructor* and *Apis mellifera* drones, as its host, were also identified in another study. According to the literature results, saturated and unsaturated fatty acid percentages were not significantly different between *Varroa destructor* and the honeybee. However, in contrast to *Varroa destructor*, γ-linolenic, stearidonic, and eicosadienoic acids were exclusively detected in honeybees (Dmitryjuk et al. 2015). In another study, total fatty acid content and fatty acid composition changes after the *Ascosphaera apis*, a fungal pathogen, infection in honeybees were investigated, and it was demonstrated that while there was a slight decrease in saturated fatty acid content, unsaturated fatty acid content increased approximately twofold after the infection. The methyl laurate and methyl palmitoleate were not detected in infected honeybees, whereas ethyl oleate and methyl linolelaidate were not identified in healthy honeybees. Moreover, the methyl stearate, methyl palmitate, and methyl oleate amounts were higher in infected honeybees. The changes were explained with the different dynamics on the metabolic mechanisms,

such as the use of fatty acids as antifungal agents, cell membranes stabilization, and/or degradation of the cuticular lipids by pathogen enzymes (Elhoseny et al. 2024).

It has also been reported that radiation may impact biological molecules and lipid metabolism of honeybees, like many other insects. From this point of view, a study by Girgis Sawires et al. (2024) investigated the impact of gamma radiation on honeybee fatty acid composition by applying five different doses ranging from 20 to 200 rad. While lauric and myristic acids were detected in unirradiated control honeybees, they were not found in irradiated honeybees. In contrast, palmitic, stearic, lignoceric, and calendic acid levels increased with gamma-ray exposure. This differentiation in fatty acid profile was associated with the potential activity of the malate dehydrogenase enzyme, which was only detected in irradiated honeybees. It was evaluated that this enzyme might play a role in altered lipid metabolism under radiation stress and function as a potential adaptive metabolic response of honeybees (Girgis Sawires et al. 2024).

4. METHODOLOGIES FOR FATTY ACID ANALYSIS IN HONEYBEES

Analyzing fatty acids in materials with biological origin, including honeybees and beekeeping products, typically involves four main steps: sample pre-preparation, extraction, esterification, and identification/quantification. An overview of the different methodologies used in the literature to evaluate fatty acid composition in honeybees is summarized in Table III.

The preparation procedure may vary depending on the sample matrix, and various techniques can be employed to obtain homogenized samples, including mixing with acid-catalyzed hot water, milling, pressing, ultrasonication, microwave treatment, and enzymatic processing (Saini et al. 2021). For honeybees, to obtain a homogenized sample for the extraction, the honeybees must be frozen (Dmitryjuk et al. 2015; Ghosh et al. 2020a), or freeze dried (Ghosh et al. 2021; Haber et al. 2019), and ground with a homogenizer (Dmitryjuk et al. 2015; Ghosh et al. 2020a; Haber et al. 2019), using a tissue homogenizer device (Mackei et al. 2023), or mixing with ceramic beads (Martin et al. 2019), stainless steel beads (Arien et al. 2020), or zirconium beads (Bennett et al. 2022).

The lipid extraction methods for food/biological materials include Soxhlet extraction, solvent extraction, solid-phase microextraction, microwave-assisted extraction, ultrasonic-assisted extraction, and supercritical fluid extraction (Hewavitharana et al. 2020). Soxhlet extraction (Haber et al. 2019) and solvent extraction with basic mixing (Arien et al. 2020; Mackei et al. 2023; Martin et al. 2022; Wang et al. 2021) have been used for fatty acid extraction in honeybees. The commonly selected extraction solvents are methanol, chloroform, hexane, or the mixture chloroform-methanol (2:1, v/v) combination specified in the Folch method (Domínguez et al. 2024; Hewavitharana et al. 2020; Kovalskyi et al. 2018). The extraction time and temperature conditions in the reported studies with honeybees were not mentioned, but if Soxhlet extraction is used, those parameters are linked to the extraction solvent boiling temperature and an extraction time long enough to allow the full extraction. Some exceptions mentioned that lipid extraction was carried out by mixing for 1 h at 4 °C with methyl tert-butyl ether and ammonium acetate (Martin et al. 2022), while another study refers to honeybee brain tissue that was subjected to a mixture of methanol and NaOH and heated at 80 °C for 1 h for the fatty acid extraction procedure (Mackei et al. 2023).

The derivatization step is crucial, especially for identifying fatty acids by gas chromatography. This procedure enables the detection of fatty acids, as they are converted into methyl esters. The most commonly used derivatization methods can be classified as basic or acidic derivatization techniques (Hewavitharana et al. 2020). Basic derivatization typically involves a methanol-KOH solution (Giri and Dillon 2012), while acidic derivatization includes methanol-HCl (Arien et al. 2020; Bennett et al. 2022), methanol-H_2SO_4 (Haber et al. 2019; Wang et al. 2021), methanol-boron trifluoride (BF_3)

Table III Methodologies for fatty acid analysis in honeybees

Honeybee	Extraction method	Derivatization method/agent	System conditions	References
Apis mellifera drone-prepupae	Solvent extraction with chloroform/methanol (2:1, v/v) including of 0.005% butylated hydroxytoluene	Methanol-KOH solution	System: GC-FID Column: Rtx 2330 chromatography column (105 m × 0.25 mm) Gas: Helium Flow rate: 0.65 mL/min Temperature: 180–250 °C with gradual increase	(Dmitryjuk et al. 2015)
Native bees from *Andrena, Bombus, Hylaeus, Lasioglossum, Megachile,* and *Osmia* genera	Solvent extraction with methanol-hexane	Methanol-KOH solution	System: GC-FID Column: DB-23 fused silica capillary column (60 m × 0.25 mm × 0.25 µm) Gas: Hydrogen from purified air Split ratio: 50:1 Split flow: 35.5 ml/min Temperature: 75–230 °C with gradual increase	(Giri et al. 2018)
Apis mellifera larvae and pupae	Soxhlet extraction with chloroform	Methanol-H_2SO_4 solution	System: GC-FID Column: SP 2560 capillary column (100 m × 0.25 mm × 0.20 µm) Gas: Hydrogen Split ratio: 1:1 Temperature: 150–250 °C with gradual increase	(Haber et al. 2019)
Apis mellifera broods	Solvent extraction with hexane	Methanol-HCl solution	System: GC–MS Column: DB-23 column (60 m × 0.25 mm × 0.25 µm) Gas: Helium Flow rate: 1.0 mL/min Split ratio: 10:1 Temperature: 175–250 °C with gradual increase	(Arien et al. 2020)
Apis mellifera—*not specified*	Solvent extraction with chloroform–methanol	1% H_2SO_4 in methanol	System: GC–MS Column: HP-88 capillary column (60 m × 0.25 mm) Gas: not specified Split ratio: 19:1 Temperature: not specified	(Wang et al. 2021)
Apis mellifera worker honeybees	Solvent extraction with chloroform–methanol (2:1, v/v)	Methanol-HCl solution	System: EI GC–MS Column: HP-5MS column (30 m × 0.25 mm × 0.25 µm) Gas: Helium Flow rate: 1.2 mL/min Temperature: 35–320 °C with gradual increase	(Bennett et al. 2022)

Table III (continued)

Honeybee	Extraction method	Derivatization method/agent	System conditions	References
Apis mellifera adult worker honeybees	Solvent extraction with methanol, methyl-tert butyl ether, and ammonium acetate	Methanol-toluene, acetyl chloride and potassium carbonate solutions	System: GC–MS Column: Varian fused silica column (50 m × 0.25 mm) Split ratio: 25:1 Temperature: 150–232 °C with gradual increase	(Martin et al. 2022)
Apis mellifera	Solvent extraction with methanol-NaOH-HCl-chloroform	BF_3-methanol reagent (14%, w/v)	System: GC–MS Column: Zebron BPX-70 column (30 m × 0.25 mm × 0.25 µm) Gas: Helium Flow rate: 1.03 mL/min Temperature: 60–240 °C with gradual increase	(Mackei et al. 2023)

(Mackei et al. 2023), and methanol-benzene/toluene-acetyl chloride solutions (Ghosh et al. 2020a; Martin et al. 2022). These reagents typically contain functional groups that react with target compounds. However, the reliability of derivatization depends on several critical factors, including reaction efficiency, the stability of derivatized products, the availability of reagents and equipment, and overall analysis time. For successful derivatization, the reaction should be complete or nearly complete, proceed rapidly, and produce minimal by-products. Therefore, it is essential to compare different derivatization strategies in relation to the sample matrix and the anticipated fatty acid profile to select the most appropriate approach for accurate detection and quantification (Moldoveanu and David 2018). In terms of bee-related studies, the type of derivatization reagents and time–temperature conditions vary significantly according to the study. In a work involving Asian honeybee species, *Apis cerana* and *Apis dorsata*, the extracted fatty acids were derivatized with acetyl chloride at 100 °C for 1 h (Ghosh et al. 2020a). Haber et al. (2019) prepared fatty acid methyl esters from isolated fatty acids of larvae and pupae of *Apis mellifera* using 3% H_2SO_4-methanol solutions during 1 h. In addition, Bennett et al. (2022) carried out a derivatization with 8% HCl-methanol solution at 45 °C for 16 h. Giri et al. (2018) reported different derivatization procedures for native bee species using methanol-HCl and methanol-KOH solutions under varying conditions (80–85 °C for 45–60 min). They suggested that direct transesterification via 1.4 M KOH in methanol (65 °C for 60 min, with mixing every 5–10 min) is suitable for bee samples as small as 0.004 g dry mass, reducing the time of analysis and increasing resolution.

Gas and liquid chromatography are widely used techniques for the identification and quantification of fatty acids. However, due to certain limitations of liquid chromatography, such as lower selectivity and high chemical requirements, gas chromatography has become the more prominent method (Chiu and Kuo 2020). The gas chromatography system with detectors such as flame ionization detection (FID) or mass spectrometry (MS) was the most frequently observed for determining the fatty acid composition of honeybees (Bennett et al. 2022; Ghosh et al. 2020a; Haber et al. 2019; Martin et al. 2022; Wang et al. 2021). It seems evident that the GC-MS system is the most preferred over the GC-FID system since it provides additional structural knowledge linked to well-established databases for compound identification, while ensuring better efficiency and selectivity (Chiu and Kuo 2020). Furthermore, a study comparing GC-MS and GC-FID methods for fatty acid

analysis in algae concluded that GC-MS is the more efficient technique, offering higher fatty acid recovery, easier identification, and lower detection limits (Kchech 2017).

Another critical factor in identifying fatty acids is the choice of columns. High-polarity columns are generally suitable for separating fatty acids with varying carbon chain lengths (Chiu and Kuo 2020). However, there are differences in the separation and identification properties of columns with high polarity. For example, the DB-23 column delivers good results for fatty acid methyl ester mixtures in complex matrices, though it has limitations in separating cis-trans isomers. In contrast, the HP-88 column can better separate these isomers (David et al. 2005). Other studies comparing the efficiency of the DB-23 and BPX-70 columns in fatty acid analysis found that the DB-23 column produced lower R^2 values for the quantification of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) compared to the BPX-70 column (Shanta 2023). Therefore, column selection should be based on the properties of the targeted fatty acids or the fatty acids expected in the sample matrix. Several columns have been used for fatty acid analysis in honeybees, including Rtx-2330, SP-2560, DB-23, HP-88, HP-5MS, and Zebron BPX-70 (Table III). These columns contain highly polar phases, such as biscyanopropyl cyanopropylphenyl polysiloxane (Rtx-2330), bis(cyanopropyl)siloxane (SP-2560), and cyanopropyl polysilphenylene-siloxane (Zebron BPX-70), while for HP-5MS the column contains (5%-Phenyl)-methylpolysiloxane.

Helium is the most commonly used carrier gas for fatty acid determination studies for honeybees, but hydrogen gas was also applied in some studies (Table III). This option may be a good alternative to helium in terms of lower price, availability, ease of supply, and its ability to produce similar ion profiles (Galletta et al. 2022). However, its reactivity raises concerns, as it can be explosive and may induce catalytic hydrogenation in the presence of certain metals (Thomas et al. 2016). In addition, different temperature ranges (35–320 °C) and programs were applied in the fatty acid analysis of honeybees. Despite differences in materials, chemicals, and experimental conditions used across studies, the internal standard method is widely recommended for optimizing conditions. Common preferred internal standards for analyzing fatty acids include tridecanoic acid, margaric acid/heptadecanoic acid, nonadecanoic acid, and tricosanoic acid (Chiu and Kuo 2020). Previous studies on honeybees have utilized tridecanoic acid (Giri and Dillon 2012), margaric acid (Corby-Harris et al. 2022; Wang et al. 2021), and tricosanoic acid (Martin et al. 2021) as internal standards. These standards enable the evaluation and optimization of sample preparation, derivatization, and chromatographic conditions.

5. CONCLUSION AND FUTURE PERSPECTIVES

Many studies highlight the critical role of honeybees in sustaining biodiversity and ecological stability through their essential contributions to plant pollination. However, changing environmental conditions and external stressors have increasingly threatened honeybee populations. In response, honeybees have evolved adaptive mechanisms that enable them to persist under such challenging conditions. These adaptations often reflect changes in key biochemical components within their structures (Bordier et al. 2017; Even et al. 2012; Zhao et al. 2021). Fatty acids are critical, as honeybees adjust their fatty acid profiles to sustain their vitality and function when exposed to stressors. Monitoring these changes in fatty acid composition provides insights into honeybees' immune responses to different stressors, enabling researchers to investigate the underlying mechanisms of these stress factors. This understanding, in turn, may help mitigate or neutralize the negative impacts of these factors, for example, the adjustment of application doses of pesticides and miticides that would minimize harm to honeybees. Despite this potential, the basic mechanisms that influence fatty acid composition in honeybees are still not fully understood, as the studies examining the

physiological dependence of honeybees on fatty acids remain limited, which delays the development of specific mitigation or neutralization strategies. Possible approaches could include optimizing dietary fatty acid profiles, such as maintaining an appropriate linoleic/linolenic acid ratio, and improving environmental management practices to reduce exposure to stress factors such as temperature, pesticide exposure, or pathogens. Nonetheless, any strategy must be validated through experimental studies conducted in controlled environments.

Methodological variability further complicates research in this area. Differences in the body parts selected for extraction (e.g., inclusion or exclusion of the digestive system) and differences in extraction and derivatization procedures can affect both qualitative and quantitative fatty acid profiles, especially in the analysis of low-mass, low-lipid samples. While the technical issues associated with extraction and derivatization procedures can be overcome with comparative studies, sample preparation requires special attention to avoid meaningful conclusions: the impact of stress factors in the fatty acid composition of honeybees cannot be elucidated if we cannot guarantee that the lipid content in the digestive system or on the body surface of bees was eliminated from the sample or at least can be clearly identified. This underscores the necessity of developing reliable standardized analytical protocols for fatty acid analysis in honeybees and other insects, thereby ensuring the repeatability and reproducibility of findings.

Overall, studying fatty acid composition in honeybees is crucial for expanding our understanding of the relationship between immune pathways and lipid metabolism. This review highlights the need for further research on the fatty acid makeup of honeybees, their physiological roles, the effects of stressors, and the analytical methods used to analyze their composition.

AUTHORS' CONTRIBUTIONS

CM: investigation, conceptualization, writing—original draft preparation, reviewing and editing. MVB: investigation, conceptualization, and writing, reviewing and editing

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DATA AVAILABILITY

Data sharing is not applicable to this review as no new data was created or analyzed.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

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