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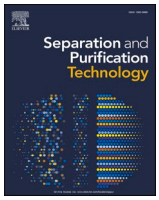

Recent progress on the recovery of bioactive compounds obtained from propolis as a natural resource: Processes, and applications

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ABSTRACT

Propolis is a functional food ingredient classified by its physical–chemical characteristics, vegetal source, bee species, and geographical origin. Due to its complex nature and variable composition, the massive use of propolis extracts would require standardization. Several techniques for recovering bioactive compounds from propolis have been reported, varying according to their complexity, degree of automation, and energy dependence. The recovery of propolis target compounds must be carried out using the most appropriate extraction process and the optimized conditions, guaranteeing a better use of the raw material, lower process costs, and good extraction selectivity. This work presents and discusses conventional and alternative techniques developed in the last 20 years. The extraction efficiency, selectivity, the type and amount of solvent, and the conditions to optimize and guarantee safe products (e.g., toxicity and biocompatibility) were reviewed. The current trends were reported, and the technological advances, future perspectives, and applications of the natural bioactive compounds obtained from propolis were discussed. A comprehensive determination of the main advantages and disadvantages of the extraction techniques and the impact of operational conditions on the processes' performance was assessed. Based on the available evidence, some future strategies will be scrutinized, not only regarding the future of using propolis and its products but also considering the impact of a propolis-business model crossing the concept of a circular economy and combining it with the idea of resource efficiency.

1. Background

Propolis is an excellent source of natural bioactive compounds (NBC), such as a complex mixture of resinous, gummy, and balsamic substances harvested by honey bees from buds, flowers, and plant exudates [1-3]. Additionally, propolis is naturally added by bees' salivary secretions, which makes them a unique natural product [4]. Therefore, Propolis is classified due to its physical-chemical characteristics, vegetal source, bee species, and geographical origin. In general, propolis comprises resins and balms, waxes, essential oils, pollen grain, and several microelements (aluminum, calcium, strontium, iron, copper, manganese, and a small concentration of vitamins B1, B2, B6, C, and E). However, its chemical composition varies mainly according to the geographic location, climate, and edaphic conditions (soil type, chemical composition, and microbiota) [3,5]. At least 300 organic compounds have already been identified in different propolis samples, including fatty and phenolic acids & esters, flavonoids, terpenes, β-steroids, aldehydes, aromatic alcohols, stilbene derivatives, and amino acids [6]. The major compounds found in propolis, their chemical structure, and their respective health benefits are shown in Table S1-Supplementary Material.

According to the World Health Organization (WHO), propolis can be combined with other drugs without any risk of inactivation of the conventional treatment [7,8]. Due to its antioxidant and pharmacological properties, propolis has attracted attention in several research areas with broad applications. The use of propolis as a food ingredient has been increasing yearly, driven by the current consumer trend towards functional foods [9]. However, the incorporation of propolis in food is a

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major technological challenge, as it has low solubility in water and strongly alters the sensory characteristics due to its unpleasant taste and odor, generally compromising the food' acceptability. Furthermore, the desired effect of propolis can be drastically changed during food processing. Since the antioxidant capacity of propolis is not just given by the sum of the antioxidant powers of each component, it also depends on the compound's microenvironment [9].

Several techniques for recovering bioactive compounds from propolis have been reported, varying according to their complexity, degree of automation, and energy dependence. In general, for any chosen extraction technique, the independent process variables, such as solvent type and its concentration, extraction time, temperature, and the solid–liquid ratio (SLR), directly influence the extraction performance. Thus, the recovery of target compounds must be carried out using the most appropriate extraction process and the optimized conditions, guaranteeing a better use of the raw material, lower process costs, and good extraction selectivity (in this case, by removing waxes) [10]. Therefore, the extraction step is crucial for complex raw materials like propolis, and the massive use of its extracts would require standardization [63].

Despite being considered a reference for recovering natural bioactive compounds from propolis, conventional techniques such as shaker homogenization, magnetic stirring, or Soxhlet, are also regarded as poorly selective. In this sense, other extraction techniques have been developed in the search for alternatives allowing to reduce the amounts of solvent; besides optimizing the extraction selectivity and yield (maximizing the extraction of the main target compounds) while guaranteeing the extract safety in terms of toxicity and biocompatibility. Indeed, the convergence between the extracted compounds displays numerous biological effects of propolis extracts, especially regarding antioxidant and immunomodulating effects [13]. However, some commercial brands intend to remove the waxes to improve the consumer's acceptance. In addition, when it is possible to obtain extracts with different chemical compositions, there are possibilities for designing new applications than those already performed. Additionally, suppose the propolis extract has high amounts of beeswax; in that case, the separation upon the polar phase during the manufacturing process could lead to technological standardization problems [14], mainly regarding uniformity and solubility of desired bioactive compounds. Furthermore, such goals may be achieved by using more eco-friendly solvents, replacing the conventional ones that are not recommended due to the final purpose of propolis extracts as nutraceuticals or phytomedicines [15,16]. Thus, new techniques were developed in the last 50 years and gained prominence for being less harmful to the environment due to the lower use of chemicals, the better yields achieved, and the high quality of the extract [15,16].

Whereas the previous literature shows some of these techniques applied to the extraction of propolis compounds, this work aims to discuss the studies from the last 20 years reporting the current trends, technological advances, future perspectives, and applications of the natural bioactive compounds obtained from propolis. A comprehensive analysis of the main advantages and disadvantages of the extraction techniques and the impact of operational conditions on the processes' performance will be assessed. Moreover, based on the available evidence, some future strategies will be scrutinized, not only regarding the future of using propolis and its products but also considering the impact of a propolis-business model crossing the concept of a circular economy [17] and combining it with the idea of resource efficiency [18].

2. Extraction techniques for the recovery of bioactive compounds from propolis

The contact between a solid material (usually grounded) and the solvent characterizes the solid–liquid extraction to maximize the solubility of the target compound(s) and minimize the solubilization of the remaining matrix components. It is essential to highlight that propolis is a particulate matrix and is not a vegetal tissue composed of cells. Vegetal cells can retain target compounds, requiring a long diffusional stage to transfer them to the extraction solvent. Although there are still interactions between the matrix components, the mass transfer during extraction in propolis is faster than in other materials and is primarily based on the solubility of compounds in the solvent [19,20]. Additional external forces such as mixing and heating are applied to speed up the extraction process. As a result, the selected technique and conditions should reach higher yields and extract concentrations, maximizing the most valuable compounds [21].

However, most extraction techniques are not selective, where not only target compounds are removed from the raw material, and several other matrix components are simultaneously extracted, resulting in an extract with a low concentration. For example, Trusheva et al. [22] reported a higher ratio of flavonoids in the extract obtained with ultrasound-assisted extraction (UAE) compared to the maceration approach. Similarly, Margeretha et al. [23] recorded that microwave-assisted extraction (MAE) recovered more phenolic compounds than those obtained by maceration (using the same solvent). Thus, despite the synergism between phenolic and non-polar compounds (mainly resins and balms) in the improvement of biological effects of the propolis extracts, some applications are designed to recover a phenolic-rich fraction, making necessary further purification steps to remove waxes (that in this case are undesired compounds), which implies spending more solvents, money, and energy. Even though conventional extraction techniques have been widely used to extract NBC from propolis, like maceration, shaker homogenization, Soxhlet, and magnetic stirring [24-26]. On the other hand, several alternative techniques have been proposed and applied to overcome the drawbacks of the conventional methods, such as high hydrostatic pressure (HHP), MAE, UAE, supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE), that are alternatives to the development of more eco-friendly processes, reaching a higher yield of extraction, saving time, and in some cases, better phenolic compounds' selectivity [27-30].

Regardless of the extraction technique, some process variables may now impact solvent extraction performance [31]. For example, the solvent type or mixtures play a crucial role mainly in the manipulation/control of the yield and selectivity of the process. Moreover, characteristics of the raw material such as granulometry, material structure, plasticity, and composition are also important [32]. Additionally, the solvent affects the microwave energy absorption and the ultrasound waves propagation for specific extraction steps like those based on microwave and ultrasound. Besides, the solvent physicochemical changes can also occur by changing temperature and pressure, such as in PLE, SFE, and HHP [21,33]. For that reason, most developed methods must evaluate different solvents and processing conditions to maximize the process efficiency.

For recovering the natural bioactive compounds from propolis, the polarity of the solvent, solute/solvent interaction, temperature, and time of contact are key parameters to optimize [34]. Since propolis is a chemically complex matrix, it is essential to find the appropriate equilibrium between the temperature and extraction time to apply. If in one hand, propolis has a large amount of waxes in its composition that can solidify during the extraction, in the other hand, it is also composed of thermolabile molecules (e.g. the phenolic compounds such as flavonoids) that can be easily degraded if temperature and the time of contact between them and the solvent(s) are not judiciously controlled [35].

In the end, a careful study of the extraction technologies and process parameters is critical to guarantee the highest efficiency while designing a more sustainable process, which contemplates, among other strategies (to be discussed in this work in section Future Perspectives), the minimization of energy consumption and mitigation of synthetic and non-renewable raw materials [33].

2.1. Conventional extraction of propolis

Despite being considered simple techniques, with an accessible

Table 1
Summary of the publications reported in the last 20 years applying conventional extraction techniques to extract bioactive compounds from propolis from different geographic regions. The extraction conditions (method, solvent, concentration, SLR, time, temperature), extraction yields, and the main class of recovered compounds are detailed.

Propolis origin	Method	Solvent	Concentration (v/v)	SLR	Time	T (°C)	Yield*	Class of Compounds	References
Lithuanian	Maceration	PEG 400 + Water	100%	0.1	15 min	70	12.7 mg. mL^{-1}	Hydroxycinnamic acids and derivatives; Hydroxybenzoic acids; Flavonoids	[37]
Lithuanian	Maceration	Ethanol	100%	0.05	120 min	50–60	175.6 mg. g^{-1}	Total phenolic compounds	[38]
Malaysian	Maceration	Ethanol	70%	0.2	7 days	RT	29.09 mg. g^{-1}	Total phenolic compounds	[24]
Korean	Maceration	Ethanol	50–60%	0.1	24 h	RT	12 24 $g.g^{-1}$	Total phenolic content	[36]
Italian	Maceration	Ethanol	70%	0.1	72 h	RT	55%	Flavonoids	[20]
Croatian	Maceration	Ethanol	80%	0.1	60 min	RT	1035.41 g. mL^{-1}	Total phenolic content	[35]
Brazilian green propolis	Soxhlet	Ethanol	100%	–	24 h	60	57.65%	Hydroxycinnamic acids and derivatives	[39]
Brazilian green	Soxhlet	Chloroform	100%	–	360 min	60	73%	Hydroxycinnamic acids and derivatives; Flavonoids	[33]
Brazilian green	Soxhlet	Ethanol	100%	–	180 min	78	65%	Hydroxycinnamic acids and derivatives; Flavonoids	[22]
Canadian	Soxhlet	Ethanol	100%	–	360 min	RT	9.68 $mg.g^{-1}$	Flavonoids	[26]
Argentine	Stirring	Ethanol	85%	0.1	3 × 30 min	30	80.5 $mg.g^{-1}$	Flavonoids	[40]
Brazilian green	Turbo-extraction	Ethanol	96%	0.3	15 min	RT	6.8%	Flavonoids	[23]

PEG: polyethylene glycol; **RT:** room temperature; **SLR:** solid–liquid ratio. * Units depend on the publication.

operation mode (technical staff specialized and/or expensive equipment are not required), the so-called conventional techniques usually require high volumes of organic solvents (commonly volatile organic solvents - VOS) to reach a high extraction yield. Still, they are frequently associated with low selectivity values [36].

While maceration and Soxhlet are the methodologies more commonly reported, ethanol is the solvent most used in the extraction of natural bioactive compounds from propolis since it is easily accessible on a large scale, cheap, eco-friendly, industrially approved, and compatible with a significant part of the target compounds present in this matrix [36]. Table 1 summarizes the works published in the past 20 years dealing with conventional solvents to extract different compounds from propolis by conventional techniques.

In the last 20 years, numerous publications reported using maceration and optimization of the method by studying the influence of different parameters, like the type of solvent, SLR, time of extraction, and temperature. In this sense, a study optimized Croatian propolis extraction by maceration and evaluated the independent variables temperature and concentration of extraction solvent. The final conditions for the extraction were established using ethanol 80% for 60 min at room temperature [37]. Woo et al., [38] observed that total phenolic content extracted by maceration from the Korean propolis was higher when an ethanolic solution of 50–60% was used (SLR 0.1, 24 h at room temperature). The authors showed that ethanol concentrations higher than 60% could increase the extracted phenolic content. A study reinforced these findings by testing ethanol (70%) and water (100%) (SLR 0.2, maceration up to 7 days at room temperature) to obtain phenolics from the Malasyan propolis. It concluded that ethanolic extracts (70%) resulted in the highest phenolic and flavonoid content (29.09 $mg.g^{-1}$) compared to the aqueous extract (7.75 $mg.g^{-1}$) [26].

Chen et al., [32] confirmed ethanol extraction's success by stirring at SLR 0.1, 25 °C, for 48 h, applying different concentrations of ethanolic solutions (60, 70, 80, 95, and 99.5%) in Taiwanese green propolis. The authors revealed that the extraction yield is directly proportional to the ethanol concentration, i.e., ethanol 95% and 99.5% provided the maximum dry extract yield compared to the other solvent mixtures composed of lower ethanol concentration, and even better when pure water is used. Thus, the literature suggests that propolis's phenolic compounds are better extracted with ethanol or ethanol and water mixtures of around 70%. This trend can be expected considering the relatively hydrophobic nature of the main components of propolis.

On the other hand, Kubiliene et al. [39] analyzed the influence of different solvents in maceration and compared the chemical composition of extracts from Lithuanian propolis. The solvent tested were pure water, 70% ethanol, pure polyethylene glycol 400 (PEG), olive oil, and mixtures composed of water + PEG and water + olive oil + PEG; the process was carried out at room temperature at SLR equal to 0.1 for 5 h. Adding olive oil to water provided less polar compounds extraction, and adding PEG in water (up to 70%) increased the phenolic compounds' extraction, mainly phenolic acids (10.7 $mg.mL^{-1}$). Also, these extracts, PEG in water (up to 70%), have higher antioxidant capacity than those obtained with pure water and ethanol.

Other solvents were tested by Ramanauskiene et al. [40] using the Lithuanian propolis, namely, pure water, ethanol (70 and 96.3%), PEG (100%), and mixtures containing ethanol + propylene glycol and water + PEG. Magnetic stirring over short-term heating (120 min) and maceration for seven days were applied as conventional extraction methods (SLR 0.05 at 50–60 °C). Water showed the lowest efficiency of extraction (17.0 $mg.g^{-1}$), followed by ethanol 100% (175.6 $mg.g^{-1}$) and PEG (118.6 $mg.g^{-1}$).

The Soxhlet extraction method is also often used to extract propolis' bioactive compounds. Monroy et al. [24] tested Soxhlet-mediated extraction with a recirculation time of 180 min at 78 °C, using different solvents (100% water, 30%, 50%, 80%, and 100% ethanol). The results indicated that ethanol promoted higher extraction yield (65%) and phenolic compounds yield (65 $mg.g^{-1}$) from Brazilian green propolis, namely, artepillin C, *p*-coumaric acid, and kaempferide.

Biscaia and Ferreira [35] also used the Brazilian green propolis as a phenolic compound source. Different solvents (ethanol, ethyl-acetate, chloroform, hexane, and water) were investigated using fixed temperature and time (60 °C for 360 min). The higher yield was achieved by chloroform (73% w/w), followed by ethanol (60% w/w), and ethyl acetate (59.7% w/w). Sambou et al. [28] also evaluated the extraction performance of different types of pure solvents or mixtures (water, methanol, ethanol, acetone, ethyl acetate, dichloromethane, ethyl acetate: hexane (1:1, 4:1, and 1:4) and hexane. In addition, the authors evaluated the Soxhlet technique in Canadian propolis with solvent reflux of 36 min. The study showed that methanol, ethanol, and acetone

Table 2
Summary of the publications using propolis as source of bioactive compounds and applying alternative extraction methods and respective conditions (methodology, solvent of extraction, concentration, SLR, time, temperature, extraction yield and class of compounds obtained).

Propolis	Method (condition)	Solvent	Concentration (v/v)	SLR	Time	T (°C)	Yield *	Extracted compounds	References
Chinese	HHP (5000 bar)	Ethanol	75%	0.54	1 min	RT	5.10%	Flavonoids	[25]
Chinese	HHP (500 bar)	Ethanol	75%	0.54	1 min	RT	230.4 mg. g^{-1}	Flavonoids	[45]
Chinese	HHP (5000 bar)	Ethanol	75%	0.35	1 min	RT	290.4 mg. g^{-1}	Total phenolic and flavonoids content	[43]
Indonesian	MAE 9 (495 – 520 W)	Ethanol	61 – 65%	0.1	24 – 31 min	N.S.	0.4%	Total phenolic content	[21]
Canadian	MAE (70 W)	Ethanol	100%	0.25	10 min	RT	9.865 mg. g^{-1}	Flavonoids	[26]
Brown	MAE (300 W)	Ethanol	70%	0.2	20 min	115	16 mg. mL^{-1}	Flavonoids	[48]
European	MAE (800 W)	Ethanol	70%	0.1	2_{cycles}x10s	n.s	75%	Flavonoids	[20]
Italian	MAE (300 W; 2450 mMHz)	Ethanol	80%	0.1	15 min	106	120.8 mg. g^{-1}	Hydroxycinnamic acids and derivatives; Hydroxybenzoics acids; Flavonoids	[49]
Brazilian green	Ultrasonic bath (28 $W.L^{-1}$)	Ethanol	99%	0.03	20 min	25	1614.80 $mg.g^{-1}$	Hydroxycinnamic acids, Flavonoids	[55]
Beijing	Ultrasonic bath (100 W)	Ethanol	75%	0.05	300 min	40	47.60%	Flavonoids	[54]
Thai	UAE (n.e)	Ethanol	70%	0.1	30 min	N.S.	14,07 mg. g^{-1}	Polyphenols	[32]
Romania	UAE (20 kHz)	Ethanol	70%	0.02	15 min	N.S.	271.65 $mg.g^{-1}$	Flavonoids and and dihydroflavonol	[52]
Chinese black	UAE (220 W; 40 kHz)	Ethanol	80%	0.03	16 min	53	60.34%	Flavonoids and triterpenes	[57]
Turkey	UAE (615.26 W)	Ethanol	70%	0.1	2.95 min	58.61	1.44 mg. g^{-1}	Total phenolic content	[56]
Malaysian	UAE (bath − 20 kHz)	Ethanol	70%	0.1	25 min	65	2.8998 $mg.g^{-1}$	Total phenolic content	[53]
Croatian	UAE (60 W)	Ethanol	80%	0.2	30 min	4	22.13%	Total flavonoids	[59]
Poland	SUAE (200 rpm; 210 W, 20 kHz)	Ethanol	70%	0.1	1460 min	28	104.16 $mg.g^{-1}$	Total phenolic content	[58]
Brazilian red and green	UAE (50–60 Hz, 160 W)	Ethanol	80%	0.02	20 min	50	–	Total phenolic content	[65]
Chinese	UAE (140 w, 20 kHz)	Ethanol	80%	0.03	17 min	N.S.	245.84 $mg.g^{-1}$	Total phenolic content	[72]
Iranian	MAE (300 W; 2.45 GHz) + UAE (bath − 280 W; 50/60 Hz)	Methanol	80%	0.1	1.5 min MAE + 10 min ultrasonic bath	40	70.88%	Total phenolic content	[60]
Brazilian red	SFE (6 $g.min^{-1}$, 350 bar)	CO_2 (Co-solvent: Ethanol)	99% (4% Ethanol 100%)	0.13	140 min	40	63.46%	Flavonoids	[67]
Anatolia	PLE (103 bar)	Methanol:water: HCl (70:25:5) + 0.1% (tBHQ)	100%	0.04	51 min	40	99.7%	Flavonoids	[32]
Brazilian red, brown and green	SFE (6 $g_{CO2}.min^{-1}$, 350 bar)	CO_2 (Co-solvent: EtOH)	99.9% (1% EtOH 100%)	0.1	150 min	50	845.05 $\mu g.mL^{-1}$	Artepillin C and *p*-coumaric	[65]
Brazilian red	PLE (100 bar)	Hexane	100%	0.04	10 min	70	64.63%	Terpenes	[74]

RT: room temperature, **N.S**: not specified, **SLR:** solid- liquid ratio;**HHP:** high hydrostatic pressure; **MAE:** microwave assisted Extraction; **UAE** ultrassound assisted extraction; **SFE**: supercritical fluid extraction; **SUAE**: ultrasound-assisted shaking extraction; **PLE**: pressurized liquid extraction; **tBHT**: *tert*-butylhydroquinone. * Units depend on the publication.

provided the higher extraction yields (8.9, 9.68, and 8.07 $mg.g^{-1}$, respectively), and ethanol was the best option for recovering phenolic and flavonoid compounds. It is worth mentioning that extraction by Soxhlet imposes some difficulties, e.g., a mixture of solvents with different boiling points implies different solvent compositions in contact with the sample, not being advised their use; besides, as the process is based on solvent reflux, extraction time usually is longer than other techniques.

On the other hand, Cunha et al., [41] performed the extraction (maceration, at room temperature, ethanol, SLR 0.2) of phenolic compounds (caffeic acid, *p*-coumaric acid, ferulic acid, caffeoylquinic acid derivatives, pinobanksin, artepillin C, kaempferol derivates, and Kaempferide) from six different samples of Brazilian green propolis for 10 and 30 days. They concluded that despite the longer extraction time (30 days), the extraction yield was improved (reaching 10%, w/w), but the phenolic yield was not improved, promoting the worst selectivity since, in this case, the phenolics are the target compounds and the removal of waxes were desired.

Additionally, the same authors tested a Soxhlet technique using ethanol under optimized operational conditions, 60 °C for 24 h, and noticed a better extraction performance than pure water at 100 °C. Thus, these data reinforce that the combination of solvent, temperature, and SLR must be optimized to improve phenolic compounds' extraction yield and selectivity [41]. Most recently, Archaina et al., [42] compared Soxhlet (ethanol) with stirring (70, 80, and 85% ethanol, SLR 0.1 for 3 × 30 min). The efficiency of the stirring extraction process increased when the ethanol concentration also increased. However, when the extraction temperature increased from 30 °C to 40 °C, the yield decreased by

around 13%, which checks the Soxhlet approach since high temperatures can significantly decrease the content of flavonoid compounds.

Working under an optimized SLR is an appropriate step regarding the well-use of the solvents and the full use of the extractive potential from the biomass, especially when high-added-value biomass is the source of the bioactive compounds, such as propolis. For example, Cottica et al. [25] performed a turbo-extraction from green propolis using ethanolic solutions (60 and 96%) as solvents under different SLR (0.05 and 0.3) for 15 min at room temperature. The SLR 0.3 was highlighted as the most efficient when applied for ethanolic solutions. However, ethanol 96% yielded higher solid content (6.8%), while ethanol 60% had a higher phenolic content (87 $mg.g^{-1}$), which differs from some studies, where higher ethanol concentrations provide better yields of phenolic compounds.

Trusheva; Trunkova; Bankova [22] evaluated the extraction efficiency of phenolic compounds from Italian propolis when the SLR 0.1 and 0.05 were employed. The authors performed maceration using ethanolic solution (70%) for 72 h at room temperature. They concluded that SLR 0.05 does not promote better phenolic compound yields than SLR 0.1 (flavonoids, flavonols, flavones, and flavonones), saving solvent expenses.

In general, for the so-called conventional extraction methods, most studies reported that the higher concentration of solvent, significantly higher than 70% in ethanol, promoted better interaction with the solute and, consequently, a higher extraction yield, regardless of the presence or absence of light. Therefore, the SLR > 0.1 does not seem necessary to recover phenolic compounds from the propolis, and the optimum extraction time varies according to the technique employed. Room temperature is the most employed in conventional methods, with a maximum of 78 °C. In some cases, temperatures higher than 40 °C already promoted the degradation of phenolic compounds. Additionally, elevated temperatures seem to be responsible for decreasing the extraction time. However, it is essential to consider the possible degradation of thermosensitive compounds since a solvent with a higher eluting capacity, such as ethanol, would increase the extraction yield of compounds degraded by heat.

2.2. Exploiting the most recent techniques to obtain natural bioactive compounds from propolis

Due to the potential of propolis and the complexity of the sample, it is agreed by experts in the field that there is no standard and unique methodology to guarantee a successful extraction from their natural bioactive compounds. Propolis has been studied extensively to find better techniques and conditions for optimizing the recovery of their bioactive compounds with physical–chemical properties compatible with analytical and industrial purposes. Despite the advantages of using conventional solvents and techniques, some of the most common disadvantages identified (high volume of solvents used, low extraction yield and selectivity, high environmental impact, and overall costs) have enlarged the set of extraction techniques and solvents under study. Table 2 shows the respective works covering these techniques.

2.2.1. High hydrostatic pressure (HHP) extraction

HHP extraction technique (or cold isostatics superhigh hydraulic pressure) has been considered a sustainable methodology for recovering bioactive compounds from different sources since it efficiently saves energy and solvents [43]. HHP is a new technique used for high-pressure food processing, aiming to extract active ingredients using a variable isostatic hydraulic pressure (1000 bar to 8000 bar) [44]. Based on the phase behavior, HHP enhances the compounds' solubility, which improves the extraction yield compared to the processes performed at atmospheric pressure. In the HHP, there is a massive difference in the pressure inside and outside the cell, favoring solvent permeability. Furthermore, this technique allows the use of different extraction solvents, and the extraction of compounds with varied polarity can be optimized, which is advantageous for complex biomasses such as propolis.

Only three articles evaluated the potential use of HHP for recovering phenolic compounds from propolis, as shown in Table 2. The authors used the Chinese propolis as the source and tested several extraction conditions, namely ethanol concentration (35–95%), pressure (1000–6000 bar), time (1–10 min), and SLR (0.02–0.2). The results revealed that when pressure is fixed at 6000 bar, an improvement of 0.54% of the extraction yield is achieved compared to the same approach performed at 1000 bar [27,45].

However, very high pressures require greater equipment power, increasing their operation, production, and energy costs. Thus, the operational conditions were optimized at 5000 bar for 1 min, SLR 0.54 using ethanol 75% at room temperature, which promoted the higher extraction yield (5.10%) compared to the other tested conditions. Besides, this optimum condition promoted a double yield compared to the conventional stirring mediated by ethanol 100% at room temperature for up to seven days [27]. Furthermore, the same authors, in another work, using the same HHP extraction procedure (developed in 1 min), found that the compounds obtained by HHP have equivalent antioxidant activity to those obtained by heat reflux (240 min) and leaching (7 days), highlighting the quickness of the developed process [45].

Using the same methodology, Jun [46] compared the efficiency of extraction of the HHP technique with the leaching at room temperature extraction (LRT). The authors concluded that both techniques have the same extraction performance, with statistically equivalent yields. However, despite LRT operating under room temperature, it requires a couple of days until completion, which contrasts with only 1 min needed for HHP.

Thus, HHP seems to be a promising technique to be explored in future works [47], mainly due to its lower operating needed time required than the conventional methods, even when compared with the other emergent and modern techniques, such as MAE, UAE, and PLE.

2.2.2. Microwave-assisted extraction

Microwave-assisted extraction (MAE) is an advanced (emergent) method that has become popular for recovering bioactive compounds from plant matrices. Its development was prompted due to the need for fast, safe, and inexpensive methodologies [48]. Íts based on the absorption of energy from electromagnetic waves and is considered highly feasible for industrial applications due to its high potential to promote matrix disruption and consequently favor the release of compounds from the matrix [48].

There is an impetus for using new (alternative) techniques to extract natural compounds, considering the increase in energy costs and the environmental goals towards reducing CO_2 emissions. The MAE has appeared among the most efficient methods for extracting value-added compounds from plant materials [33]. However, effective MAE needs an extraction solvent that absorbs microwave energy and warms (dielectric constant property — ε'). The ε' positively changes the absorbed energy by the molecules; in other words, a higher ε' promotes a faster temperature increase and consequently reaches the ideal extraction temperature. Thus, the extraction solvent must have a high ε' to absorb the radiation and enable rapid and localized heating, increasing the pressure within the matrix, leading to a fast transfer of the compounds to the solvent [49].

Sambou et al., [28] evaluated the extraction of phenolic compounds, mainly flavonoids, from Eastern Canadian propolis under operational conditions fixed at 70 W, SLR 0.25 for 10 min in a domestic microwave at room temperature, varying only the extraction solvents with different ε', namely water, methanol, ethanol, acetone, ethyl-acetate, dichloromethane, ethyl hexane acetate (1:1, 4:1, and 1:4, v/v), and hexane. It was identified that polar solvents such as ethanol and methanol promoted similar yields, 9.87 and 8.87 $mg.g^{-1}$, respectively.

However, the extraction of the phenolic compounds by ethanol was lower than the other solvents. This fact can be justified since ethanolic

extracts have a high content of polyphenols and flavonoids (compounds with aromatic rings and hydrogen bonds), which would make them more susceptible to degradation when subjected to microwave energy. Besides, increasing the power leads to increased temperature, requiring constant control not to overheat the sample and consequently promoting the degradation of thermosensitive compounds [49,50]. In addition, microwave power determines the rate of energy transferred to the sample, which may cause overheating when inefficient temperature control is done.

Illustratively, Hamzah; Leo [50] evaluated the yield of the phenolic compound (especially flavonoids) from brown propolis (*Trigona* propolis) by MAE in an open system (without temperature control) using different powers between 100 and 400 W and extraction time varying from 5 to 25 min and compared these results with the same approach with efficient temperature control (closed system). An ethanolic solution (70%) was selected as the best extraction solvent due to its high compatibility with the compounds in the analyzed samples. The authors noticed that using 400 W in an open system increased at least 30% of the total flavonoid content compared to the same approach performed at 100 W. However, this process led to a significant loss of antioxidant activity, probably due to the degradation of some thermolabile compounds with high antioxidant potential. Thus, the process was optimized, targeting the increase of yield of flavonoids and antioxidant activity, being the optimal condition proposed in a closed system at a maximum temperature of 115 °C using SLR 0.2, microwave power of 300 W, in 15 min.

Also evaluating the influence of the microwave power and its relation with the cycles (pulses) of extraction, a study proposed an MAE performed in a closed system for recovering phenolic compounds (flavonones, flavonols, and dihydroflavonols) from Italian propolis (operational conditions fixed at 800 W, SLR 0.1, in a multimodal household microwave device), and showed that three pulses of ten seconds of extraction (3 × 10 s) led to a significant decrease in the flavonoid content (7.5 wt% flavanones/dihydroflavonols) compared to two pulses of ten seconds (2 × 10 s) (20 wt% flavanones/dihydroflavonols), suggesting that the high influx of energy leads to the degradation of phenolic compounds (including flavonoids) [22].

Thus, it is essential to highlight that for efficient temperature control, a closed system is necessary, where the extraction solvent can be used at a temperature higher than its boiling point without its evaporation, which is satisfactory for improving the mass transfer and consequently promoting high yields of extraction, besides more safety for the manipulator [49]. For example, Pellati et al., [51] extracted polyphenols from the Italian propolis using ethanolic solution (80%) by a MAE in a closed system by applying a maximum microwave power at 300 W, 2450 MHz, SLR 0.1, 106 °C for 15 min. In this study, the pressure was dynamically adjusted by controlling both temperature and power to provide continuous heating, with magnetic stirring to homogenize the sample, and concludes that comparison with other techniques, such as maceration, heat reflux extraction (HRE), and ultrasound-assisted extraction (UAE), the extraction with MAE was improved by shorter extraction time and lower volume of solvent.

Compared with other techniques, MAE showed advantages since it requires a shorter extraction time and a lower solvent volume. For example, Margeretha et al., [23] compared three methods of extraction, namely, maceration (SLR 0.1, 55–85% ethanol at room temperature for 14–82 h), reflux (SLR 0.1, in a water bath at 70 °C for 10–140 min), and MAE (SLR 0.1, 55–85% ethanol, power 420–600 W for 5–30 min), to extract polyphenolic compounds from Indonesian propolis *Trigona* spp. The results showed a clear relationship between flavonoids and total phenolic yields with the extraction parameters. The maceration and reflux techniques yielded around 0.2–4% of flavonoids and total phenolics, respectively. On the other hand, MAE recovered up to 5.8% using 4.66–164-fold lower time, besides a better selectivity.

Thus, the MAE technique seems to be also a promising strategy for recovering NBC from propolis biomass. However, a closed system is desirable to maintain the extraction temperature and preserve the extracted compounds and their bioactivity.

2.2.3. Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) is an efficient technique that promotes high extraction yields but sometimes low selectivity. Cavitation is the primary phenomenon that makes up the technique, where ultrasonic waves promote the formation of bubbles in the solvent and consequently cause the rupture of the matrix, promoting the solubilization of molecules in the extraction solution [32]. In general, the extraction of natural products by UAE last 10 to 60 min, demand for laboratory scales, for propolis, a sample amount between 1 and 30 g, 50 to 200 mL of solvent, and the temperature can reach up to 226 °C when the cavitation bubbles are compressed [49]. However, in studies using propolis, temperatures did not exceed 100 °C, which is interesting to avoid the degradation of flavonoids that can happen at temperatures higher than 110 °C, being ethanol the most used solvent [52].

There are several articles focusing on optimizing the techniques for extracting propolis compounds. Sanpa et al. [53] used the UAE to increase the extraction efficiency in a shorter time of Thai propolis (SLR 0.1, 70% ethanol for 15, 30, 45, or 60 min). The authors noted that the total polyphenol content in the ethanolic extract varied from 7.13 to 14.07 mg.g-1, with the best yield using 30 min..Oroian et al, [54] also used the UAE to increase the extraction efficiency of flavonoids and dihydroflavonol in a shorter time, 15 min, of Romania Propolis (SLR 0.02, 70% ethanol). Yusof et al. [55] focused on optimizing the UAE of phenolic compounds from Malaysian propolis in an ultrasonic bath (20 kHz). The best SLR of 0.1 was selected, and the other variables were optimized using a surface-responsive methodology (temperature: 25–65 °C; time: 5–25 min; concentration of ethanol: 50–90%). The optimum conditions were achieved using 70% ethanol at 65 °C for 25 min.

Sun et al., [56] evaluated the correlation between concentration of ethanolic solutions (25, 50, 75, 95, and 100%) under SLR of 0.05, 40 °C, 100 W for 5 h. They concluded that higher than 75% concentrations promoted better extraction yields (up to 51.03%). However, 75% ethanol might be the most suitable to extract phenolics of propolis (282.83 $mg.g^{-1}$). Cavalaro et al. [57] evaluated the influence of different ethanol concentrations (0 to 99%) to obtain total phenolics from green propolis. An increase in the total extraction yield was observed when ethanol 99% was used. The operational conditions were optimized using an ultrasonic bath (SLR 0.03, 25 °C, 20 min, 28 W/L), recovering 1614.80 $mg.g^{-1}$ and 807 mg of artepillin $C.g^{-1}$, being artepillin C the major compound (and considered as the main chemical marker from green propolis).

Recently, a study searched for an optimum condition for UAE using different solvents (70% ethanol, distilled water, dimethylsulfoxide (DMSO), and PEG) for the recovery of phenolic compounds from Turkey propolis [58]. Following most works, 70% ethanol was the best-selected solvent, yielding 1.44 $mg.g^{-1}$ at 58.61 °C, 2.95 min, and 615.26 W. However, DMSO at 60 °C, 3 min at 580 W promoted an equivalent yield (1.33 $mg.g^{-1}$). When PEG (3 min, 59 °C, and 591 W) and pure water (2.70 min, 59.19 °C, and 591.73 W) were used, the lowest yields were achieved, 0.36 $mg.g^{-1}$ and 0.44 $mg.g^{-1}$, respectively. This study suggested that the solvent and ultrasonic application significantly affected the phenolic compound extraction.

Ding et al. [59] applied an optimization methodology using UAE from the Chinese black propolis, in which ethanol concentration, SLR, temperature, ultrasonic pulse, and extraction time were selected as the independent variables. As a result, the optimal conditions were achieved using 80% ethanol, SLR 0.03, 53 °C, and 16 min with pulses of 5 s, in which the sum of flavonoids and triterpenes obtained corresponded to 60% of the initial mass representing at least the double of the extraction yield compared to magnetic stirring, besides saving solvents and time.

In the same year, Pobiega et al., [60] used Poland propolis to compare UAE (30, 20, and 10 min, 210 W, 20 kHz) with shaking (200 rpm, 28 °C for 1 or 7 days). In addition, they combined both techniques,

resulting in ultrasound-assisted shaking extraction (SUAE: 200 rpm, 28 °C for 1 or 7 days, 210 W, 20 kHz, for 10–30 min). Ethanol at 70% was used for all extraction, and the SLR was 0.1 or 0.2. The results reveal that both techniques alone gave lower extraction yields and lower phenolic and flavonoid contents, 92.36 quercetin equivalent.g^{-1}, 98.74 quercetin equivalent.g^{-1}, respectively. At the same time, SUAE obtained 104.16 quercetin equivalent. g^{-1} with 1-day shaking extraction followed by 20 min UAE. These findings reveal that combining extraction methods can be a valuable tool for maximizing extraction efficiency or even acting as a strategy to extract different compounds in different cycles, a pertinent approach considering that propolis is rich in nonpolar and polar compounds.

Jug et al., [61], compared the UAE (SLR: 0.2; 60 W, 30 min, 4 °C, using 80% ethanol) with maceration (SLR 0.2; 25 or 50 °C, 24 h, using 80% ethanol) for recovery flavonoids from Croatian propolis. In contrast to most works that report better extraction performance when UAE is done, the authors concluded that maceration extraction has an equivalent yield. However, the UAE has additional benefits, such as short duration and low extraction temperature.

Similarly, a study [62] compared the efficiency of extracting phenolic compounds from Iranian propolis by applying two different methods, UAE and maceration. Both extraction techniques were performed using 70% ethanol at an SLR 0.1, but maceration was conducted for 24 h, while UAE (80% amplitude) up to 21 min. Even in a lower extraction time, the UAE yielded at least 2-fold higher phenolic compounds than the maceration, keeping the same major compounds from the sample.

Also comparing UAE (SLR 0.02, 50–60 Hz, 160 W, 50 °C for 20 min) with maceration (80% ethanol, seven days, 25 °C), but now using 80% ethanol and the green and red propolis as sources of NBC, authors concluded that extracts obtained by UAE show an excellent inhibitory effect of gram-positive bacteria (*Staphylococcus aureus*) when compared to the traditional maceration [62].

Trusheva; Trunkova; Bankova [22] compared the UAE (300 W, 25 °C, 30 min) with MAE (800 W, 20 s, room temperature), both applying 70% ethanol at an SLR of 0.1. They showed that UAE promoted better yield and selectivity of flavones, flavanols, flavonones, and dihydroflanoids from Italian propolis than MAE (UAE_{yield}: 9.6% total flavones/flavonols, 22% total flavanones/dihydroflavonols; MAE_{yield}: 8.7% total flavones/flavonols, 20% total flavanones/dihydroflavonols). Even counterintuitive, the UAE technique promoted higher extraction selectivity than MAE. In the extracts obtained by MAE, large amounts of unwanted waxes have been found, besides a higher degradation rate. Contrasting, also comparing the extraction performance of MAE (70 W, SLR 0.25, 10 min, at room temperature), and UAE (40 W, SLR 0.25, 10 min at room temperature), Sambou et al., [28] concluded that MAE promoted at least 3-times fold higher extraction yield compared to the UAE. Also, an article published in the same year observed that UAE (15 min, 20 kHz, 40 W) recovered better yields than MAE (1 min, 140 W, 60° C), and maceration + stirring (24 h, at room temperature and 250 rpm), all using 70% ethanol as the solvent with SLR of 0.02. In all the extracts, the major extracted compound was *p*-coumaric acid (271.65 mg.g^{-1}), besides other twelve phenolic compounds (flavonoids and phenolic acids).

Mixing MAE (100–450 W, 30–150 s) with UAE (30–70 °C, 5–45 min), [62] optimized the extraction of flavonoids, mainly quercetin, from the Iranian propolis, and compared the results with those obtained by the conventional techniques, namely Soxhlet (SLR 0.04, 80% methanol, at 60 °C for 6 h), and maceration (SLR: 0.1; methanol 80%, at room temperature for 24 h). It was reported that the best extraction yield (total flavonoids content: 70.88%, total quercetin content: 44.53%) was achieved by MAE + UAE under the operational conditions at 300 W, 1.5 min for microwave, and 10 min of cavitation at 40 °C, which represented a 5-fold higher yield compared to the conventional extraction techniques.

Using ultrasound to obtain bioactive compounds from natural sources is not new. It is well known that UAE provides high yields, allows fast batches, permits using green solvents, has simple operation, and is safe to run. Therefore, the doubt that hangs over us is why UAE is not yet widespread in the various regions of the planet. The technology still has some gaps to overcome, probably regarding energy consumption and proper optimization between cavitation power and temperature variation. Moreover, on these gaps, we invite the scientific community to pay attention and focus on developing works to create technical solutions that help the technology break boundaries.

2.2.4. Supercritical fluid extraction (SFE)

SFE is a well-established technology that has been extensively used in the past two decades to obtain bioactive compounds from various raw materials [63]. The technology presents several advantages over conventional extraction techniques; however, the high selectivity mediated by temperature and pressure control is undoubtedly one of the most important. Besides, SFE uses low operational temperatures, is efficient in solvent use (with possible recyclability), allows the extract recovery free of solvent by easy pressure drop, and demands low energy consumption [64,65].

Carbon dioxide (CO_2) is the most widely used supercritical solvent (SC-CO_2) due to its low cost, low toxicity, and easy achievement of the critical point (31.1 °C and 75 bar). In addition, SC-CO_2 is a nonpolar substance able to dissolve low-polar or moderately polar compounds, usually the main target compounds extracted using this technique [66]. However, in SC-CO_2 extraction, high molecular weight compounds, predominantly those hydrophilic, have poor solubility and are not well extracted [32].

Adding a polar co-solvent is usually adopted to overcome such drawbacks, increasing the interactions between the target compound and solvent and improving the SFE performance [66]. For example, in the Brazilian green propolis, Machado et al. [67] applied SC-CO_2 extraction (flow rate of 6 g_{CO2}.min^{-1}, 350 bar, 50 °C, for 30 min). They noticed that adding 1% ethanol (80% v/v) promoted better extraction performance (yield: 80.3 mg.g^{-1}) compared to the same processes without co-solvent (yield: 66.21 mg.g^{-1}), being artepillin C and *p*-coumaric acid were the major extracted compounds.

Similarly, [29] extracted artepillin C, 3-prenyl-4-hydroxycinnamic acid, *p*-coumaric acid, and kaempferol from green propolis by SFE, also using SC-CO_2 as the solvent (solid/feed ratio of 0.17, 1 g_{CO2}.min^{-1}, 150–350 bar, 35–60 °C, for 30 min). They showed that adding 15% ethanol improved the extraction performance from 7.3% to 51% at 250 bar and 35 °C, promoting energy and solvent savings in the process.

It is no surprise that temperature and pressure are critical parameters in SFE. Changes in both affect the target compounds' solubilization into the SC-CO_2. For example, Fianco et al. [68] compared the influence of different pressures (9.0, 15.0, and 300 bar) to obtain benzophenones from Brazilian red propolis (solid/feed ratio 1, 10 g_{CO2}.min^{-1}, 40 °C, for 120 min, and 1% of 70% ethanol as co-solvent). According to the authors, the global extraction yield was directly proportional to the increase in pressure, from 8.6% to 39.9%, which showed that a higher pressure was advantageous in this case.

Reis et al. [69] also determined the best operational condition for obtaining red propolis extract by SFE (SC-CO_2 + ethanol). This condition was found within the investigated parameters: SLR equal to 0.13, 4% ethanol as the co-solvent, and 350 bar at 40 °C, which resulted in higher extraction yields, being formononetin the primary biomarker (and major compound) of the red propolis. The combination between pressure and temperature also modulates extraction performance in SFE.

Biscaia and Ferreira [35] studied the interaction of different temperatures (30, 40, and 50 °C) and pressures (100 to 250 bar) for recovering artepillin C and quercetin from Brazilian green propolis at 5 g_{CO2}.min^{-1} and 5% ethanol as the co-solvent. Under fixed temperature (40 °C), the increase in the pressure from 100 to 250 bar improved the extraction yield by 3-fold. However, under constant pressure (10 MPa), increasing the temperature from 30 to 50 °C, the extraction yield decreased up to 4-fold. Additionally, from 200 bar, regardless of

temperature, the extraction yield did not vary significantly.

Chen et al. [106] applied SFE for recovery NBC from the Brazilian green propolis fixing pressure at 207 bar, using different temperatures (40, 50, and 60 °C) and different concentrations of ethanol as the co-solvents (2, 4, and 6%), being the higher temperature the best condition to achieve a higher extraction yield (41%). The rise in solvent density favors the extraction due to the reducing distance between molecules, enhancing the interaction between CO_2 and raw material. But, density is not the only property that drives the SFE; above a region known as crossover pressure, the extraction becomes to be governed by the vapor pressure of the solutes, and therefore, the increase in the temperature may increase the desorption and solubilization of the extract [71].

However, the extraction yield is not the only relevant outcome; the extract concentration should be considered, especially for NBC production. Given this, SFE can be explored to produce concentrated extracts due to its selectivity power. For example, [67] obtained extracts from different types of Brazilian propolis (red, green, and brown) by SFE (S/F ratio 0.1, 350 bar, 50 °C, 1% ethanol, 6 $g_{CO2}.min^{-1}$, for 150 min) and shaking homogenization (80% ethanol, SLR equal to 0.13, 70 °C, for 30 min, and 710 rpm). They showed that the conventional extraction recovered the highest total phenolic yield (300.36 $mg.g^{-1}$), while SFE yielded 97.97 $mg.g^{-1}$. However, the highest concentration of artepillin C and *p*-coumaric acid were identified in the SFE extracts, suggesting a better selectivity of the SFE to extract the primary compounds from propolis.

Sequential extraction is another exciting approach to recovering extracts. The authors used a moderate SC-CO_2 density (0.772 $kg.m^{-3}$) in the first extraction stage that promoted the recovery of nonpolar compounds, such as essential oils and waxes (present in large amounts in propolis). In comparison, in the second stage, high SC-CO2 density (0.880 kg.m-^{3}) extracts more polar substances, such as phenolic compounds and flavonoids [35,66].

Following this strategy, a recent study carried out the SFE in two stages and concluded that sequential extraction has a high potential to improve the SFE performance [72]. The first stage, a "pre-extraction step," was performed using 50 g sample, 50 g CO2, 150 bar, 50 °C for 60–80 min, and successfully applied for recovery waxes. In the second stage, named "dynamic extraction," using the same biomass extracted in the pre-extraction step, SFE was performed using different operating conditions for changing the density of the SC-CO_2 (18–33–66.82 °C, 166–334 bar, flow rate 6.59–23.41 $g_{CO2}.min^{-1}$, for 240 min).

The optimum condition was identified as 50 °C, 250 bar, 15 $g_{CO2}.min^{-1}$, SLR equal to 0.013, which promoted 14.4% of total phenolic compounds yield, with galangin, *p*-coumaric acid, phenyl ester, and caffeic acid. At higher temperatures, the extraction yield was higher compared to lower temperatures. The extract solubility increased as the temperature increased from 40 to 60 °C. At constant temperature, the increase in pressure led to a higher yield due to the increase in the CO_2 density. However, this effect was seen up to 250 bar, corroborating with the previous works that reported the effect of temperature and pressure. The results indicated that a flow rate above 15 $g_{CO2}.min^{-1}$ decreased the yield. Increasing the flow rate occurs until the intraparticle resistance becomes the dominant mass transfer; additional increments can reduce the extraction rate due to the low CO_2 residence time in the extraction vessel [72].

Other authors have compared SFE with different techniques. De Zordi et al. [73] optimized the SFE process considering an optimum condition of pressure (317 bar), temperature (45 °C), flow rate (2 $L_{CO2}.min^{-1}$), and extraction time (6.5 h), achieving a yield of phenolic compounds around 15%. The authors also noticed that despite SFE yielding a lower concentration of phenolic compounds than the UAE, it promoted better lipophilic compounds, such as waxes, which can be considered interferents or contaminants in some cases.

Similarly, [74] compared UAE (S/F ratio 0.03, 80% ethanol, 140 W, 20 kHz, pulse for 17 min) with SFE (methodology by [73]) and maceration (SLR equal to 0.04, 80% ethanol, at room temperature, for 6 h), besides the biological activities of Chinese propolis. The results showed that propolis extracted by UAE contained more phenolic compounds and showed the highest total phenolic content (245.84 ± 6.41 $mg.g^{-1}$), total flavonoids content (198.82 ± 5.74 $mg.g^{-1}$), and stronger *in vitro* antioxidant activity (DPPH: 1.03 ± 0.04 $mmol_{Trolox}.g^{-1}$, ABTS: 2.19 ± 0.05 $mmol_{Trolox}.g^{-1}$ and FRAP: 1.48 ± 0.12 $mmol_{FeSO4}.g^{-1}$) than SFE and maceration.

Thus, SFE could also be considered a clean-up technique to promote a previous removal of lipophilic unwanted (in this case) compounds before extracting phenolic ones, considered the primary target (bioactive) compounds from propolis. Indeed, integrating extraction processes toward the biorefinery concept is regarded as a promising trend to use the raw materials fully. In this sense, previous stages of SFE may provide lower yields but high concentrated extracts that originate the first products; the degreased raw material may be subjected to other extraction processes targeting the most polar substances and giving other products.

2.2.5. *Pressurized liquid extraction (PLE)*

PLE allows fast extraction of analytes/compounds in a closed and inert system, under high pressures (33–203 bar) and temperatures (40–200 °C). A great benefit of PLE over conventional extraction techniques (conducted at atmospheric pressure) is that pressurized solvents remain in the liquid state above their boiling points, allowing for extraction at high temperatures without losing solvent for the atmosphere. Unlike HHP extraction, the PLE works under lower pressure, but it is possible to extract using a dynamic solvent flow and high temperatures, which facilitate the full extract potential of the matrix [75]. In addition, these conditions improve the target compounds' solubilities and the matrices' desorption kinetics, making the extraction solvents, including water, usually considered a low-performance solvent for extracting phytochemicals at low temperatures more efficient in PLE [75].

In the case of propolis applications, Erdogan et al. [34] applied the PLE technique for the recovery of catechin derivatives, phenolic acids, and flavonoids from Anatolia propolis, reaching a 99.7% yield using methanol:water:HCl; (70:25:5, v/v/v) containing 0.1% *tert*-butylhydroquinone (tBHQ), under the operational conditions optimized at 40 °C, 103 bar, with three cycles of extraction using the same biomass to optimize/recover all the NBC as possible. The same authors also tested the temperature effect using an acidified solution of methanol (70%) as the extraction solvent at 103 bar for 45 min. The most efficient temperature for recovery NBC was optimized at 40 °C since higher temperatures (>80 °C) decreased the extraction performance. Besides, after 45 min, the yield of extraction remains practically unchanged.

On the other hand, De Carvalho et al., [76] proposed a new solvent accelerated extraction method based on PLE (100 bar, SLR 0.04) to obtain active compounds of low polarity and high cytotoxic profile from the Brazilian red propolis. They evaluated the influence of the temperature, the number of cycles, and time. The results showed that one cycle of 10 min of extraction at 70 °C recovered a yield of around 64% of terpenes (mainly lupeol and lupeol acetate), which promoted a high cytotoxic activity against the tested cell lines.

It is known that elevated temperatures are reported to improve extraction efficiency due to the higher diffusion rate and better solubility of analytes in solvents [75,77,78]. However, degradation of thermosensitive compounds could happen, which makes necessary the effective monitoring and/or optimization of this variable. In this sense, some online PLE-mediated extractions have been proposed for some authors, as well as made for pomegranate peel [79], apple pomace [80], and *Yerba mate* [81], that represent an essential source of phenolic compounds of medicinal interest, such as propolis. The online (or even in-line) approach is considered a modern and desired technology since it assists in improving the yield of extraction and improves the processes' cost-benefit and sustainability [82].

Table 3
Summary of the works reported to date focusing on the extraction of bioactive compounds from propolis using alternative solvents.

Propolis origin	Method	Solvent	Concentration	SLR	Time	T (°C)	Yield*	Compounds	References
Brazilian green	Magnetic stirring	Cholinium chloride: propylene glycol or lactic acid:water	1:2 (mol.mol^{-1}) or 85:15 (w/w)	0.02	3 h	50	2.13 or 1.99%	Artepillin C	[93]
Chinese	UAE (300 W, 60 s)	[C_{12}mim]Br	100 mg.mL^{-1}	0.05	Maximum speed for 60 s	25	82.74–97.88%	Pinocembrin, chrysin and galangin	[90]
Chinese	Silica-supported ionic liquid-based matrix solid phase dispersion extraction	Dispersant: [C_6mim]Cl; Washing solvent: n-hexane; Elution solvent: methanol	10%; 20 mL; 15 mL	0.75	20 min	40	19.2–74.0 ng. mL^{-1}	Phenolics acids	[92]
Brazilian red	UAE (probe) 400 W, 20 kHz,	[C_6mim]Cl	1:2 (mol.mol^{-1})	0.33	5 min	N.S	394.39 mg.g^{-1}	Total flavonoids	[88]
Popular type	UAE (bath – 60 min)	Citric acid – 1,2-propanediol 1:4 (CAPD)	1,2-propanediol 1:4 (CAPD)	0.03	60 min	R.T	36.7%	Total flavones and flavonols	[94]

N.S: not specified. **R.T:** room temperature; **UAE** ultrassound assisted extraction; **IL:** ionic liquids; **MCC**: microcystalline cellulose; **SPE**: solid-phase extraction; **[C_{12}mim] Br:** 1-dodecyl-3-methylimidazolium bromide; **[C_6mim] Cl:** 1-Hexyl-3-methylimidazolium chloride. * Units depend on the publication.

Indeed, integrated systems to extract, separate, and online monitor the chemical composition of natural matters are already available. For example, [83] reported developing an automated system composed of PLE inline coupled with solid-phase extraction and high-performance liquid chromatography to monitor in real-time the extraction and comprehensively analyze phenolic compounds from natural products.

In summary, PLE is a technique able to produce high extraction yields quickly. However, it is not a highly selective technique as SFE is. Given what we present in this work section, we can affirm that PLE is still unexplored for obtaining propolis extract, despite its potential. In terms of processes integration, PLE is a versatile technique; it can be carried out after SFE to obtain polar compounds from the degreased raw material; it can be coupled with ultrasound to speed up extraction; and still, it can be coupled to SPE to concentrate the extract on the compounds of interest. These possibilities of process integration can be an alternative to leverage the application of PLE in propolis.

3. Use of alternative solvents to obtain bioactive compounds from propolis

Traditionally, ethanolic solutions are the most conventionally used in extracting bioactive compounds from propolis, which is advantageous considering that ethanol is widely used in various industrial processes, including the food industry. Besides, ethanol has a low-carbon footprint in its production (biosolvent) and is generally recognized as safe (GRAS). Like ethanol, water is regularly applied as an extraction solvent, mainly for the most hydrophilic compounds. Water is the most sustainable solvent, however, with low selectivity, which means that using water, only most of the hydrophilic compounds are recovered.

However, propolis is a complex biomass composed of several hydrophilic and hydrophobic compounds (such as waxes and resins); Thus, aqueous solutions present severe limitations as extraction solvents. Some authors have considered of utmost interest non-volatile alternative solvents, especially using ionic liquids (ILs) and eutectic solvents/mixtures to overcome the drawbacks of using ethanol or water to develop sustainable development downstream processes. These solvents are used for replacing (or reducing) the use of organic solvents while presenting the possibility of being recovered and recycled during the processes [84].

ILs are salts composed of organic or inorganic cations and anions of different sizes, which imposes to the IL different physicochemical properties. In the extraction processes, ILs are commonly dissolved in water (or, in a few cases, in ethanol) and submitted to contact with the biomass. IL-mediated extracts have been recognized as promoting better characteristics to the extracts and, in some cases, also improving/strengthening some biological effects of the biomolecules, namely their antioxidant activity and bioavailability [85,86]. Additionally, ILs are recognized as high-performance solvents since they usually promote a higher extraction yield than the processes performed using organic solvents [85,86], although sometimes requiring more costs.

Eutectic solvents are low-transition-temperature mixtures that in specific proportions have the eutectic temperature below that achieved by an ideal liquid mixture [87]. They are easily formed by mixing two or more compounds, usually dissolved in a specific amount of water. They are easy to prepare, non-reactive in water, and versatile. Furthermore, much more knowledge is being produced regarding their application and the understanding of interactions between eutectic mixtures/eutectic solvents with the liquid phase [88].

Both ILs and eutectic solvents are recognized as design solvents, which places them in an excellent position to be applied as solvents. Some authors have already covered the successful extraction performance and the main advantages of the ILs [84] and eutectic solvents [89] in extraction processes. However, up to now, only five works have been referenced as using alternative solvents on the extraction of bioactive compounds from propolis, as presented in Table 3. Besides the methodologies used, the extraction conditions employed in each method (namely the concentration of the alternative solvent, SLR, time, and temperature) are also described.

Recently, dos Santos et al. [90] recovered flavonoids from the Brazilian red propolis using ILs and eutectic solvents and compared the results with those obtained using water and ethanol as solvents. The authors performed a screening assay using imidazolium-based ILs (1-butyl-3-methylimidazolium, chloride ([C_4mim]Cl), 1-butyl-3-methylimidazolium hexafluorophosphate ([C_4mim][PF_6], 1-butyl-3-methylimidazolium tetrafluoroborate ([C_4mim][BF_4]), and 1-hexyl-3-methylimidazolium chloride ([C_6mim]Cl)), and eutectic solvents (cholinium chloride:1,4-butanodiol, cholinium chloride:levulinic acid, and cholinium chloride:glycerol, in a molar ratio 1:2). The extraction was done using UAE (probe) under the operational conditions fixed at SLR of 0.33, 400 W, 20 kHz, for 5 min. And concluded that [C_6mim]Cl allowed the best extraction yield among the alternative solvents tested, even better than water and ethanol. After proper optimization, a total of 394.39 mg.g^{-1} flavonoids were obtained, with total antioxidant capacity evaluated up to 7595.77 $\mu mol_{Trolox\ equivalent} \cdot g^{-1}_{dried\ biomass}$, besides inhibiting the growth of *Staphylococcus aureus* and *Salmonella enteritidis* bacteria. Despite an alcohol-free method being developed, and the good results reported regarding the IL positive influence on the antimicrobial activity, the final extract rich in flavonoids was obtained using a proportion of IL:water at 10:1, which means that ~ 90% of the solvent was IL. This fact represents a high financial cost for the process and the impossibility of applying the extract rich in flavonoids as a phytomedicine or nutraceutical product, especially considering that imidazolium

based-IL have several toxically potential. Therefore, a strategy for recovering the used IL (polishing) is still necessary for this work to lower the final cost and decrease the environmental impact of the process by applying the recycling of the raw materials, as performed in [91].

Earlier, long-chain imidazolium-based ILs were investigated as solvents to extract phenolic compounds from the Chinese propolis. By applying the tensoactive IL 1-dodecyl-3-methylimidazolium chloride - [C_{12}mim]Cl [92], the authors developed an IL-based micellar extraction combined with microcrystalline cellulose-assisted dispersive micro SPE methodology (ME-DMSPE) at 200 mM, 25 °C, 300 W, 20 min. At least 20 different compounds between phenolic acids, flavonoids, and methyl-ethers were recovered and characterized by mass spectrometry, showing a precise fingerprint of the key compounds usually present in many types of propolis. Despite interesting, the developed approach performed in this work, by coupling different techniques in an integrated way, represents a poorly explored strategy in the works dealing with propolis. However, the authors did not recover the IL from the extract, which deeply compromises the sustainability of the process but also the safety of the final product due to the toxicity of the tensoactive IL applied [93].

Wang et al., [94] performed a SPE using a silica-supported IL (S-IL) to analyze eight bioactive compounds from the Chinese propolis (caffeic acid, ferulic acid, morin, luteolin, quercetin, apigenin, chrysin, and kaempferide). The procedure was performed using 10% of [C_6mim]Cl immobilized by direct impregnation on the silica gel surface and immersed in a methanol solution. The optimum ratio of S-IL to propolis was 4:1, using 15 mL methanol as eluent for 20 min. The extraction yield of the S-IL process (1.16 mg.g^{-1}) was equivalent to those obtained by UAE (1.19 mg.g^{-1}, 20 mL methanol in 50 min), and Soxhlet (1.035 mg.g^{-1}, 100 mL acetone in 180 min). Despite the extraction yields being statistically equivalent for the three different processes, the volume of organic solvent used and the time-effectiveness when using the S-IL was lower than those performed in UAE and Soxhlet. Unlike the others, the study focused on the analytical procedures, not the extraction performance. The authors discussed that, with the proper adjustments, the S-IL method developed could be applicable for the simultaneous extraction and analysis of phenolic acids and flavonoids in different medicinal plants.

Funari et al. [95] proposed an alternative methodology based on 29 eutectic solvents (formed using cholinium-chloride, L-lysine, L-proline, fructose, sucrose, propylene glycol, glycerol, 1,4-butanodiol, lactic acid, and malic acid in different molar ratios and water content) for the extraction of bioactive compounds from the Brazilian green propolis. The extraction was performed by conventional magnetic stirring at room temperature for 3 h at 50 °C. Cholinium chloride:propyleneglycol (1:2), and cholinium chloride:lactic acid: water (1:1:1) have been shown as the best candidates for replacing hydroethanolic and aqueous solutions of propyleneglycol, with extraction yield higher than those obtained when water, ethanol, honey, or an aqueous solution of L-lysine were used as solvents. Additionally, the authors reported the appearance of some crystals in the extract at room temperature (25–30 °C), indicating the impossibility of using chromatography as the analytical method and the final product in most of the applications, a drawback only surpassed, as the authors suggested, by slightly increasing the temperature.

Poplar propolis, typical propolis from Bulgaria, was subjected to UAE using eutectic solvents as extractants [96]. The procedure was performed using an ultrasonic bath, under operational conditions fixed at SLR of 0.03, room temperature, for 60 min. Citric acid:1,2-propanediol (1:4) was set as the best eutectic solvent, recovering 36.7% of total phenolics, an equivalent result obtained by using 70% of ethanol under the same extraction conditions. Typical cinnamic acids, esters, flavonones, and dihydroflavonols were identified by GC–MS in the extract obtained with the selected eutectic solvent. They showed high antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

After analyzing the few studies reported so far, some authors consider that only using alternative solvents gains the "seal" of sustainability, which is untrue. Additionally, "toxicity-free" credentials are often used for these solvents, giving a false impression that alternative solvents are always better than organic solvents, another untruth. Eutectic mixtures are usually labeled as "natural", which creates a false prerogative that they are safe just because they are "naturally-based" or "derived from natural sources". Like the ILs community did for years, the researchers working with eutectic mixtures tend to overgeneralize eutectics' sustainable and non-toxic credentials. Again, depending on the nature of the starting materials used and the molar ratios applied, they can be (cyto)toxic [97] or might have synergistic toxic effects when compared with their starting materials alone [98].

Indeed, non-volatile solvents have a vast potential to be applied as sustainable alternatives; since they can be designed with desired properties (viscosity, density, miscibility, hydrophobicity, just to mention a few) to suit a particular application. However, designing a sustainable process is not just using a new solvent. Future articles must cover more than the extraction performance of the solvent (we already know that ILs and eutectic solvents promote higher extraction yield); now, we need to explore efficient strategies for the recovery of the solvent from the extracts and evaluate their efficiency when recycled [91,99]. However, when the eutectic solvent is free of toxicity, or improve some characteristic of the obtained extract, they can be incorporated in the final product. As well as reviewed by Murador et al. that reported that extracts obtained with eutectic solvent could increase the bioavailability of the extracted compounds compared to those obtained with organic solvents [85]. Another example, in this case out of the food sector, is represented when the eutectic solvent act as stabilizing agent in cosmetic formulations [100]. Thus, if a substantial advantage is demonstrated, the eutectic solvent could be kept in the final extract.

4. Analysis of propolis extracts and process' monitoring

Since propolis is a complex sample, different analytical techniques and methods can be used to quantify the phenolic compounds with precision in the samples. Standardized extracts are particularly relevant since the information about the bioactivity of specific components of propolis with different origins is growing in amount and detail. This information will be helpful to identify markers for specific applications and set values (i.e., quality) based on the concentration of active components [9].

In this context, the analysis of components found in propolis can be achieved with several methods. However, due to the growing need to characterize the chemical composition and determine the individual components of propolis [101], chromatographic techniques coupled with spectrophotometric detection are preferred due to their ability to quantify individual components, relatively low detection levels, and cost. Liquid chromatography (LC) is the technique most used for separating and quantifying phenolic compounds from propolis and other biological samples [102].

Chromatographic analysis of the propolis extract is usually carried out in reverse phase conditions using a C18 column, acidified water, and another organic solvent, such as methanol and acetonitrile [103,104]. However, due to propolis's wide variety of phenolics, the developed method can exceed 100 min, implying a considerable mobile phase and energy consumption [105,106]. However, some authors performed more eco-friendly strategies. For example, Funari and co-authors [107] developed a chromatographic fingerprinting methodology for propolis analysis using bioethanol from sugar cane as the mobile phase. The analysis time was 50 min, with a high-chromatographic resolution, reproducible with propolis from different countries (Brazil, United States, England, and Australia). However, even with a long chromatographic method, the authors used ethanol in the mobile phase, which is even more difficult considering the complexity of propolis samples. Furthermore, despite the good results obtained by the authors, it is possible to assume that a universal chromatographic method for

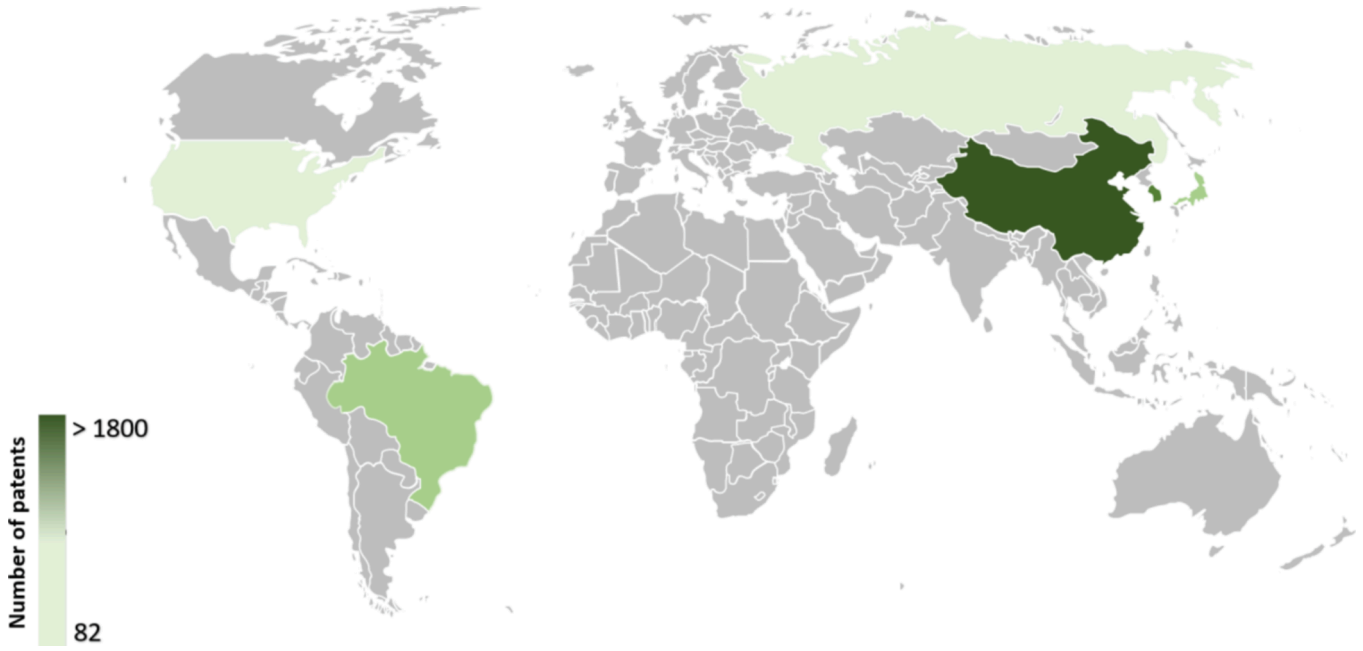

Fig. 1. Countries with the highest number of patents including propolis. Russia = 82; United States of America = 89; Brazil = 102; Japan = 222, South Korea = 584 and China = 1845 [129].

propolis is quite improbable (and an ambitious goal) considering the vast diversity of compounds present in the different propolis worldwide (yellow, brown, green, red, and so on).

In this sense, the UPLC (ultra-performance liquid chromatography) technique is becoming a critical analysis tool since it reduces the expenses with mobile phases and allows the development of faster analytical methods [108]. Most recent advances to optimize the separation process include using smaller fused-core particles while reducing operating systems pressures and analysis time by 3–4 times compared to conventional methods [109]. However, it is essential to highlight that the coupling of classical and modern techniques for analyzing complex samples is increasingly common. Also, chromatographic methods can not provide complete information on the compounds present in the extract without the combined analysis of an LC with MS and UV detectors [51]. Thus, to improve identification and quantitation, more than one analysis technique can be used, such as online UV combined with mass spectrometry (MS) [110,111] and Nuclear Magnetic Resonance (NMR). Indeed, NMR detection is the spectroscopic technique with the most precise and accurate detection system for natural compounds. However, the maintenance cost of such equipment is expensive and requires highly specialized labor. Another point is the difficulty of coupling NMR with LC systems. The receiver of the NMR detector cannot handle the intense solvent signals and the weak substance signals simultaneously [112], which for propolis is a very difficult task that needs a complete and long optimization step. Furthermore, as we already discussed, propolis is a complex chemical matrix with a high diversity of NBC. So, in this case, NMR analysis, although promising, complicated, complex data are obtained, which impairs the identification of the compounds [113]. However, despite the difficulty, some authors use NMR to elucidate new chemical compounds from propolis, especially in a metabolomic approach [113,114]. Thus, considering the difficulty and cost of NMR analysis, the preferable technique performed for propolis analysis is based on MS. The information provided by MS is a valuable tool for complex natural mixtures like propolis, ensuring the molecular fingerprinting of similar extracts [110,111]. Nevertheless, these techniques require some precautions regarding the sample preparation, making the analysis longer than when the biomass could be directly analyzed.

Alternatively, some authors have studied infrared spectroscopy (IR) [115-121]. The IR technique generally does not require very elaborate sample preparation steps, which increased the interest of the authors in recent decades. Besides, it is considered a low-cost, fast, and non-destructive technique [122]. Although advantageous, it is not very sensitive, which raises concerns about its applicability in complex samples. Lately, in most studies, IR was coupled with other methods of analysis, making it difficult to criticize its influence on the propolis analysis.

Nevertheless, the studies show that it is possible to distinguish specific signals for a given organic compound based on extensive literature data on the chemical propolis composition with proper calibration. Ku et al., [120] used Fourier transform mid-infrared spectroscopy (FTIR) to obtain a complete chemical profile of the Mediterranean propolis. The authors argue that the method can evaluate the phenolic composition with correct calibration. In addition, recent studies also showed advantages of the IR (especially FTIR) method in predicting phenolics and bioactive propolis properties, like antioxidant activity [116,121]. For example, Vivar-Quintana and co-workers [117] analyzed the phenolic composition of 50 propolis extracts from Spain and Chile, comparing the results obtained by high-performance liquid chromatography (HPLC) with those obtained with Near-Infrared Spectroscopy (NIR), and concluded that there are no differences between the data. Thus, IR is quite promising, yet more research is needed to investigate its full potential.

Another technique being explored is Capillary Electrophoresis (CE) ([123]. Analysis by CE has the advantage of using reagents considered greener than those used in liquid chromatography (sodium tetraborate buffer, for example) and of easy treatment for disposal. The CE method developed allowed better detection of artepillin C (a most significant component of the Brazilian green propolis) in comparison to the other methods that used the HPLC-UV and HPLC-MS techniques (100 and 22 times greater, respectively), pointing to an attractive perspective for the use of CE in future studies with propolis.

It is also interesting to note the high number of techniques applied to analyze phenolic compounds, but it still lacks optimization for a complex sample such as propolis. This optimization must be done to understand the sample regarding the quality control of products derived from propolis since it is used for several important biological applications. Another critical point to be evaluated is the Green Analytical

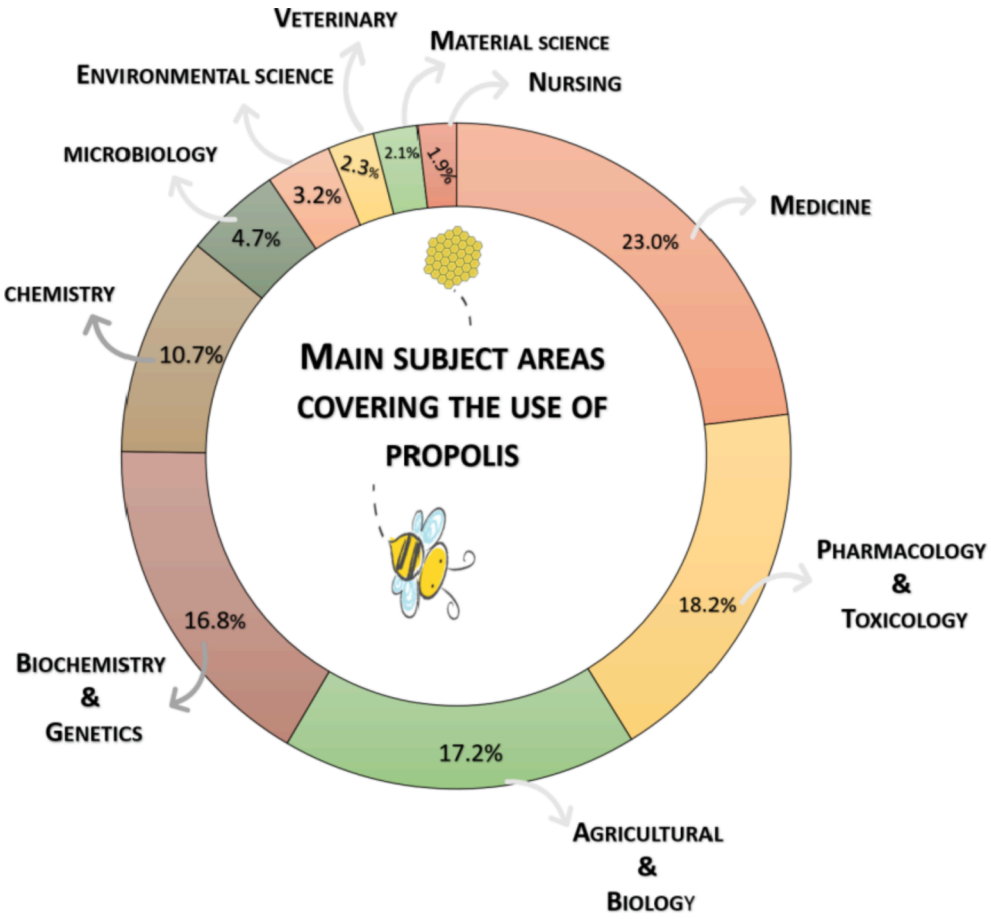

Fig. 2. Main subject areas of application of propolis. The percentage values are approximations calculated based on a Scopus 2021 search.

Chemistry (GAC) concept, relevant to industries dealing with biomaterials. Green analytical chemistry can take many forms, but most segments of this emerging field will need to minimize the wastes associated with sample preparation or analysis and use less toxic and renewable solvents [124].

In this aspect, coupling analytical systems and detectors to the extraction process is an attractive strategy to provide real-time information and a valuable standardization tool. Analytical systems can be coupled in different ways with the extraction equipment. Recent examples of coupled techniques for extracting and analyzing phenolics include on-line coupling with HPLC-DAD and in-line UV detection [125,126]. It can be expected that other analytical techniques and detection systems will be explored to obtain detailed information about the process, the composition of the extract, and biological activity, leading to product standardization.

5. Applications of propolis and their products

Since the end of the last century, the number of publications dealing with propolis has expanded, increasing the researchers' knowledge about its biochemistry, botanical origin, and biological properties [127]. And its pharmacological properties, immunomodulating, anti-inflammatory, antiobesity, and antitumor activities have instigated the technological innovation performed by companies and research institutions [6].

According to the most recent data, the top 5 countries working with propolis applications are China, South Korea, Japan, Brazil, the United States, and Russia (Fig. 1), responsible for most of the patents in the field [128].

In terms of products, propolis has been studied in the production of capsules, mouthwash, lotions, throat pastilles, and wax-free products to improve manipulation and use [129]. The main areas of application of propolis include medical, pharmaceutical, nursing, veterinary, biology, agriculture, and environmental science (Fig. 2). The most frequent subareas are dental, daily care, food, non-alcoholic beverages, and preservatives [128-130].

The side effects of chemical drugs and toxicity are why a natural product like propolis is gaining popularity in treating various medical conditions [128-130]. As a way forward, optimization of propolis composition, standardization, and further investigations into the mechanisms by which the bioactive compounds from propolis exert their biological effects are other research areas of interest.

There are various methods to optimize propolis in health and medicine by utilizing its biological and chemical properties for many applications, like gastric disorders, bronchial asthma, antidiabetic, dentistry, anticancer, antifungal, and antibacterial activity [128-131]. Furthermore, the developments in this field, including animal and human clinical trials, will pave the way for more innovative treatment strategies, especially in complicated wound healing and skin regeneration [127,130,132].

Propolis has also been used to prepare dermatological products, while the cosmetic industry has invested in its use mainly for treating acne [127,132]. In veterinary medicine, propolis has been used as a functional food, for example, in chicken diets [133], but also in the treatment of skin wounds, in the protection of epithelial cells against pathogens in mammals [134-136], acting on rumen ciliated protozoa populations [137] and reducing the need of milk, by supplementing dairy cows diets with propolis extract [138].

Another area of intensive research in the last few years is the application of propolis in food products. Most products on the market

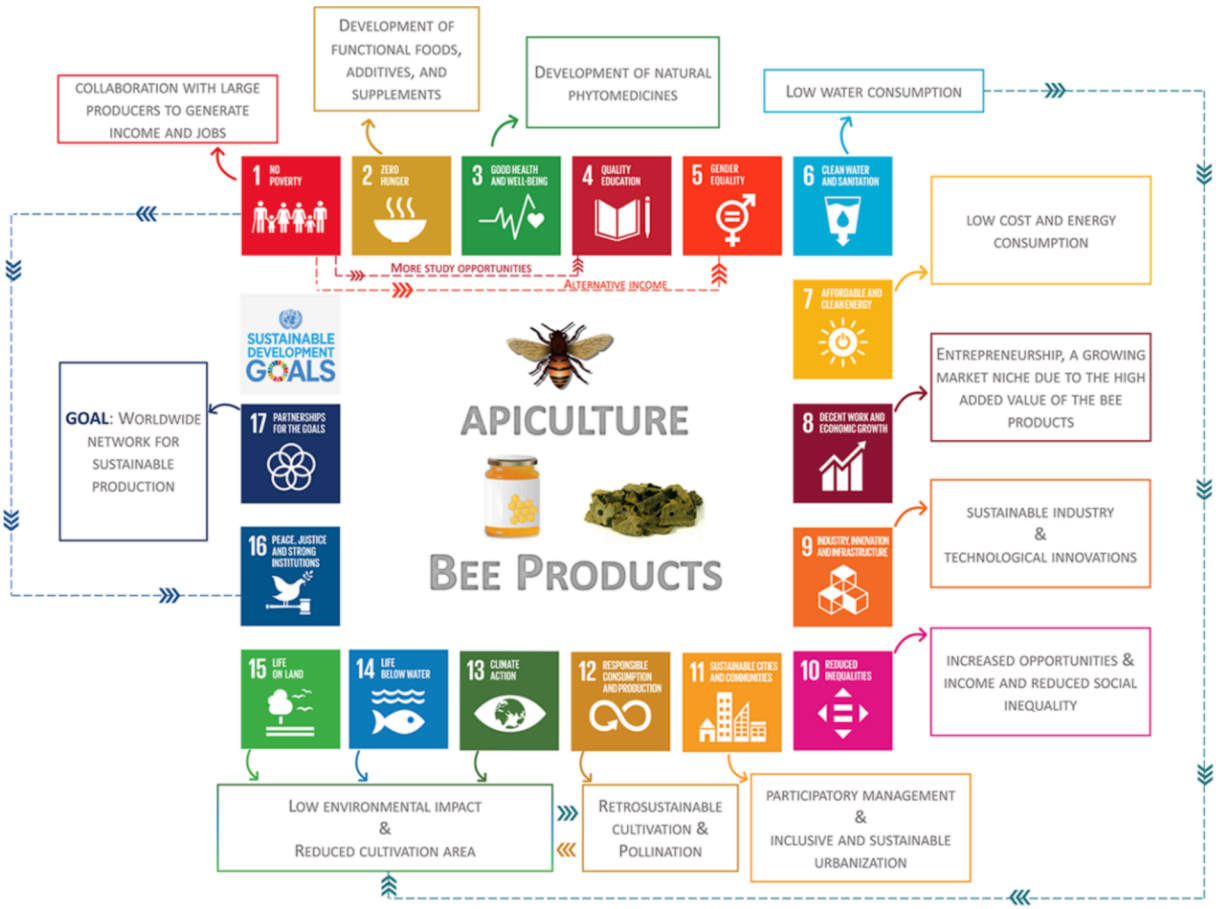

Fig. 3. Schematic representation of the relation between apiculture and bee products with the 17 SDG defined by the United Nations.

today are intended to provide health benefits (e.g. powdered milk, tea, and fermented beverages) [139]. In addition, some products use propolis in their formulations as a preservative, thus avoiding altering their microbiological quality [139-141]. Food preservatives include mainly antimicrobial and antioxidative agents to control natural spoilage of food and prevent/control contamination by (pathogenic) microorganisms [139-141]. The antioxidant and antimicrobial characteristics of propolis are essential for the food industry due to their potential to delay lipid oxidation and increase product shelf life [142].

Although the advantages of using propolis in food formulations, some drawbacks need to be pointed out, namely its characteristic flavor and odor, which are responsible for altered food sensory properties [143,144]. Some authors also reported incorporating propolis extracts into films or food coatings, using these membranes to prevent microorganisms dissemination but avoiding its incorporation in the food formula [143,144].

The different propolis applications increase the interest of the consumer market for these products. This aspect promotes the emergence of new industries, products, and markets [144]. Thus, propolis's application is vast, and its potential is increasingly explored.

6. Sustainability in beekeeping-related chain

Since sustainability can only be achieved with intergenerational decision-makers [145], searching for new downstream processes to recover bioactive compounds from propolis is required. Moreover, a powerful strategy for providing a high-quality natural product is also needed to respect the current market demands aiming for a better and more sustainable society. Thus, sustainable cultivation with good agricultural practices is one way to increase production while meeting world demands and maintaining the conscious and rational use of natural resources to help climate change mitigation and improve human health [146]. It is well known that bees help to preserve the environment by pollinating native plant species. Indeed, circa 75% of the crops consumed worldwide need insect pollination, mainly done by bees [146,147].

Furthermore, pollination allows for sustainable agriculture, promotes social and environmental actions, helps the conservation of natural environments and their local populations, and boosts the creation of new public policies for social development and income generation [148]. Beekeeping surpasses the rural setting by providing natural products with unique characteristics, with high- commercial, -industrial, -nutritional, and -pharmacological interest, going towards a green & social economy. Besides, bee products, such as honey and propolis, provide alternative (additional) income generation while improving the dietary health of the consumers [149]. Compared to other agricultural activities, beekeeping aids in protecting biomes due to the absence of deforestation, being performed as an integrated cultivation approach [150]. In addition, the production of bees is relatively simple, and does not require highly specialized labor, a large land area, or even a high amount of other natural resources and raw materials, namely water, fertilizers, pesticides, or energy.

The 2030 Agenda for Sustainable Development represents the action plan developed by the United Nations based on 17 Sustainable Development Goals (SDGs) [151]. As shown in Fig. 3, all 17 SDGs directly or indirectly correlate with the apiculture market chain. However, an international collaboration network envisioning integrative communication is still necessary since bee-products demand a holistic view enclosing the environment preservation, which is currently threatened due to the high rate of deforestation and unrestrained use of natural

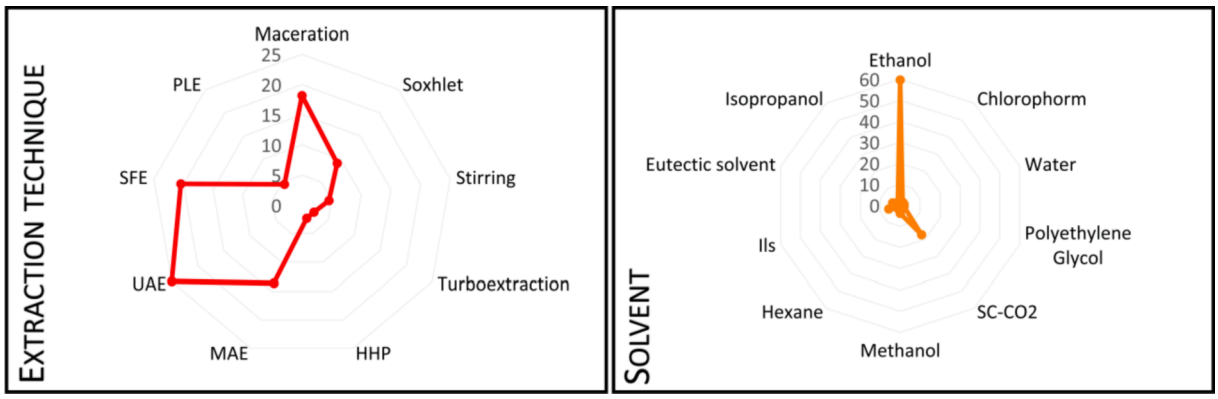

Fig. 4. Distribution of the works dealing with different extraction techniques and solvents.

resources. Thus, sustainable methods for recovering propolis compounds based on the Green Chemistry principles could integrate (even more) this scenario, favoring a low environmental impact compared to conventional approaches.

7. Concluding remarks and future perspectives

In this work, we gathered a collection of works from a critical perspective regarding the extraction processes developed so far to obtain propolis bioactive compounds, especially phenolics, and many strategies were available. Fig. 4 gathers the main extraction variables used in the processes evaluated here.

The most used extraction techniques employed in the works here analyzed applied to extract bioactive compounds from propolis were UAE (25%) and SFE (20.4%), consistent with that used industrially by several leading brands in the market. Usually, to produce propolis extracts without wax, the biomass is previously submitted to SFE extraction (SC-CO_2), followed by UAE. Furthermore, ethanol is the most used solvent for extraction (~60%), even in SFE as the co-solvent.

Most published works quantify compounds extracted by spectrophotometric techniques, providing low-resolution quantitative information. Although these techniques are recognized by the scientific community and provide a rapid response, it is not recommended that these approaches standardize extracts, mainly considering the high chemical complexity of the propolis. Therefore, a comprehensive analysis method, preferably performed by chromatography coupled with mass spectrometry (or another detector that provides minimal identification of the extracted compounds), is highly recommended, mainly because it will help control the commercial samples' quality. An interesting strategy would be to couple the extraction steps with online monitoring, which would provide a faster and more accurate response for the quality control of natural extracts compared to those performed offline (i.e. extraction step is separated from the analysis of the extract). Besides, online monitoring (or even inline or at-line) would enable the fractionation of molecules (or set of molecules) of interest, which has not yet been done for propolis extracts. All extraction processes reported in this work were performed offline, which impairs effective standardization of the extract and increases the environmental impact of the process.

Green analytical methods and coupled methods of extraction and analysis are scarce nowadays, especially for complex matrices like propolis. In this context, this work intends to open the door to discussing the need to create integrated platforms to mitigate the environmental impact inherent to the extraction process and the resolution and accuracy of the analysis. Unfortunately, the real environmental impact of the developed processes reported here was not evaluated, even though some

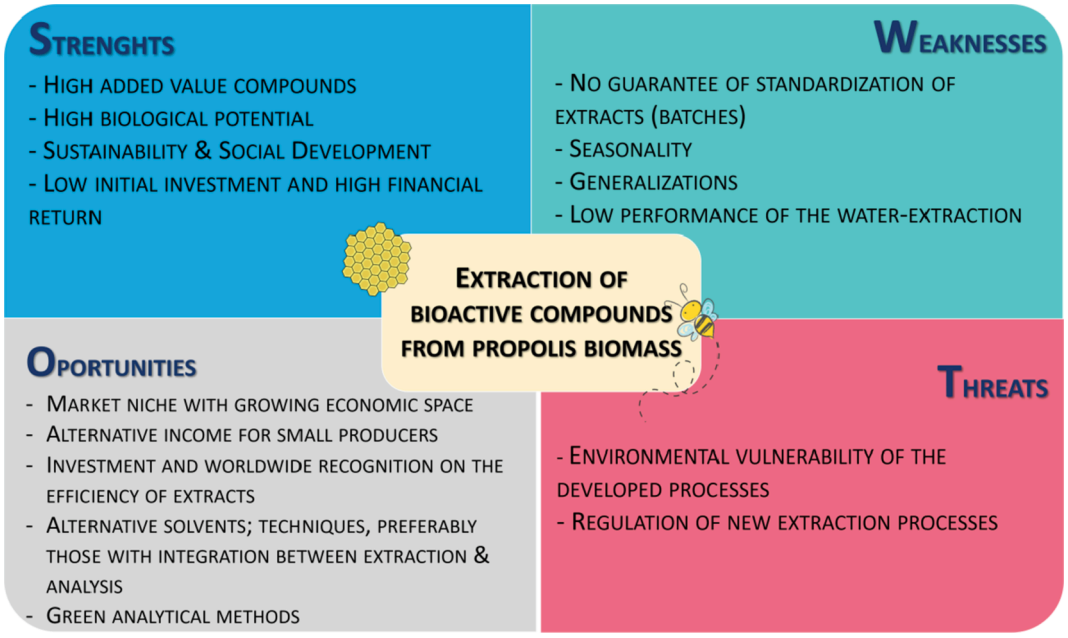

Fig. 5. SWOT analysis towards valorization of propolis.

authors have presented some preliminary studies for using alternative solvents. So far, we cannot say there is a sustainable process for obtaining propolis compounds.

ILs and eutectic solvents were explored for extracting a large plethora of phenolic compounds, like those obtained in propolis. However, despite the countless combinations of possible IL and eutectic solvents, the works reported so far have only explored the imidazolium family of ILS and cholinium chloride-based eutectic solvents, which is incipient considering the fundamental necessity for obtaining alternative strategies for replacing ethanol or improving the efficiency of the water as solvent. Additionally, and up to now, the combination of alternative solvents and high-pressure techniques, such as PLE and SFE, was neglected, although it may be a promising strategy to consider. Thus, despite the several works reporting downstream process platforms for the recovery of bioactive compounds from propolis, much more needs to be addressed to reach a successful proposal for propolis valorization. In this context, we have prepared a SWOT (Fig. 5) analysis to initiate the discussion on what should be done. We encourage young scientists to continue developing alternative processes based on sustainability principles and fast and green analytical analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2022.121640.

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