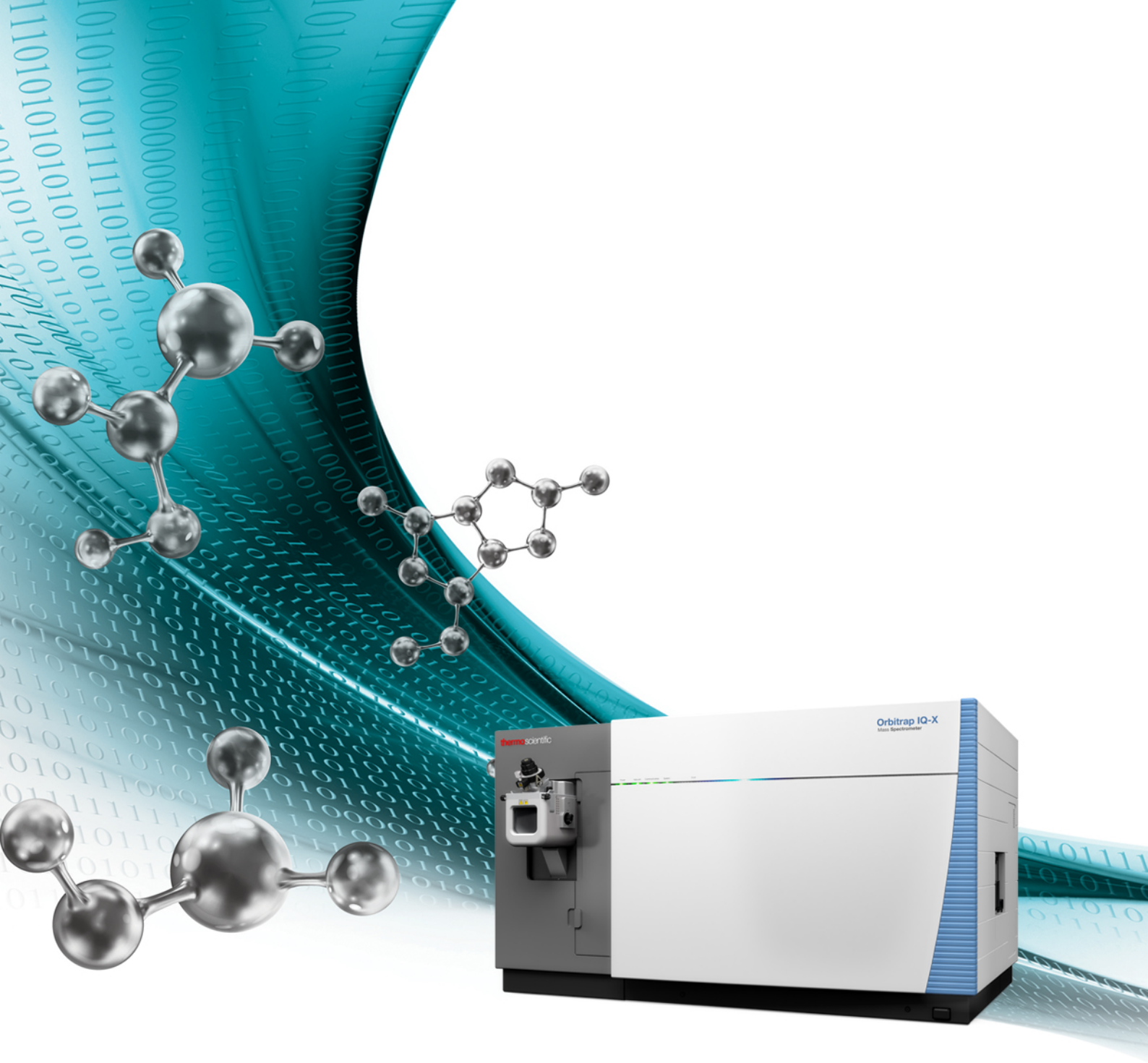

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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

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Conventional and emergent technologies for honey processing: A perspective on microbiological safety, bioactivity, and quality

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Abstract

Honey is a natural food of worldwide economic importance. Over the last decades, its potential for food, medical, cosmetical, and biotechnological applications has been widely explored. One of the major safety issues regarding such applications is its susceptibility to being contaminated with bacterial and fungi spores, including pathogenic ones, which may impose a hurdle to its consumption in a raw state. Another factor that makes this product particularly challenging relies on its high sugar content, which will lead to the formation of hydroxymethylfurfural (HMF) when heated (due to Maillard reactions). Moreover, honey's bioactivity is known to be affected when it goes through thermal processing due to its unstable and thermolabile components. Therefore, proper food processing methodologies are of utmost importance not only to ensure honey safety but also to provide a high-quality product with low content of HMF and preserved biological properties. As so, emerging food processing technologies have been employed to improve the safety and quality of raw honey, allowing, for example, to reduce/avoid the exposure time to high processing temperatures, with consequent impact on the formation of HMF. This review aims to gather the literature available regarding the use of conventional and emergent food processing technologies (both thermal and nonthermal food processing technologies) for honey decontamination, preservation/enhancement of honey biological activity, as well as the sensorial attributes.

KEYWORDS
antibacterial activity, antioxidant activity, emergent technologies, honey, nonthermal processing, physicochemical quality, thermal processing

Nomenclature: ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (free radical scavenging activity); DA, diastase activity; DN, diastase number; DPPH, 2,2-diphenyl-1-picrylhydrazyl (free radical scavenging activity); FRAP, ferric reducing antioxidant power (antioxidant capacity); HMF, hydroxymethylfurfural; HPP, high pressure processing; MGO, methylglyoxal; ORAC, oxygen radical absorbance capacity; TPC, total phenolic content; UV light, ultraviolet light

1 | INTRODUCTION

Honey has been used as food and natural remedy since ancient times (Eteraf-oskouei & Najafi, 2013). Currently, honey consumption is continuously growing, mainly due to the increase in the world population and the growing demand for natural and healthy products (García, 2018). Likewise, honey is used in the pharmaceutical and cosmetic industries, mainly due to its effectiveness in wound healing and burns (McLoone et al., 2020).

Due to its natural antioxidants and their synergic interaction with other components, honey has the potential capacity to serve as an important source of antioxidants in human nutrition and can exhibit beneficial effects on human health, as antitumor and cardiovascular protection (Erejuwa et al., 2014; Olas, 2020). The antibacterial activity of honey is one of the most important findings that was first recognized in 1892 by the Dutch scientist Van Ketel (Scepankova et al., 2017). Recent research indicates that the effectiveness of honey in many of its medical uses is due to its antibacterial activity that can inhibit Gram-positive and Gram-negative bacteria, including multidrug-resistant strains *Staphylococcus aureus* (MRSA) (Combarros-Fuertes et al., 2020), and some species of fungi (Almasaudi, 2021; Fernandes et al., 2021; Manyi-loh et al., 2010; Nolan et al., 2019; Roberts et al., 2019) and viruses (Abedi et al., 2021; Hossain et al., 2020; Semprini et al., 2019; Watanabe et al., 2014).

Thermal processing is an important step in honey production, which directly affects its essential composition and quality. Honey is subjected to thermal processing to delay crystallization, to remove the contaminating microorganisms, to reduce viscosity, and to prevent crystallization and fermentation (Singh & Singh, 2018). The major concern arising from thermal processing comes from the formation of compounds that are not naturally present in foods but may develop during heating. Such compounds may possess harmful effects such as mutagenic, carcinogenic, and cytotoxic effects, due to the presence of neoformed contaminants. Hydroxymethylfurfural (HMF) is formed upon thermal processing and/or long-term storage as an intermediate product of Maillard reaction (Shapla et al., 2018) and is a well-known neo-formed contaminant and its safe level of daily consumption is not well clarified (Husøy et al., 2008). However, some studies reported tolerable daily intake of HMF in doses ranging from 80 to 100 mg/kg body weight (Shapla et al., 2018). It is important to notice that HMF in honey is extensively studied as an indicator of honey quality and freshness. Indeed, overheated honey (as a result of uncontrolled and excessive thermal processing) can contain very high HMF levels (Biluca et al., 2014). Özkök and Silici (2016) concluded that high HMF content of honey may affect the human health adversely and thus HMF level must be controlled. Therefore, International Honey Commission (IHC) established the content of HMF as well as diastase activity (DA) as the international standards of quality used to control the honey freshness and limit for thermal processing of honey (Bogdanov, 2009). Likewise, thermal processing can result in severe physical and chemical degradation of honey, with a significant impact on color, flavor and antioxidant and antibacterial properties (Chaikham et al., 2016; Chua et al., 2014; Zarei et al., 2019). Nevertheless, honey must be delivered to the consumers with its essential composition and minimally altered quality (Doménech et al., 2010).

Considering that honey is a raw food product, it may contain pathogenic microorganisms, such as bacterial spores (*Clostridium botulinum* strains), which, despite having a small effect on honey degradation, constitute severe concerns regarding its safe consumption, especially by immunosuppressed people or by children and pregnant woman. Honey is the only dietary vehicle so far definitively linked to infant botulism (European Commission, 2002). As recommended by the World Health Organization (WHO), for the prevention of infant botulism, it is essential not to use honey as a sweeter in preparations for infants <12 months of age (World Health Organization, 2018). Although conventional thermal processing is effective in reducing viscosity and microbial decontamination, the lack of this technique lays in the inefficiency to inactivate bacterial spores such as the *C. botulinum*.

Currently, there is increased consumers' demand for minimally processed foods with improved nutritional and sensorial characteristics. Emergent technologies for food processing, especially nonthermal technologies, are increasingly replacing the conventional thermal pasteurization techniques (Jan et al., 2017). Emergent processing technologies may constitute a promising alternative to ensure honey microbiological safety with preserved fresh-like quality (Lee et al., 2019).

Therefore, the present paper aims to review the effect of conventional thermal processing and emergent food processing technologies (both thermal and nonthermal) on honey quality, microorganisms, and bioactive properties.

2 | EFFECT OF CONVENTIONAL THERMAL PROCESSING ON HONEY'S QUALITY

Thermal processing of food is still considered the simplest and most effective method for preventing foods from microbial spoilage. In the honey industry, thermal processing is widely applied for the pasteurization. Even though bacteria cannot reproduce in honey (due to low moisture, pH, water activity, and high acidity as well as other

antibacterial components), they can be transmitted when honey is used as an ingredient in the preparation of other foods and multiply until they deteriorate the product (Serreira et al., 2010). For instance, Iurlina and Fritz (2005) found high levels of aerobic mesophilic bacteria in commercial honey (1200 CFU/g), artisanal honey (1100 CFU/g), and bulk honey (500 CFU/g) from Argentina. Those samples exceeded the maximum limit established by the Southern Common Market (MERCOSUR) regulations (100 CFU/g) (Finola et al., 2007).

Despite this, the osmophilic yeasts (mainly *Saccharomyces*) are found to be the dominant yeasts fermenting honey, able to withstand the concentrated sugars and limited level of water (Belitz et al., 2009). Their presence in honey is unavoidable because bees collect them together with the nectar, pollen, and honeydew. Also, inappropriate honey handling and cross-contamination with equipment or other products also contribute to the microbial contamination of honey. Honeys with a high initial yeast count and borderline moisture content (between 17.1 and 20%) may start to ferment after crystalizing (Snowdon & Cliver, 1996). The spontaneous crystallization attributes to phase separation, which increases the water concentration in the liquid phase and the water activity up to the normal levels (above 0.61), which is suitable for yeasts multiplication, thus promoting honeys' fermentation (Ma et al., 2017; Zamora & Chirife, 2006). Commonly, the fermentation of honey occurs on the top of the barrel or jar, where the water content is increased and such honey is not likely to be palatable or marketable (Snowdon & Cliver, 1996). Unlike milk or other dairy products, pasteurization of honey is done not only to eliminate the spoilage microorganisms but also to remove any chance of fermentation, as it brings down the moisture content to a safe limit and delays crystallization (Singh & Singh, 2018).

There are various thermal processes for maintaining the liquid consistency of honey for a longer time, increasing shelf life. The high-temperature, short-time heating is a basic method of pasteurization widely used in industry, for flash pasteurization. The flash pasteurization of honey at 80°C for only 30 s, followed by rapidly cooling, has been reported to eliminate all fungi and yeasts (Tosi et al., 2004). In addition, the process melts glucose microcrystals and removes air bubbles, delaying postprocessing crystallization—as required by large honey retailers. Although milk can be pasteurized by high-temperature, short-time heating at a temperature of 72°C for only 15 s (Lucey, 2015), this procedure did not reduce yeast counts in honey (Schvezov et al., 2020). On the other hand, Gonnet et al. (1964) suggested 78°C and a longer time (6–7 min) is the best pasteurizing condition. It is important to consider, however, that spores are resistant to pasteurization, but sterilization could cause severe changes in the honey quality. According to Codex Alimentarius (2001), honey should not be submitted to an extreme heating temperature, as it would impact honey's essential composition and/or lower its quality. To date, no guideline is available regarding the use of temperature and time for pasteurization of a particular type of honey (Chua et al., 2014).

The quality of honey is vital for national and international markets to ensure competitive prices and human health. HMF and DA are important parameters used for evaluating honey quality and freshness. These parameters express the ability to maintain original chemical–physical and sensorial features of honey over time (Baglio, 2018). Indeed, when the DA (expressed as diastase number [DN]) drops below the acceptable limit of 8 DN (except for honey with a low natural enzyme content, not less than 3 DN) and/or HMF exceeds 40 mg/kg (except for honey from tropical zones, where the cutoff is 80 mg/kg), the quality of honey is considered degraded, and the product should be designated as baker's honey (Thrasyvoulou et al., 2018). Codex Alimentarius and European Directive require that HMF and DA contents should be determined after honey processing and blending (Thrasyvoulou et al., 2018). Table 1 summarizes HMF content and DA changes in honey after thermal processing. Chaikham et al. (2016) reported that the conventional thermal processing at 90°C for 5 min sufficiently reduced the total plate counts and yeast molds in all honeys and kept the HMF within the standard limit (40 mg/kg). However, in this study, the DA was lower than 8 DN, indicating excessive thermal processing. Kowalski et al. (2012) reported no DA in honeydew honey after increasing the heating time above 30 min at 90°C. In the same line, several studies pointed out that prolonged heating at high temperatures causes a high concentration of HMF in honey. For instance, the honey processed at the 75°C for 24 h presented too high values of the HMF (from 173.4 to 226.35 mg/kg) (Karabournioti & Zervalaki, 2001). In comparison, Brazilian *Apis mellifera* honey processed at the same conditions (75°C for 24 h) had an even higher HMF concentration (695.40 mg/kg) (Biluca et al., 2014). On the other hand, when honey is submitted to higher temperatures but during reduced periods of time, the HMF levels tend to be much lower, being within the stablished limits. This has been shown by several authors including Czipa et al. (2019), when honey was heated at 80°C for 60 min, and Akhmazillah and Farid (2015), for 10 min of processing at 70°C.

Therefore, the basic concept of honey pasteurization is to heat the honey only to the lowest temperature and for the shortest period of time (Escriche et al., 2009; Tosi et al., 2004). Currently, industrial heating, up to 55°C, is the most accepted process for the prevention of honey crystallization and de-crystallization (liquefaction) (Bucekova, Juricova, Di Marco, et al., 2018). The International Honey

TABLE 1 Effect of conventional thermal processing on changes of the physicochemical quality standards (HMF and DA) of honey

Honey			Conventional heating Conditions					
Name	**Botanical origin**	**Geographical origin**	**Temperature (°C)**	**Time**	**Heating method**	**Parameter**	**Findings**	**Reference**
Lotus, thyme, multifloral	*Ziziphus lotus*, *Zataria multiflora*	Iran	63	≤30 min	WB	HMF	HMF increased with increasing heating time. After 30 min increased by 81.3, 85.6, and 108.3%, respectively. The established limit of HMF (40 mg/kg) was not exceeded.	Zarei et al. (2019)
Multifloral honey	*	Romania	50, 80, 100	≤5 h	WB	HMF	The established limit of HMF (40 mg/kg) was not exceeded. The max. level of HMF was achieved at 100°C after 5 h of heating (26.69 mg/kg), with an increased ratio of 1147.19%.	Cozmuta et al. (2011)
						DA	Complete inactivation of DA in the range of 3–5 h at 50 and 80°C and in the range of 3–5 h at 100°C heating.	
Tualang, gelam, acacia	*	Malaysia	90	60 min	WB	HMF	HMF content increased gradually with increasing time. Honey, with higher initial content of HMF, showed a faster rate of HMF formation.	Chua et al. (2014)
						DA	A slight decrease in DA.	
Acacia	*Robinia pseudoacacia*	Hungary	≤80	60 min	WB	HMF	HMF content in raw honey was 0.31 mg/kg, heating at 60, 70, and 80°C increased the content of HMF to 4.45, 7.37, and 10.2 mg/kg, respectively. The established limit of HMF (40 mg/kg) was not exceeded. Heating at 40 and 50°C did not increase the HMF content significantly.	Czipa et al. (2019)
						DA	Reduction of DN by 5 DN at 80°C.	
Pine, orange, sunflower, cotton, thymus	*	Greece	≤75	24 h	WB	HMF	Long-time heat treatment at 65°C exceed the established limit of HMF content (≤40 mg/kg) in sunflower, cotton, and thymus honey, being 87.60, 52.70, and 48.20 mg/kg, respectively, and at 75°C, the content of HMF was extremely high in all honey, except in pine (43.40 mg/kg).	Karabournioti and Zervalaki (2001)
Apis mellifera	*	Brazil	75 75	15 min 24 h	WB; Oven	HMF	An extremely high content of HMF after 24 h, being the value 695.40 mg/kg, whereas after 15 min, it was only 8.05 mg/kg.	Biluca et al. (2014)

(Continues)

TABLE 1 (Continued)

Honey			Conventional heating Conditions					
Name	**Botanical origin**	**Geographical origin**	**Temperature (°C)**	**Time**	**Heating method**	**Parameter**	**Findings**	**Reference**
Orange, eucalyptus, sulla, chestnut	*Citrus aurantium* L., *Eucalyptus camaldulensis* L., *Hedysarum coronarium* L.), *Castanea sativa* L.	Italy	50 70 100	144 h 96 h 60 h	thermostatic oven	HMF	At 50°C, the content of HMF increased by increasing heating time, except for the Chestnut honey that did not form any HMF even after 144 h (6 days) of heating. At 70°C, the content of HMF increased by increasing heating time and exceeded the limit of 40 mg/kg, after 24 h orange honey (47.5 mg/kg), after 48 h eucalyptus honey (290 mg/kg) and sulla honey (96.8 mg/kg), and 84 hr chestnut honey (56 mg/kg). At 100°C, all honey showed exceeded limit (40 mg/kg) of HMF after 4 h of heating. With increasing time of heating, all honey showed extremely high content of HMF.	Fallico et al. (2004)
Citrus, multifloral, pine honeydew	*	Turkey	≤75	≤60 min	–	HMF	HMF content increased with increasing temperature and reached maximum at the processing temperature of 75°C.	Sahinler (2007)
						DA	DA decreased with increasing temperature in all honey except pine honeydew honey, which was the most resistant honey.	
Rape	*	Poland	≤80	15 min	WB	HMF	Did not significantly increased the HMF content.	Kedzierska-Matysek et al. (2016)
						DA	Did not significantly reduced the DA.	
Zantaz' honey (monofloral)	*	Morocco	121	30	–	HMF	Significantly increase in the HMF content. The mean values changed from 7.70 ± 5.82 to 20.11 ± 11.91 mg/kg (increase around 55%). Both values are below the limit (40 mg/kg).	Elamine et al. (2020)
						DA	Value of DA changed from 21.31 ± 5.94 shade unit/g in raw samples to values below the detection limits following processing.	
Honeydew and floral	*	Turkey	75, 90, 100	≤90 min	TC-WB	HMF	HMF contents exceed the limit in honeydew honey at 90°C after 75 min and at 100°C after 15 min; floral honey at 100°C after 60 min.	Turhan et al. (2008)

(Continues)

TABLE 1 (Continued)

Honey			Conventional heating Conditions					
Name	**Botanical origin**	**Geographical origin**	**Temperature (°C)**	**Time**	**Heating method**	**Parameter**	**Findings**	**Reference**
Citrus, rosemary, multifloral, honeydew	*	Spain	(L) 45 (P) 80	48 h 4 min	(L) TC-oven; (P) flow-through silicon tubing in TC-OB	HMF	Pasteurization (P) caused higher content of HMF than liquefaction (L). Values (mg/kg) for (L) and (P) of citrus honey were 6 (L) and 11 (P), for rosemary honey 5.1 (L) and 7.2 (P), for multifloral 7.6 (L) and 9.9 (P), and honeydew 6.2 (L) and 9.5 (P). The established limit of HMF (40 mg/kg) was not exceeded.	Escriche et al. (2009)
Coriander	(*Coriandrum sativum*)	India	47.5	9 min pH 4.7	WB	HMF	The optimum conditions that can keep the HMF concentration (7.78 mg/kg) within the standard limit.	Kamboj et al. (2019)
						DA	The optimum conditions that can keep the DA (17.95 DN) within the limit of standard.	
Sunflower	*Helianthus annus*	India	47.5 ± 1	9.5 ± 1 min pH 5.2 ± 0.15	WB	HMF	The optimum conditions that can keep the HMF concentration within the limit of standard.	Nanda et al. (2006)
						DA	The optimum conditions that can keep the DA within the limit of standard.	
Lime buckwheat, acacia, and honeydew	*Tilia* sp., *Fagopyrum* sp., *Robinia pseudoacacia*	Poland	90	≤60 min	WB	HMF	HMF content increased with increasing heating time. The HMF values (mg/kg) in raw buckwheat, lime, acacia, and honeydew honey were 53.77, 33.85, 22.36, and 2.04, respectively. After 60 min at 90°C, the content of HMF (mg/kg) increased to 94.33, 45.99, 31.14, and 6.13, respectively.	Kowalski (2013)

(Continues)

TABLE 1 (Continued)

Honey			Conventional heating Conditions					
Name	**Botanical origin**	**Geographical origin**	**Temperature (°C)**	**Time**	**Heating method**	**Parameter**	**Findings**	**Reference**
Honeydew	*	Poland, Italy, Turkey	90	≤60 min	WB	DA	Heating up to 30 min did not cause a decrease below the normative limit (DN = 8). However, the increasing heating time above 30 min inactivated the DA.	Kowalski et al. (2012)
Longan flower, lychee flower, wildflower	*	Thailand	90	5 min	WB	HMF	HMF content in all honey increased but did not exceed the established limit. The content of HMF in the raw longan, lychee, and wildflower honey was 4.13, 5.01, and 9.97, and after heating increased to 20.09, 23.65, and 28.14 mg/kg, respectively.	Chaikham et al. (2016)
						DA	DA of all honey after heating was lower than 8 Units/g, indicating the excessive heating of honey. The values of DA (Units/g) in the raw longan, lychee, and wildflower honey were 15.12, 13.87, and 13.98, and after heating decreased to 7.95, 6.61, and 7.01 (Units/g), respectively.	

Abbreviations: L, liquefaction; P, pasteurization; TC-OB, temperature-controlled oil bath; TC-WB, temperature-controlled water bath; WB, water bath.
*The authors did not provide the information.

Commission recommends that the crystallized honey may be liquefied by heating at no more than 40°C (International Honey Commission, 2002). According to of Organic Federation of Canada, heating of honey for extraction shall not exceed 35°C and the de-crystallization temperature shall not exceed 47°C; in case the product is heated above such temperatures, it should only be used as an ingredient in a multi-ingredient product (Organic Federation of Canada, 2020). Despite this, the duration of liquefaction at mild temperature is not uniform and the honeys are often heated until complete dissolution of crystals. Moreover, it starts to liquefy at the sides of the container, leading to overheating of honey in some zones (Kretavičius et al., 2010). In order to prevent this situation, honey should be homogenized during in-package heating or processed in continuous followed by hygienic packaging.

The origin of honey has also been referred to be an important factor affecting the formation of HMF, particularly when honey is processed at low temperatures (Fallico et al., 2004). The study carried out by Bucekova, Juricova, Di Marco, et al. (2018) showed that samples of wildflower honey (50–72% *Brassicaceae* pollen) completely de-crystallized within 14 h of heating at 45°C, whereas rapeseed honey (80–91% *Brassicaceae* pollen) did not liquefy even within 120 h. Cozmuta et al. (2011) demonstrated that after 5 h of heating multifloral honey at the temperature of 50°C, the formation of HMF increased by 192.05%, and the DA was completely inactivated. Therefore, the same duration of the liquefaction process at constant temperatures may decrease the quality in some honeys while preserving the characteristics of others; further studies are needed to clarify this question. The changes in the concentration of HMF and the DA during thermal processing (pasteurization and liquefaction) are also related to other factors including pH, free acidity content, lactone content, and mineral content, which, in turn, are related to the botanical origin of the honey (Shapla et al., 2018).

Some authors advocate that the concentration of HMF is a thermal index rather than a quality standard, and that other variables should be introduced in the evaluation of honey quality (Bucekova et al., 2020; Fallico et al., 2004).

2.1 | Changes in the biological activity of honey after conventional thermal processing

During thermal processing, changes occurring in physicochemical quality indicators of honey (i.e., HMF and DA) are merely significant from compliance with national and international legal norms. However, from the nutritional point of view, the decrease of the biological activity during the thermal processing of honey is of particular importance because it may affect honey's nutritional quality and health benefits (Zarei et al., 2019). This section discusses thermal processing's effect on changes in honey's antioxidant properties (Table 2) and antibacterial activity.

2.1.1 | Changes in antioxidant properties of honey

Phenolic compounds of honey behave as antioxidants in a variety of ways, including the direct trapping of reactive oxygen species (ROS), inhibition of enzymes responsible for producing superoxide anions, chelation of transition metals involved in the process of forming radicals, and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals (Pyrzynska & Biesaga, 2009). Several studies observed a correlation between total phenolic content (TPC) and antioxidant activity, suggesting that phenolic compounds are the main compounds responsible for that bioactive property. However, due to the complex composition of honey, the interaction among the different nonphenolic compounds and the possible synergism between them may also play an important role (Kędzierska-Matysek et al., 2021). Among those compounds are proteins, amino acids, peptide inhibitors of oxidative enzymes, enzymes such as catalase or glucose oxidase, vitamins such as ascorbic acid and α-tocopherol, and organic acids, which may chelate metals and favor polyphenols' action. In addition, owing to the complex nature of the matrix and the involvement of multiple reaction characteristics and mechanisms, different antioxidant assays provide different results. Each assay assesses diverse mechanisms, in which a wide variety of phytochemicals take part (Combarros-Fuertes et al., 2019; Scepankova et al., 2017). Therefore, the determination of the antioxidant activity of honey is generally performed by using a combination of different in vitro antioxidant activity assays (2,2-diphenyl-1-picrylhydrazyl [free radical scavenging activity] [DPPH], ferric reducing antioxidant power [antioxidant capacity] [FRAP], 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) [free radical scavenging activity] [ABTS], and oxygen radical absorbance capacity [ORAC]) together with the quantification of TPC, total flavonoid compounds, and individual phenolic compounds (Karabagias et al., 2018).

During thermal processing, the antioxidant activity of different types of honey is changed. Zarei et al. (2019) recently investigated the effect of thermal processing at 63°C for 30 min on the lotus, thyme, and multifloral honey from Iran. They found a significant reduction of TPC in all honey, being the values for the unprocessed and processed lotus honey 609 and 482 mg/kg, for thyme 538 and 447 mg/kg, and multifloral honey 462 and 404 mg/kg, respectively. Thyme honey showed a decrease in DPPH

TABLE 2 Effect of conventional thermal processing on changes of antioxidant properties of honey

Geographical origin	Type of honey	Processing conditions(temperature, time)	Antioxidant properties of honey				Observations and conclusions	Reference
			Total phenolic content (TPC)	Total flavonoid content (TFC)	Antioxidant activity	Other compounds with antioxidant activity		
Turkey	Astragalus (crystallized)	(L) 55°C/12 h (P) 90°C/15 s	L: no SD; P: no SD.	*	L: No SD P: No SD	P: ↓ caffeic acid (4.46 ± 0.13 mg/kg); Unprocessed: caffeic acid (6.40 ± 0.07 mg/kg).	The impact of L was lower than the P process in terms of quantitatively phenolic compound changes.	Aydogan-coskun et al. (2019)
	Sunflower–cornflower mix (crystallized)		L: no SD P: ↑ (1.34 ± 0.00 mmol TE/kg); Unprocessed: (0.75 ± 0.03 mmol TE/kg).	*	L: No SD P: ↑	P: ↓ Kaempferol-3-glucoside (3.48 ± 0.73 mg/kg) Unprocessed: Kaempferol-3-glucoside (18.09 ± 4.68 mg/kg)		
Spain	Citrus, rosemary, multifloral, and honeydew	(L) 45°C/48 h (P) 80°C/4 min	*	*	L: ↓ (56.68%) P: ↑ (63.18%) Unprocessed (59.34%).	P and L: ↓ *p*-coumaric acid and kaempferol P: ↓ galangin and myricetin.	The impact of P and L did not modify the phenolic profile to the point of affecting discrimination of honey botanical origin.	Escriche et al. (2014)
Mexico	Tasquillo, Acaxochitlan, Huehuetla, El Arenal, and Orizatlan	40–80°C/45 min	↑ (mg GAE/100 g) in Tasquillo and Huehuetla at 70°C, but at 80°C ↓; no SD in other three honeys.	↑ (13.7% increase) in Acaxochitlan at 70°C but at 80°C ↓; ↑ (mg QE/100 g) in Huehuetla at 50°C but ↓ tendency up to 80°C.	DPPH (mg AAE/100 g honey) ↑ in all honey up to 80°C; ABTS (mg AAE/100 g honey): ↑ in Orizatlan and El Arenal, but ↓ Tasquillo, Acaxochitlan, and Huehuetla at 80°C.	The high correlation coefficient ($r = .8–.9$) between DPPH and HMF, and between TPC and ABTS for all samples.	TPC and TFC reacted differently to the heat treatments depending on the origin of the honey.	Pimentel-González et al. (2016)

(Continues)

TABLE 2 (Continued)

Geographical origin	Type of honey	Processing conditions(temperature, time)	Antioxidant properties of honey					
			Total phenolic content (TPC)	Total flavonoid content (TFC)	Antioxidant activity	Other compounds with antioxidant activity	Observations and conclusions	Reference
India	Cherry and saffron	60–80°C/10–15 min/pH 3–6	↑ with ↑time and temperature and ↓pH (from 6 to 3)	↑ with ↑time and temperature and ↓pH (from 6 to 3)	↑ with ↑time and temperature and ↓pH (from 6 to 3)	*	The increase of AA was more noticeable at 80°C than at 60 and 70°C but decreased at ↓ pH from 6 to 3; AA increased with the formation of browning pigment (Maillard reaction products [MRPs])	Nayik and Nanda (2016) Nayik and Nanda (2015)
Thailand	Longan flower (LOF), lychee flower (LF), and wildflower (WF)	90°C/5 min	↓ in all honey (mg GAE/100 g): LOF: from 95.16 ± 1.04 to 80.74 ± 2.37; LF: from 60.13 ± 0.73 to 47.21 ± 1.66; WF: from 81.66 ± 2.74 to 64.75 ± 1.52.	↓ in all honeys (mg QE/100 g): LOF: from 53.58 ± 1.93 to 42.69 ± 2.11; LF: from 45.14 ± 1.05 to 31.52 ± 2.04; WF: from 46.01 ± 3.10 to 35.80 ± 2.00.	↓ FRAP in for all honeys; ↑ DPPH (%) in all honeys: LOF: from 42.95 ± 2.05 to 48.16 ± 1.88; LF: from 30.85 ± 1.00 to 35.90 ± 2.20; WF: from 34.11 ± 0.50 to 38.62 ± 1.02.	↓ of vitamin C (mg/kg) LOF: from 8.48 ± 0.62 to 1.02 ± 0.53; LF: from 5.14 ± 0.41 to 0.97 ± 0.28; WF: from 4.50 ± 0.32 to 0.51 ± 0.13.	Thermal processing darkened honey, which negatively influenced the consumer's acceptance. Over 80% reduction of vitamin C.	Chaikham et al. (2016)
Thailand	Longan flower	50, 70, and 100°C/up to 5 min	(mg GAE/100 g) ↑ at 50 and 70°C; ↓ at 100°C.	(mg QE/100 g) No SD at 50 and 70°C; ↓ at 100°C.	DPPH (%) and FRAP (mM $FeSO_4$/g): ↑ 50 ↑ (5 min), no SD at 70°C; ↓ at 100°C.	*	By increasing processing temperature up to 100°C, antioxidant properties showed a decreasing tendency.	Chaikham and Prangthip (2015)

(Continues)

TABLE 2 (Continued)

Geographical origin	Type of honey	Processing conditions(temperature, time)	Antioxidant properties of honey					
			Total phenolic content (TPC)	Total flavonoid content (TFC)	Antioxidant activity	Other compounds with antioxidant activity	Observations and conclusions	Reference
Iran	Lotus, thyme, and multifloral	63°C/up to 30 min	(mg GAE/kg) ↓ after 30 min.	*	DPPH (%): ↓ after 30 min in thyme honey; no SD in others; FRAP (μM Fe(II)): ↓after 30 min in thyme and lotus; no SD in multifloral honey.	*	A decrease in antioxidant activity and phenolic compounds at increasing processing time (thyme honey and lotus honey).	Zarei et al. (2019)
Greece	Fir, pine, thyme, and orange blossom	40, 45, and 80°C/30 min	*	*	DPPH ↑ in all samples up to 45°C; ↑ in thyme and orange at 80°C; ↓ in fir and pine at 80°C.	Color transformation at 80°C due to nonenzymatic browning (Maillard reaction).	Mild heat treatment (40–45°C) increased antioxidant activity in all honey, whereas heating at 80°C was not considered a tool for increasing antioxidant activity.	Karabagias et al. (2018)
Poland	Lime (L), buckwheat (B), honeydew (HD), and acacia (A)	90°C/60 min	(mg GAE/100 g) L: ↑ from 69.11 to 75.67; B: ↓ from 121.06 to 120.58; HD: ↓ from 109.22 to 84.68; A: no SD.	*	DPPH (%): ↑ in all honey; ABTS (%): L: ↑ from 24.69 to 28.42; B: ↑ from 76.21 to 81.52; HD: ↓ from 63.00 to 38.70; A: no SD.	*	The changes in antioxidant properties of honey after heating have been botanical origin dependent.	Kowalski (2013)

(Continues)

TABLE 2 (Continued)

Geographical origin	Type of honey	Processing conditions(temperature, time)	Antioxidant properties of honey					
			Total phenolic content (TPC)	Total flavonoid content (TFC)	Antioxidant activity	Other compounds with antioxidant activity	Observations and conclusions	Reference
Morocco	Zantaz' (monofloral)	121°C/30 min	(mg GAE/100 g) No SD	(mg CE/100 g) ↓ around 14%	↑ DPPH (IC_{50} mg/ml); ↑ ABTS (IC_{50} mg/ml); ↓ Iron chelating power (max abs); No SD Reducing power (max abs); ↓ Nitric oxide (IC_{50} mg/ml); No SD Superoxide (IC_{50} mg/ml).	The color was the most affected parameter after heating;	Antioxidant activity was dependent on the method used for assessing this property.	Elamine et al. (2020)
Canada	Buckwheat, manuka, and dandelion	121°C/30 min	*	*	*	Transformation of color to dark hues and increase the content of melanoidins by 2.5-fold and 1.4-fold for light-colored and medium honey, respectively.	The initial concentration of melanoidins was a factor influencing the formation or reduction of melanoidins during heating.	Brudzynski and Miotto (2011)

Abbreviations: AAE, ascorbic acid equivalents; CE, catechin equivalents; GAE, gallic acid equivalents; L, liquefaction; P, pasteurization; SD, significant difference; QE, quercetin equivalents.
*The authors did not provide the information.

radical scavenging activity, and the FRAP values decreased in both thyme and lotus honey, whereas no changes in multifloral honey were observed. Thyme honey, even as it is light-colored honey, is the honey with a great antioxidant capacity due to its characteristically high content of vitamin C (up to 170 mg/100 g) (Leon-Ruiz et al., 2011) than in other botanical honey (average content is 25 mg/kg) (Majkut et al., 2021). However, thermal processing negatively affects the vitamin C content in honey. Chua et al. (2014) found about 11–14% decline of vitamin C in tualang, gelam, and acacia honey, whereas Chaikham et al. (2016) reported an even higher decline of 80% in longa, lychee, and wildflower honey after thermal processing. The decrease in honey's antioxidant activity in some honeys may be related to reducing vitamin C content caused by thermal processing. Majkut et al. (2021) observed that short-time exposure (100°C/15 min) reduced vitamin C content (≤50%) but increased TPC (≥27%) and antioxidant activity (FRAP; ≥106%) of honey.

Several studies pointed out that the changes in antioxidant properties of honey after thermal processing are botanical origins dependent. For instance, the thermal processing at 80°C for 30 min decreased the antioxidant activity (ABTS) of honey with the highest initial pigment content (fir and pine honey). In contrast, it increased the antioxidant activity in honey with lower initial pigment content (thyme and orange honey) (Karabagias et al., 2018). According to Elamine et al. (2020), heating light-colored honey, honey with lower amounts of polyphenols, or lower antioxidant activity induces more accentuated changes than those observed in darker honey or those already possessing higher amounts of polyphenols or higher antioxidant activity. For instance, Kowalski (2013) demonstrated that TPC and antioxidant activity in light-colored lime honey increased after thermal processing (90°C for 60 min), whereas in dark, phenolic compounds-rich honeydew honey the opposite effect was observed. Also, the change in the antioxidant activity of honey depends on the pH of honey. Generally, different kinds of honey have different pH, depending primarily on the honey's floral origin. Nayik and Nanda (2015) found that increasing processing temperature (from 60 to 80°C) and decreasing pH of honey from 6 to 3 (increase in acidity) increased the antioxidant activity of saffron honey — comparable results were found for cherry honey (Nayik & Nanda, 2016). Pasteurization at 90°C for 5 min is effective in all floral honeys (while keeping the HMF within the standard limit of 40 mg/kg); however, the antioxidant activity can increase or decrease, depending on the type of honey (Saric et al., 2013). Some authors observed a selective increase of individual phenolic compounds during the short-time pasteurization conditions. For instance, Aydogan-coskun et al. (2019) demonstrated that industrial pasteurization at 90°C for only 15 s almost doubled the concentration of kaempferol in sunflower–cornflower honey. These authors observed that the pasteurization process increased the amount of kaempferol-3-glucoside and increased the TPC and antioxidant activity. On the other hand, Chaikham and Prangthip (2015) observed decreased antioxidant properties (TPC, DPPH, and FRAP) in longan flower honey after short-time heating (up to 5 min) at 100°C. Nevertheless, some honeys showed a significant reduction of TPC and total flavonoids after pasteurization (these values are summarized in Table 2), yet the antioxidant activity varied, depending on the assay employed in the analyses.

Besides the honey's botanical origin, the intensity of the thermal processing, such as the processing temperature and time, is also an essential factor affecting the antioxidant activity of honey. The study by Karabagias et al. (2018) reported that mild temperature increased antioxidant activity in all tested honeys. On the other hand, when honey was processed at 80°C, the antioxidant activity decreased in some samples and increased in others. Further, the prolonged thermal processing at 50, 70, and 90°C for 12 h improved the antioxidant potential of honey by gradually increasing the antioxidant compounds (phenolic acids and flavonoids) and antioxidant activity (DPPH and FRAP) (Jahan et al., 2015). This may happen because heat promotes phenolic compounds' extraction (Chaikham & Prangthip, 2015) but also the formation of Maillard reaction products (Brudzynski & Miotto, 2011) and HMF (Ota et al., 2019). Melanoidins are high-molecular-mass components responsible for radical scavenging capacity. They are present in unheated honey, but their content increases during thermal processing, particularly at elevated processing temperature, at the Maillard reaction's advanced stage (Brudzynski, 2012). Brudzynski and Miotto (2011) have stated that the initial concentration of melanoidins in honey influences honey's antioxidant activity during thermal processing. They found that at a low initial concentration of melanoidins, the thermal processing at extreme temperature (121°C for 30 min) accelerated the formation of new melanoidins and increased the antioxidant activity (ORAC values). In contrast, at high initial concentration, the radical scavenging activity decreased. Elamine et al. (2020) observed increased content of melanoidins at similar processing conditions (120°C, 30 min). However, such extreme processing conditions darkened the honey. Depending on the assays employed to estimate the antioxidant activity (Table 2), the DPPH and ABTS free radical scavenging activity increased; but NO scavenging activity drastically decreased, indicating the reduced ability of the processed honey to scavenge this critical mediator of inflammation; and the chelating ability of honey removed after the thermal processing.

The antioxidant activity of thermally processed honey can be reduced or enhanced. However, the changes in antioxidant activity during thermal processing depend primarily on honey's botanical and geographical origin and the temperature and duration of thermal processing (Pimentel-González et al., 2016). Prolonged thermal processing and processing at high temperatures (≥80°C) can enhance the antioxidant potential of honey, probably due to the compensation of loss of natural antioxidants by the formation of non-nutrient antioxidants, such as the Maillard reaction products (Jahan et al., 2015).

2.1.2 | Changes in antibacterial activity of honey

Due to the increased antibiotic resistance among pathogens, many infections become harder to treat as the antibiotics used to treat them become less effective. The World Health Organization (WHO) acknowledged that a post-antibiotic era, in which common infections and minor injuries can kill, is a real possibility for the 21st century (World Health Organization, 2014). Honey has been recently investigated due to its potential to aid fight against multi-resistant infectious agents (Combarros-Fuertes et al., 2020). Moreover, microbial resistance to honey has never been reported, making the honey an up-and-coming antimicrobial agent against the infection of antibiotic-resistant bacteria (Mcloone et al., 2016). Remarkably, in vitro research showed that combinations of honey with antibiotics have synergistic and additive actions against bacterial biofilm (Nolan et al., 2019). A recent study by Oliveira et al. (2017) demonstrated that combined delivery of honey with phages is a promising antimicrobial alternative, particularly in the treatment of chronic wound infections with *Escherichia coli*, and can be an alternative for other bacterial strains that do not respond to antibiotic therapy (Oliveira et al., 2017).

Nowadays, products based on medical-grade honey, mainly manuka honey, are utilized for topical treatment of infected wounds (Scepankova et al., 2017). However, there is a plethora of scientific papers reporting in vitro as well *in* vivo antibacterial activity of several types of honey, equivalent or better than the medical-grade manuka honey, being those tualang honey, heather honey, kanuka honey, buckwheat honey, and others (Bucekova et al., 2020; Dezmirean et al., 2015; Mcloone et al., 2016; Semprini et al., 2019). The antimicrobial efficacy of these honeys is primarily due to the hydrogen peroxide (H_2O_2) (H_2O_2-dependent activity honey) (Oliveira et al., 2017), whereas manuka honey displays significant antibacterial effects due to the presence of non-peroxide compounds, such as methylglyoxal (MGO) and phenolic compounds (non-peroxide activity honey) (Girma et al., 2019).

Only a few reports focused on honey's thermal stability regarding its antimicrobial activity during thermal processing. A recent study carried out by Kato et al. (2021) investigated the changes in the antibacterial essential compound MGO in manuka honey during heating at 90°C. The results demonstrated that the level of MGO gradually decreased with prolonged heating time (up to 120 min), being the loss of MGO more than 55% after 2 h. Moreover, thermal processing also caused a significant decrease of 2′-methoxyacetophenone (MAP), one of the most important markers for the official definition of manuka honey in New Zealand (Kato et al., 2021).

Chen et al. (2012) have examined the effect of commercial thermal processing (45°C/8 h with filtering) on honey derived from three native Australian floral sources associated with H_2O_2-dependent activity. Four from a total of 17 unprocessed honey samples showed antibacterial activity against *S. aureus*. The mild thermal processing (45°C/8 h) significantly reduced the antibacterial activity against *S. aureus* in three red stringybark honey, dropping activity from 2.7 to 7.2% of phenol equivalence, and inhibited the antibacterial activity in red stringybark–canola blend honey. Similarly, most processed honey also had lower antifungal activity against *C. albicans* than the unprocessed honey.

Bucekova, Juricova, Monton, et al. (2018) investigated the effect of mild (45°C) and moderate (55°C) liquefying conditions (for 8, 24, and 48 h) on the honey antibacterial compound, such as bee defensin-1 content and H_2O_2 production in crystalized rapeseed (*Brassica napus*) honey. The H_2O_2 production significantly decreased (at 55°C after 48 h), whereas defensin-1 showed only a slightly decreasing trend. Also, the glucose-oxidase enzyme significantly decreased at 55°C for 8 h. Kretavičius et al. (2010) documented that glucose-oxidase activity in de-crystalized buckwheat honey decreased at higher temperature intervals, such as at 55–70°C. Although the thermal processing has a deleterious effect on honey's antibacterial components, the study by Bucekova, Juricova, Di Marco, et al. (2018) reported no significant changes in antibacterial activity toward *S. aureus* and *P. aeruginosa* between unprocessed and processed honey at 45, 55, and 65°C.

Pimentel-Gonzalez et al. (2017) investigated the effect of thermal processing at temperatures up to 80°C (45 min) on the antibacterial activity of multifloral honey from different Mexico regions. Honey exposed to a high processing temperature of 80°C for 45 min did not show significant changes in the antibacterial activity against *Bacillus subtills*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. However, different behavior in antibacterialactivity against *S. aureus*

and *E. coli* was found. The antibacterial activity against *S. aureus* and *E. coli* enhanced in all honey processed at temperatures up to 60°C. However, a higher processing temperature caused a reversed behavior, being the maximum decrease of the antibacterial activity at 80°C.

Majkut et al. (2021) found that increasing temperature to 42°C for 15 min did not change the antibacterial activity of multifloral, lime, rapeseed, and buckwheat honey against most Gram-positive and Gram-negative bacteria (*S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa*). However, at 62°C, most of the tested honeys exhibited a loss of antibacterial properties, and at 100°C total loss of bactericidal properties was observed in all tested honeys. Moreover, prolonged thermal processing (2 h), even at nonexcessive temperatures (i.e., 42°C), drastically reduced antibacterial properties in all tested honeys.

The antimicrobial activity of honey decreases by the application of conventional thermal processing. Mainly, prolonged and/or high-temperature processing conditions can cause loss of honey's antimicrobial activity, yet depending on the honey's floral sources and the kind of bacteria they inhibit (Jahan et al., 2015). Therefore, studies indicated that honey's curative use as an antimicrobial is recommended with no or limited thermal treatment (Majkut et al., 2021). Recently, there is a demand for honey's antibacterial activity to be used as an additional quality standard parameter reflecting its biological properties (Bucekova et al., 2020).

3 | EMERGENT TECHNOLOGIES FOR RAW HONEY PROCESSING

In this section, the use of emergent thermal and nonthermal food processing technologies in combination with moderate/high temperatures, for honey decontamination, and the main effects on the overall quality parameters are presented in Table 3–6 and discussed in this section.

3.1 | High-pressure processing

High-pressure processing (HPP) is a nonthermal food pasteurization technique that uses elevated hydrostatic pressures for short periods (commercially up to 600 MPa and a few minutes) to inactivate both spoilage and pathogenic vegetative microorganisms, as well as to inactivate some enzymes. As no heat is applied, most of the nutritional value remains unchanged, mainly by keeping thermolabile compounds on foods. HPP is reported to have little to no impact on microbial loads of foods with low water activity due to the protective effect of microorganisms (Pinto et al., 2020), which can be surpassed by honey dilution (Akhmazillah et al., 2017).

Nevertheless, when combined with moderate/high temperatures, it is possible to reduce the microbial loads of raw manuka honey below detection limits, as observed by Akhmazillah et al. (2012) for hydrostatic pressures of 300 MPa onward and temperatures higher than 30°C.

The application of HPP in honey can enhance several biochemical aspects, namely, the TPC, which will increase antioxidant activity and, consequently, antimicrobial activity, which seems to be more pronounced at higher pressures, with temperature having a minor effect (Akhmazillah et al., 2013; Akhmazillah & Farid, 2015). Considering that the current HPP equipment is operated at and below room temperatures (4–25°C, water inlet temperature), nonthermal HPP could be an exciting approach to enhance honey's bioactivity for market niches or other biomedical and cosmetic applications.

The main effects of nonthermal HPP and its combination with moderate/high temperatures are displayed in Table 3. As previously mentioned, the formation of HMF is a consequence of thermal processing and long storage periods, being an indicator of honey quality. HPP seems to have a positive effect on HMF formation, as the combination of pressures (≤330 MPa) with high temperatures usually results in lower HMF levels compared to thermal processing alone, as observed by Önür et al. (2018) for Turkish honey (canola, cotton, and sunflower) and by Akhmazillah and Farid (2015). The authors stated that dwell time has a more significant impact on HMF formation than the pressure levels, that is, longer processing times increase the formation of HMF for a given pressure level, whereas HMF levels present lower variations when a processing time is set, and the pressure level varies. This fact can be explained by the inhibition exerted by HPP on Maillard reactions, which normally occur at high temperatures. Also, the amount of oxygen in the matrix/package to be processed seems to increase the formation of brown pigments due to the formation of melanoidins (Isaacs & Coulson, 1996), so this is a parameter to be considered when setting up for HPP-based applications with honey.

The color of honey is immensely affected by thermal processing due to brown pigments' formation caused by Maillard and caramelization reactions. As HPP usually results in lower levels of HMF, an intermediate in the Maillard reaction's chain, it will have a lower impact in processed honey compared to conventional thermal processing, as observed by Akhmazilah Fauzi et al. (2014); notwithstanding, high dwell times tend to decrease color parameters of honey, which is related to the increase of HMF.

TABLE 3 Effects of nonthermal HPP and thermal-assisted HPP on microbiological quality indicators and antioxidant properties of honey

Honey origin	pH/°Brix	Processing conditions	Results	References
Clover	*	100–600 MPa, 15–90 min, 25.75–30.18°C	No evident conversion of dihydroxyacetone (DHA) to methylglyoxal (MGO) and hydroxymethylfurfural (HMF).	Grainger et al. (2014)
Kelulut	*	200–600 MPa, 5–15 min, ambient temperature	Increase of antioxidant activity (similarly to thermal processing at 60°C at atmospheric pressure). Diastase activity and total color differences remained unchanged for HPP-treated samples.	Önür et al. (2018)
	*	600 MPa, 10 min, ambient temperature	Increase in TPC that inhibited the probiotic activity of *Lactobacillus acidophilus* and *L. brevis*.	Razali et al. (2019)
Longan flower	*	300–500 MPa, 5–20 min, 25°C	Significant increase of antioxidant activity, TPC, and flavonoids.	Chaikham and Prangthip (2015)
Manuka	*	100–800 MPa, 15–120 min, 25°C	Antimicrobial activity increased with pressure increase. No effect on HMF levels and diastase number.	Al-Habsi and Niranjan (2012)
	4.3/79	270–450 MPa, 3–37 min, 25–60°C	Microbial load reduction below detection limits for honey processed at 300 MPa onward (except at 30°C).	Akhmazillah et al. (2012)
	4.3/79	200–600 MPa, 5–15 min, 26.80–70°C	Increase of 47.16% of TPC after a pressure treatment at 600 MPa for 10 min. No significant differences at higher processing temperatures.	Akhmazillah et al. (2013)
	*	100–600 MPa, 15–90 min, 25.75–30.18°C	No evident conversion of DHA to MGO and HMF.	Grainger et al. (2014)
	4.3/79	200–600 MPa, 10 min, 25–74°C	Antioxidant activity increased about 30% after 10 min under 600 MPa at room-like temperatures. No effect on color. No beneficial effects when HPP was combined with high temperatures.	Akhmazilah et al. (2014)

(Continues)

TABLE 3 (Continued)

Honey origin	pH/°Brix	Processing conditions	Results	References
	*	200–600 MPa, 10–30 min, 25–74°C	Increase of brown pigment formation at higher pressures correlated with the increase of antimicrobial activity (against *Staphylococcus epidermidis*). No significant changes in HMF levels (except at 600 MPa at 70°C).	Akhmazillah and Farid (2015)
	4.3/80	200–600 MPa, 2–30 min, 26.80–32.60°C	Less than 1 log unit reduction of *Saccharomyces cerevisiae* after 20 min under 600 MPa.	Akhmazillah et al. (2017)
	3.8/80	600 MPa, 10 min, 30°C	Increase of antioxidant activity, TPC, and brown pigment formation during 1 year of storage of HPP-treated honey. Flow behavior remained for HPP-treated samples.	Akhmazillah and Farid (2017)
Mexican multiflowered	3.8/80	600 MPa, 0–15 min, <37°C	HPP reduced microbial loads below detection limits after 15 min under 600 MPa. Increase of antioxidant activity and TPC. Decrease of violaxanthin and diastase activity. No effect on HMF content.	Leyva-Daniel et al. (2017)
Turkish (canola, cotton, and sunflower)	*	220–330 MPa, 23–106 min, 50–60°C	Lower HMF levels compared to thermal processing at atmospheric pressure. At 60°C, HPP reduced the liquefaction time.	Önür et al. (2018)

Abbreviations: DHA, dihydroxyacetone; HMF, hydroxymethylfurfural; MGO, methylglyoxal; TPC, total phenolic content.
*The authors did not provide the information.

TABLE 4 Effects of US on microbiological quality indicators and antioxidant properties of honey

Honey origin	pH/°Brix	Processing conditions	Results	References
Acaxochitlán, Arenal, Huehuetla, Orizatlan, and Tasquillo	*	42 kHz, 5–15 min, 20°C	Smaller crystal sizes. Improved color, antioxidant activity, and TPC. No impact on HMF.	Quintero-Lira et al. (2017)
Blossom and honeydew honey from Pine forest	3.9/*	23 kHz, 18–24 min, 76–82°C	Lower content of HMF, higher diastase activity, and no effect on moisture, pH, and electrical conductivity compared to thermal processing. Recrystallization of honey was slower for samples treated with ultrasounds.	Thrasyvoulou et al. (1994)
Buckwheat, granulated multiflowered, and lime honey	*	40 kHz, 0–30 min, 20–30°C	Higher exposure times yielded higher crystallization indexes, which correlated with higher hardness values.	Stasiak and Dolatowski (2007)
Indian (Shillong, Sohra, Mawsynram, Jorhat, and Tezpur)	*	30 kHz, 0–120 min, 50°C	Low HMF for Jorhat and Sohra honey after sonication. General increase of antioxidant activity and TPC.	Mahnot et al. (2019)
Indian (Hisar)	*	20 kHz, 1–15 min, <40°C	Total coliforms and yeasts and molds loads reduction below detection limits. Improved diastase activity and lower HMF values for the optimized conditions compared to thermal processing.	Janghu et al. (2017)
Kelulut	*	25 kHz, 30–120 min, 45–90°C	TPC, viscosity, antioxidant activity, and color intensity increase. The decrease in moisture and water activity. HMF remained in acceptable levels but was higher for sonicated honey, compared to thermal processing.	Chong et al. (2017)
Longan	*	20 kHz, 5–20 min,*	Total phenolic and flavonoid content and antioxidant activity increased after ultrasonic processing, especially at higher amplitudes and treatment times.	Chaikham and Prangthip (2015)
Longan, Lychee, and Wildflower	≈3.5/*	20 kHz, 30 min, 52.63–75.09°C	Microbial load reduction below 1 log CFU/g. Ultrasonic treatment improved antioxidant activity, along with TPC, total flavonoids, and higher diastase activity. Reduced impact on HMF formation.	Chaikham et al. (2016)
Rosemary	*	40 kHz, 20–60 min, 40–60°C	Ultrasound treatments allowed to speed up honey liquefaction, mostly below 50°C. A lower amount of crystals (and smaller), clearer and transparent honey compared to control samples.	Kabbani, Sepulcre, and Wedekind (2011)
	*	40 kHz, 40–120 min, 40–50°C	Microbial loads reduction, especially at 50°C and longer treatment times (120 min). Enhanced antimicrobial activity against *Saccharomyces cerevisiae*.	Kabbani, Sepulcre, Gastón, et al. (2011)
Salvation Jane	*	7000 J, 434 s, 77.3°C**	US treated delayed honey crystallization compared to the thermally treated samples. Lower HMF levels compared to thermal processing.	Rajapakse (2011)
Turkish (Canola, Cotton, and Sunflower)	*	24 kHz, 0–100 min, 15–90°C	The probe thickness influenced the overall quality parameters of honey. Less impact on color, diastase activity, and HMF levels compared to thermal processing.	Önür et al. (2018)

Abbreviation: HMF, hydroxymethylfurfural; TPC, total phenolic content.

*The authors did not provide the information.

**Optimized conditions from a set of conditions tested previously.

TABLE 5 Effects of microwave irradiation on microbiological quality indicators and antioxidant properties of honey

Honey origin	pH/°Brix	Processing conditions**	Results	References
Acacia, buckwheat, and lime	*	1.26 W/g, 3 min, 48–69°C	HMF levels remained stable after microwave processing and after 3 months of storage, along with diastase number and invertase activity decrease.	Kowalski and Lukasiewicz (2017)
Acacia, buckwheat honeydew, and lime	*	1.26 W/g, 2–6 min, 90–100°C	HMF formation in honey was faster in microwave-heated samples than conventional water-bath heating. No evident pattern on TPC behavior. General increase of antioxidant activity.	Kowalski (2013)
Aegean, forest, and honeydew	*	1.26 W/g, 2–6 min, 25–83°C	Faster diastase inactivation by microwave heating compared to isothermal heating. No specific kinetics for diastase inactivation was found compared to first-order kinetics for isothermal heating.	Kowalski et al. (2012)
Argentina	*	800 W, 45–90 s, 83–108°C	Inactivation of diastase and glucose oxidase after microwave heating. Yeasts and molds and *Paenibacillus larvae* reduced below detection limits. Severe browning due to high HMF levels.	Moline et al. (2015)
Czech	*	90–800 W, 15–45 s, 28–90°C	No significant increase of HMF levels, despite the variations between honey's botanical origins.	Bartáková et al. (2011)
Eucalyptus lanceolatus and *Helianthus annuus*	*	*	HMF formation was accelerated by increasing microwave power and treatment time, which was more evident in honey from *Helianthus annuus* than *Eucalyptus lanceolatus*.	Bath and Singh (2001)
Flower and honeydew	4.41–4.76/*	80 W, 2–4 min,*	HMF levels increased with microwaving time, especially for flowered honey. Diastase activity slightly decreased for all honey varieties. Significant decrease of invertase and glucose oxidase (H_2O_2) for longer microwaving times.	Reynolds (2019)
Forest/Rock bee	*	3.5–16 W/g, 15–90 s, 20–110°C	More intense treatments at shorter times were more effective in terms of HMF levels and diastase activity. Yeast counts varied considerably with treatment time and power intensity.	Hebbar et al. (2003)
Latvia	*	180–800 W, 10 s,*	A linear relationship between microwave power intensity and HMF concentration. Invertase activity decreased significantly at potencies of 600 W onward.	Diminš et al. (2019)
Rapeseed	*	80–800 W, 10–180 s, 24.0–95.5°C	Microwave heating eliminated antibacterial activity of honey samples through depletion of glucose oxidase and defensin-1.	Bucekova, Juricova, Monton, et al. (2018)
Starfruit	3.6/*	600 W, 205 s, 71°C	Honey darkened when stored at room temperature (compared to cold storage at 4°C). A slight variation on pH along with storage. Reduction of diastase activity and yeast counts.	Ghazali et al. (1994)

Abbreviation: HMF, hydroxymethylfurfural; TPC, total phenolic content.

*The authors did not provide the information.

**The temperatures referred in the processing conditions regard the maximum temperature reached because of a microwave heating experimental run.

TABLE 6 Effects of irradiation on microbiological quality indicators and antioxidant properties of honey

Honey origin	pH/°Brix	Received dose (kGy)	Results	References
China	3.94–4.49/*	6–25	No effect on glucose oxidase. Reduction of amylase activity (from 19 to 43%, except for one batch). Reduction of *Clostridium botulinum* and *Bacillus subtilis* endospores below detection limits after an irradiation dose of 25 kGy.	Postmes et al. (1995)
Honeydew	4.7	10–30	Bacterial counts reduced below detection limits. No impact on total protein content and HMF. A slight increase in free acidity. Minor differences on antibacterial and antibiofilm activities between irradiated and unirradiated samples.	Horniackova et al. (2017)
India	*	1–15	Microbial decontamination of honey at doses of 10 and 15 kGy. Minor differences in antioxidant activity, HMF, TPC, and sensorial attributes.	Saxena et al. (2010)
	*	5–15	No effects on rheological characteristics of honey.	Saxena et al. (2014)
Lukotan	*	10–30	A decrease in antioxidant activity. No effect on total flavonoid content.	Verzola et al. (2019)
Manuka, clover, and nodding thistle	*	25–50	*Clostridium perfringens* and *C. tetani* were reduced below detection limits with 25 kGy dose. No loss of antibacterial activity against *S. aureus* after irradiation.	Molan and Allen (1996)
Monofloral Gelam and Nenas	3.87/*	25	Significant decrease of moisture, vitamin E, and HMF. Increase of color intensity and vitamin C. No effects on pH, acidity, minerals, and sugar content.	Hussein et al. (2014)
Multiflowered	4.16/*	10	Lower HMF and viscosity (measured at 35°C) for irradiated honey samples, along with acidity increase. No significant differences on ash, sugars, and viscosity (measured at 25°C).	Bera et al. (2008)
Parana region	*	0–10	Viscosity was not affected by irradiation. Significant differences in taste were found for 10-kGy-irradiated honey compared to controls, which were irrelevant compared to 5-kGy-irradiated honey.	Matsuda and Sabato (2004)
Supermarket honey	*	10	Reduction of 99% of aerobic, anaerobic bacteria, and molds. Increase of antibacterial activity after irradiation. No changes on HMF, acidity, saccharose, water content, and diastase activity.	Migdal et al. (2000)
Tualang	3.7–4.1/*	25	Increase of total protein and proline content. Increase of antioxidant activity and color intensity.	Khalil et al. (2015)
Urmia region	3.96/*	0–25	Similar antibacterial activities against *S. aureus*, *E. coli*, and *P. aeruginosa* for irradiated and unirradiated honey samples.	Jalali et al. (2007)

Abbreviation: HMF, hydroxymethylfurfural; TPC, total phenolic content.
*The authors did not provide the information.

Diastase, an enzyme considered a quality indicator of honey, seems to be generally unaffected by HPP (at room-like temperatures), as reported by Önür et al. (2018) and Al-Habsi and Niranjan (2012) for kelulut and manuka honey, whereas Leyva-Daniel et al. (2017) reported a decrease in DA in Mexican honey. These results may be related to the honey variety (origin), which will inherently influence honey's chemical composition.

3.2 | Ultrasounds

The primary use of high-intensity ultrasounds (in the range of 10–1000 W/cm^2 and a frequency of 20–100 kHz) in food processing aims microbial inactivation by disruption of the cell walls of bacteria and fungi (Yao et al., 2020; Yu et al., 2020), as a consequence of the cavitation process.

The first known report of ultrasound processing of honey is attributed to Thrasyvoulou et al. (1994), who reported the possibility of using ultrasounds for honey liquefaction compared to conventional thermal liquefaction, with the advantage of lower HMF content and higher DA, thus resulting in honey with higher quality. The authors also reported that the sonication process slowed down honey recrystallization. Indeed, especially when combined with mild temperatures, honey liquefaction can be accelerated by ultrasonic treatments (Kabbani, Sepulcre, & Wedekind, 2011). Interestingly, after the sonication processes, the recrystallization presents smaller crystal sizes compared to those thermally liquefied (Kabbani, Sepulcre, & Wedekind, 2011; Quintero-Lira et al., 2017).

When it comes to the feasibility of ultrasounds as a decontamination technique, it depends on the operation setup (probe diameter), temperature, and treatment time. Indeed, higher temperatures and treatment times and bigger probe diameters seem to improve microbial load reduction in honey (Chaikham et al., 2016; Kabbani, Sepulcre, Gastón, et al., 2011; Önür et al., 2018); nevertheless, the application of ultrasounds combined with high temperatures can have an amplified detrimental effect when compared to thermal treatment alone (Chong et al., 2017). For so, when considering the use of ultrasounds for honey processing, a balance must be achieved between the desirable microbial load inactivation and the inherent impact that the processing conditions will have on the sonicated honey.

The main effects of ultrasounds and their combination with moderate/high temperatures are displayed in Table 4.

3.3 | Microwave

Microwaves consist of electromagnetic waves that fit in frequencies between 300 MHz and 30 GHz, corresponding to the region between infrared and radio frequencies in the electromagnetic spectrum (Chandrasekaran et al., 2013). The feasibility of microwave heating relies on the food product's moisture content, being more feasible for foods with high water content. Indeed, microwaves can penetrate the food product to a certain depth and interact with polar molecules (such as water), which are quickly heated due to the molecular friction generated by dipolar rotation in the presence of an alternating electric field. Dissolved sugars are also susceptible to microwave irradiation, making honey (due to its water content and high amounts of dissolved sugar) a suitable candidate for microwave processing to increase safety (due to the temperatures reached, allowing to inactivate pertinent vegetative pathogenic microorganisms) and bioactivity (by increasing the content of melanoidins) (Subramanian et al., 2007).

The first known report covering the application of microwave processing in honey is attributed to Ghazali et al. (1994). They reported a decrease in yeast counts and lower DA after honey microwaving at 600 W for 205 s (the temperature reached 71°C at the end of the microwaving process), along with accelerated darkening for honey afterward stored at room temperature, compared to that kept at 4°C (which did not darken), possibly due to browning reactions.

The heating process by microwaves occurs quickly than conventional isothermal heating, and it allows a lower impact on HMF values of the processed honey due to the lower times required to achieve a specific temperature set point. Nevertheless, the HMF formation in honey seems to increase at higher potencies for the same processing time, because it results in higher final temperatures and lower diastase activities (Kowalski, 2013; Kowalski et al., 2012).

The feasibility of microwave processing for honey decontamination depends on honey origin, potency, treatment time, and achieved temperature. More intense treatments (at higher potencies, longer treatment times, and higher temperatures) seem to be more effective in inactivating honey's indigenous microorganisms (Bath & Singh, 2001; Hebbar et al., 2003).

An additional effect of microwave heating seems to be the loss of antimicrobial activity, mainly due to the inactivation of enzymes, such as glucose oxidase, which play a significant role in honey's antimicrobial activity (Bucekova, Juricova, Monton, et al., 2018; Moline et al.,

2015). Other effects of microwave heating are summarized in Table 5.

3.4 | Irradiation

This methodology consists of an in-package (or not) sterilization procedure, primarily when performed at higher doses (above 10 kGy), wherein ionizing radiation in the form of gamma-rays, X-rays, or electronic beams penetrates the food product and, consequently, microorganisms. The radiation then penetrates the cells and creates DNA dimers, which will hurdle vital cellular functions such as protein synthesis and DNA replication. It can also target the lipids from the membranes by oxidizing them. Irradiation is generally recognized as a safe practice to decontaminate food products (Feliciano, 2018).

The first known report on gamma radiation for honey decontamination was performed by Huhtanen (1991). In this preliminary study, honey cans irradiated with ^{137}Cs at a dose of 6.0 kGy reduced *C. botulinum* endospores less than 0.70 log units and *Bacillus subtilis* between 1.5 and 1.8 log units in honey, compared to complete inactivation of both spores when suspended in water. The authors stated that higher doses would be needed to achieve the same microbial inactivation levels due to the radio-protective effect conferred by ascorbic acid, fumarate, and glutamate present in honey.

The success of irradiation is widely reported, especially at 10–15 kGy for vegetative microorganisms (Saxena et al., 2010) and at 25 kGy onward for bacterial spores (Molan & Allen, 1996; Postmes et al., 1995). Despite the lack of studies covering the effects of irradiation against fungi spores in honey, it is expected that the same dose for bacterial spores' inactivation is also suitable for fungi spores, as they are generally more sensitive than bacterial spores (Pinto et al., 2020).

The impact of irradiation in honey's rheological behavior is entirely dependent on honey origin and temperature of analysis. Indeed, Matsuda and Sabato (2004) reported no viscosity changes in Parana honey after being irradiated at 5 and 10 kGy, whereas Bera et al. (2008) reported viscosity decrease (measured at 35°C) in multiflowered honey after receiving a dose of 10 kGy, but, if measured at 25°C, it was similar to unirradiated honey, clearly showing that temperature is an essential parameter to control upon viscosity measurement.

The effects of irradiation in raw honey regarding physicochemical and enzymatic parameters, antioxidant activity, HMF formation, among others are entirely dependent upon honey origin (and composition, as expected), as seen in Table 6.

3.5 | Ultraviolet light

Literature covering the application of ultraviolet light (UV light) for nonthermal pasteurization of honey is very scarce. Either way, some studies are reporting the effects of pulsed and continuous UV light treatments on raw honey.

Hillegas and Demirci (2003) evaluated the effectiveness of pulsed UV light against *Clostridium sporogenes* endospores inoculated in clover honey, varying the number of the UV light pulses, treatment time, the distance between the UV light source and the honey, and the depth of honey. The authors reported a reduction of 89.4% in endospore counts after 540 pulses for 180 s at a distance of 20 cm from the UV light source with a honey depth of 8 mm. It was also observed that the treatment time and the distance between the honey and the UV light source were the main parameters with more influence on endospore inactivation. Different results were obtained by Roig-Sagués et al. (2018), who studied the effects of UV light in *E. coli* and endospores of *B. subtilis* and *C. sporogenes* and reported 5 log units' inactivation of *E. coli* and approximately 2.5 log units' reduction of both endospores' species. The authors also evaluated the effects of short exposition time to UV light (4.5 to 36.0 J/ml, from 15 to 120 s, respectively) and observed higher color variations at higher exposition times, attributing this to the temperature increase. Besides, HMF levels decreased proportionally to the UV light received dose, being lower compared to control samples (untreated honey), with the authors being unable to provide a possible explanation for this fact due to the lack of literature in this sense. A possible explanation for this observation can be stated considering the increase of color variations observed at higher UV light doses due to the formation of melanoidins, decreasing the concentration of HMF.

In another study, Fit et al. (2014) evaluated UV light feasibility to improve the antibacterial activity of four honey types (acacia, lime, sunflower, and multifloral). They observed that, after UV light treatment for 1 h, the antibacterial activity increased against *S. aureus* and *B. cereus*, along with lower values of HMF compared to thermal processing (50, 70, and 100°C for 30 min).

In addition, the use of UV light for honey decontamination can remove pesticides from honey (Marghtas et al., 2011), by photodegradation.

4 | CONCLUSION

Honey processing is a critical step when it comes to ensuring its quality and safety. Intense thermal processing can significantly change the nutritional features of this

natural product. The use of (non)thermal emergent processing technologies for honey processing is a topic of interest, considering their capacity of preserving or even enhancing antioxidant and antimicrobial activity. Although thermal emergent processing technologies allow a more homogeneous and quicker heat process, nonthermal processing technologies allow obtaining a more rawliker food product, as most chemical changes induced by thermal processing (such as diastase and glucose oxidase inactivation, honey crystallization) do not occur. In this sense, it is possible to improve the biological activities of honeys processed by nonthermal emergent technologies, considering the lower impact on phenolic compounds and enzymes. Further studies are needed to ascertain, at a higher extension and depth, the impact of such emergent technologies on the biological activity and microbial safety of honey and the integration of these technologies for the development of honey-based cosmetic and pharmaceutical applications.

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AUTHOR CONTRIBUTIONS

Hana Scepankova and Carlos A. Pinto wrote the main manuscript and constructed the tables. Vanessa Paula was responsible for the literature compilation and correction of the references. Leticia Estevinho and Jorge A. Saraiva designed this study and reviewed the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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