

Review

Applicability of Honey on Silkworms (*Bombyx mori*) and Quality Improvement of Its Biomaterials

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Abstract: Since ancient times, honey has been considered one of the most illustrious and esteemed natural products. Honey plays two key roles; specifically, it is an appreciated nutritional product, and also exhibits a wide range of beneficial properties for human health as a therapeutic agent. Furthermore, it has been shown that honey has valuable effects on the biological and physiological features of mulberry silkworms (*Bombyx mori*). *Bombyx mori* exhibits importance not only for the economy, but it also serves as an important biotechnological bioreactor for the production of recombinant proteins that have a great impact in the medical field and beyond. It also represents an important model organism for life sciences. In view of the fact that silk fibroin serves as a natural biopolymer that displays high biocompatibility with human organisms and due to honey's various and remarkable properties for human health, the two elements are currently used together in order to develop ideal biomaterials for a wide range of purposes. In this review, by discussing the applicability of honey on *Bombyx mori* and beyond, the importance of honey for life sciences and related fields is spotlighted.



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1. Introduction

Honey represents one of the most valued natural products that exhibit a great impact on human nutrition and health. It has been used since ancient times in various forms to sweeten and flavor different types of foods [1]. Furthermore, honey has been used for therapeutic purposes in order to treat or to prevent several clinical conditions, such as dermatitis, diabetes or cancer [2]. Honey possesses a wide range of curative properties due to the presence of more than 200 bioactive compounds. By using flower nectar, honeybees perform considerable work in order to obtain this appreciable natural product [3]. The nectar source dictates the physical properties of honey, such as flavor, odor and color. Furthermore, the geographical origins also exhibit an impact on honey's features. It has also been shown that the floral source and the geographical origin regulate the most important therapeutic effect of honey, specifically the antimicrobial activity [1]. Moreover, honey represents an important alternative agent against antibiotic resistant bacteria. Hydrogen peroxide (H₂O₂) is the main compound that is responsible for the antibacterial activity. However, several types of honey have this prominent feature, even if the catalase neutralizes their hydrogen peroxide, suggesting that there are more active compounds that generate antibacterial activity [2,4]. In this direction, Dezmirean et al. highlighted the key role played by heather honey against several microorganisms that represent a threat for human health. Besides the content of hydrogen peroxide, heather honey exhibits antibacterial activity due to its low acidity, the presence of polyphenolic compounds and due to its high osmotic concentration [5].

As insects that are native to China, mulberry feeding silkworms (*Bombyx mori*) represent key players for the global economy. *Bombyx mori* is of great importance due to silk production. The silk gland of *Bombyx mori* exhibits three different regions, namely

the anterior silk gland (ASG), the middle silk gland (MSG) and the posterior silk gland (PSG) [6]. Silk is an organic polymer with a wide range of extraordinary properties [7], such as elasticity, high strength, and toughness, but also displays an extraordinary level of biocompatibility with human organisms. Regarding the composition of silk, it contains two main proteins, fibroin and sericin, as well as an alcohol-soluble organic matter, in small amounts. The sericin is synthesized in the MSG, and the fibroin in the PSG [6]. Morphologically, silk involves two fibroin threads that are held together by sericin [8,9]. Owing a wide range of extraordinary features, silk fibroin is currently used to develop various biomaterials with a great impact for the medical field [10]. In this direction, silkworms exhibit a great advantage; specifically, they have a short-term replication cycle (with an incubation period of 14 days) which includes several phases of growth, namely, egg, larvae, pupa, moth [11]. Presently, extraordinary advancements in the medical field are being made and by considering this situation, there is a great need for affordable sustainable biomaterials. The classic synthetic materials that are used in the medical and pharmaceutical field do not display the same remarkable physical and biologic properties as silk. Currently, silk exhibits numerous applications in these fields; for instance, it has been used for decades as surgical mesh and suture thread [12]. In order to overcome the limitations of polymeric synthetic biomaterials, there have been described several silk-based biomaterials (Table 1).

Table 1. The advantage of using silk-based biomaterial compared to synthetic polymers.

Synthetic Polymer	Homologous Silk Based Biomaterial	Advantages of Silk Based Biomaterials	Reference
Metal-based microneedle	Silk microneedles	Controlled drug release	[13]
Gauze	Silk fibroin nanoparticles hydrocolloid dressings	Accelerated wound healing	[14]
Polypropylene	Fibroin	Increased cell adhesion and viability	[15]
Su8	Photo-cross-linkable silk fibroin	Higher-level affinity towards neurons	[16]

Silk production is not the only reason for which *Bombyx mori* receives great attention from the scientific community. *Bombyx mori* plays a pivotal role as a model organism due to its remarkable characteristics [11,17,18]. There are numerous studies that highlight the advantages of using silkworms as a model organism in life science. For instance, Zhang et al., used *Bombyx mori* to analyze the effect of several therapeutic drugs for hyperuricemia and gout treatment [19]. Moreover, silkworms are currently used for environmental monitoring due to *Bombyx mori*'s high sensitivity to pollution [17].

It is a well-known fact that *Bombyx mori*'s biological parameters are determined by the nutritional value of the mulberry leaves. In this direction, it has been shown that the use of honey increases the nutritional value of mulberry leaves, and therefore, honey exhibits a great advantage for the economic features of *Bombyx mori* [20]. Furthermore, honey plays a key role in the biomaterials field. It is currently used as an additive in silk-fibroin-based biomaterials due to its hygroscopic and antibacterial properties. There have been described a wide range of biomaterials that consist of silk fibroin and honey [21]. In this context, this review, by highlighting the impact of honey on *Bombyx mori* and its biomaterials, may contribute to revealing new directions for the applicability of honey in different fields. Thus, this could represent a starting point for future research. Even if the subject of this paper has not yet been massively approached, it is innovative and exerts extraordinary importance for the field of life science.

2. The Impact of Honey on *Bombyx mori* Biological and Technological Traits

Mulberry leaves are the only natural food source in terms of *Bombyx mori* nourishment. Mulberry leaves represent both the source of water and the source of nutrients necessary

for growth and development stages [22]. The availability of the silkworms' nourishment source is limited; specifically, it is available only in the spring–summer season. Due to the great potential of *Bombyx mori* in sericulture, agriculture or in the pharmaceutical industry, the restricted availability of mulberry leaves represents a great impediment. In this regard, there have been described several artificial diets for *Bombyx mori* in order to facilitate and to overcome the impediment of season-restricted rearing. The artificial diets are not climate-change impacted and are also pathogen-free. These two features have a significant impact on the use of silkworms in life science fields [23,24].

Honey, as a natural sweetening agent, is mainly composed of water and several types of sugars, such as glucose, fructose and sucrose [25]. The composition of honey also involves a small proportion of vitamins, enzymes, amino acids, minerals or phenolic compounds [26,27]. Regarding the enzymes that are found in honey, there are three key players, namely invertase, diastase and glucose oxidase. Of the three enzymes, the most outstanding is the invertase or the α -glucosidase. Being an exo-carbohydrase, by converting sucrose, it generates two monosaccharides, specifically glucose and fructose [28,29].

Recognizing honey's prominent effects based on its compounds, particularly due to the enzymes' presence, Bhatti et al. carried out a study in order to analyze the impact of rearing silkworms with honey-dipped mulberry leaves. The authors closely observed the effects of a honey-enriched diet on larval growth. They also hypothesized that honey could have an impact on the silk cocoon yield. There were used two groups of *Bombyx mori* larvae: one experimental group and one control group. The mulberry leaves received by the experimental group were dipped in advance in an aqueous solution that contained 2% honey. The treatment was applied for 4 days. Specifically, the larvae of silkworms received honey-dipped mulberry leaves on days 1, 3, 5 and 7, and on days 2, 4, 6 and 8, they received just mulberry leaves [30].

Surprisingly, in the first instance, after day 1, the silkworms included in the experimental group did not consume more mulberry leaves than the control group. Nevertheless, after days 3, 5 and 7, differences could be observed between the two groups. Specifically, the experimental group left fewer uneaten leaves than the control group, suggesting that *Bombyx mori* has a predisposition for eating honey-enhanced leaves [30].

Taking into consideration the complex composition of honey and the beneficial effects that its compounds manifest on a large spectrum of disorders, including digestive diseases, one can assume its important role in *Bombyx mori* nutritional aspects [31,32]. In this direction, the results of the study showed that by adjoining honey with the mulberry leaves, the digestive capacity of the experimental group was better compared with the digestive function of the control group. Furthermore, higher weight gain was observed in the experimental group, particularly of 348.23%, than in the other group that exhibited a weight gain of 204.54%. When it came to cocoon parameters, by dipping the mulberry leaves in an aqueous honey solution, it was observed that *Bombyx mori* formed the cocoon earlier, but also it was noticed that the cocoon shape was more uniform and the weight was higher in the experimental group [30].

Nevertheless, a study was performed that investigated the impact of a honey-enriched traditional diet on the *Bombyx mori* digestive metabolism, both on the digestive substrates and digestive enzymes. It was demonstrated that the *Bombyx mori* digestive metabolism is based on the Hutchinson's principle, namely that during larval development, digestive substrates, such as proteins or cellulose, are accumulated in the gut wall cells, while the activity of the digestive enzymes are reduced. This study revealed that by supplementing with honey the traditional food of *Bombyx mori*, there was a significant growth when it came to substrate accumulation in the gut wall cells and a noticeable decline in the level of digestive enzymes. Their data showed that by adding honey in the silkworms' diet during the larval stages, the amount of total proteins found in the midgut recorded a significant increase, specifically from 1.04 mg/g it to 10.25 mg/g. In addition, by observing the protease activity, they noticed that it was much lower due to the addition of honey, namely it was 0.02 μm proteins/h from 0.28 μm proteins/h. In regard to the gut wall

tissue, there was also an observed increase when it came to the total protein content, from 11.09 mg/g to 15.56 mg/g. Once again, the advantages of adding honey in *Bombyx mori*'s diet were underlined [33].

Along the same line, another study investigated the minimum effective concentration of honey as an exogenous nutrient that is needed for *Bombyx mori* to acquire optimal larval growth and a substantial metabolic rate. The authors observed that the minimum effective concentration is tissue specific. They demonstrated that the concentration of honey is a key parameter, more specifically, while 2% honey is the optimal concentration for ideal larvae growth, 1% honey is required to acquire an optimal metabolism rate [34].

Keeping in view the optimal concentration of 2% honey for ideal larval growth, [34], another study was performed in order to investigate the impact of a honey-enriched diet on several parameters of *Bombyx mori*. They examined the honey's effect on the feeding patterns and proteins profiles, but on the larval and silk gland growth as well. By comparing the silk gland weight (g) of the group that received the traditional mulberry diet and the silk gland weight of the group that was fed the honey-enriched mulberry leaves, their data showed that by adding honey into the silkworms' diet, a great improvement was observed (Table 2) [35].

Table 2. The effect of honey-enriched diet on silk gland weight (g) [35].

5th Instar Day	Control Group	Experiment Group
	Silk Gland Weight (g)	
1	0.018	0.018
3	0.092	0.105
5	0.182	0.280
7	0.350	0.430

In regard to the three distinct regions of the silk gland, the authors compared the protein profiles and concluded that honey exhibits a remarkable influence. The most outstanding impact of the honey-supplemented diet on the protein profiles was observed in the PSG. There was a great difference between the control and the experimental group. Specifically, the mean of the total protein profiles (mg/g) of the control group on day 7 was 50.75 and the mean of the experimental group was 64.27. Their results demonstrate that by including honey in the silkworms' diet, productivity and, more importantly, the quality of the silk are enhanced. On the other hand, the author investigated the honey's impact on various economic parameters, such as shell protein, floss weight, floss protein, raw silk weight and so on. As expected, the experimental group revealed increased values, compared with the control group, for each parameter, emphasizing once again the advantage of using this sweetening agent in the silkworms' diet [35].

Tamilselvi et al. carried out an experiment that aimed to analyze the impact of honey on various economic parameters of *Bombyx mori*. By supplementing the mulberry leaves with different types of honey, they analyzed the changes that occurred among the economic characteristics of *Bombyx mori* (Table 3) [20].

Their results confirmed that honey is a reliable and promising additional nutrient for *Bombyx mori*. Thus, honey exhibits a powerful influence on various economic traits of silkworms, such as growth, silk production and metabolism. When it comes to comparing the different types of honey utilized, by adding *T. iridipennis* honey, the best results were observed [20].

On the same line, another experiment, aiming to analyze the applicability and the impact of honey on *Bombyx mori*'s genetic and economic traits, revealed that honey also exhibits a protective effect for silkworms against several diseases [36]. Furthermore, it was reported that honey has a positive effect on the rate of deposited eggs. In this direction, the group that received honey as a nutritional supplement exhibited a 36.5% higher rate compared to the control group [37].

Table 3. The impact of honey produced by different honey bees on the economic parameters of *Bombyx mori* [20].

Honey Sources	Larval Weight (g)	Cocoon Weight (g)	Filament Length (m)	Shell Weight (g)
<i>Apis cerana</i>	2.98	1.34	1214.35	0.284
<i>Apis florea</i>	3.07	1.42	1235.13	0.296
<i>Apis mellifera</i>	3.22	1.45	1273.4	0.308
<i>Apis dorsata</i>	3.32	1.47	1278.38	0.33
<i>Trigona iridipennis</i>	3.44	1.59	1312.15	0.372
Control	2.89	1.34	1198.75	0.257

Additionally, another study pointed out the beneficial effects of a honey-enriched diet on silkworms' biological and reproductive characteristics. The researchers aimed to investigate the effect of this natural additive on the production yield of silk and eggs. They analyzed the impact of carob honey, citrus honey and also palm pollen. The three elements were analyzed alone but also they were blended in different combinations. Their results showed that the mixture between citrus honey and palm pollen exhibits the most significant positive impact on the silkworms' studied parameters. In this study each experiment was replicated three times and three different concentrations of each type of honey were analyzed, specifically, 1 mL, 3 mL and 5 mL. By comparing the impact of the two types of honey analyzed, in regard to the number of eggs, the citrus honey (5mL) exhibited the best results (Table 4) [38].

Table 4. The impact of different concentrations of carob honey and citrus honey on the number of eggs of *Bombyx mori* [38].

Type of Honey (mL)	Number of Eggs
Carob honey 1	122.67
Carob honey 3	178.33
Carob honey 5	263
Citrus honey 1	286
Citrus honey 3	365
Citrus honey 5	429.67
Control	417.33

In complete agreement, in regard to the number of deposited eggs, another experiment demonstrated the outstanding benefits of using honey-enriched food on *Bombyx mori*'s yield. In this case, three types of honey were studied, both alone and in different mixtures: carob honey, seder honey and black cumin honey (Table 5) [39].

Table 5. The impact of three types of honey on the number of deposited eggs of *Bombyx mori* [39].

Type of Honey	Number of Eggs
Carob honey	558.6
Seder honey	563.8
Black cumin honey	494.6
Black cumin honey/Seder honey	459
Control	393

Additionally, another research study demonstrated that the economic traits of *Bombyx mori* were enhanced when honey was included as an additive to the mulberry leaves in the silkworms' nutrition. They examined the effect of different camphor honey concentrations on several biological aspects, such as larval weight and silk gland weight, but also technological aspects were studied, such as cocoon weight, cocoon shell weight, silk content ratio and filament length [40].

Keeping in mind the great advances that have been made by using next-generation sequencing techniques, there have been performed numerous studies regarding the gut microbiome of a wide range of organisms. As silkworms represent important experimental model organisms in the life science fields, Chen et al. exhaustively characterized their gut microbiota. Surprisingly, their results showed that even if, for reproduction and survival, the silkworms are dependent on humans, their gut microbiota comprise numerous bacteria. In this direction, it has been suggested that non-pathogenic microorganisms have a positive impact on the host's growth [41]. Recently it was demonstrated that gut bacteria increase the silkworms' resistance against organophosphate insecticides [42]. In this direction, the results of the study showed that by adding honey to the mice's diet, there was observed a great impact on the amount of lactobacilli and bifido bacteria [43]. Knowing this, adding honey to the silkworms' diet may have a great impact on the number of gut members and, therefore, on the silkworms' nutrition and resistance against insecticides. By improving the silkworms' growth and resistance to certain insecticides, the economic traits might be affected in a positive way.

3. The Impact of Honey on Silk Fibroin-Based Biomaterials

3.1. Honey as a Medicine

3.1.1. Antibacterial Activity

Of the broad-spectrum of extraordinary therapeutic characteristics of honey, the most outstanding beneficial property, but also the most investigated one, is the antibacterial activity. Even though the revolutionary discovery of antibiotics decreased interest in honey in terms of medical use [44,45], nowadays, due to the high resistance of countless bacterial strains to antibiotics, this remarkable natural product has gained much more attention in the scientific community [46].

In the literature, there are mentioned several factors that are relevant to honey's strong bactericidal activity [47]. An important characteristic of honey is that it possesses a low water content [48]. This aspect exhibits great importance due to the fact that in order to grow, microorganisms require water. However, there are several strains of bacteria that are able to grow despite the reduced quantity of water. For instance, *Staphylococcus aureus* (*S. aureus*) possesses this capacity, but it has been also shown that by using honey, the bacteria's growth is inhibited [49]. In addition, Mama et al. successfully demonstrated the bacteriostatic and bactericidal effect of honey on *S. aureus* methicillin-resistant strains. This finding suggests that not only the low water content is responsible for honey's inhibitory activity [46].

Being the majority constituents in honey, the high amounts of sugars have a key role in fulfilling the antibacterial activity by dehydrating bacterial cells through the process of osmosis. However, by using honey in order to combat wound infections, the antibacterial activity is reduced due to the fact that when it comes to the human body, there are body fluids that dilute the honey [50,51].

There are small quantities of organic acids in honey's composition, and these elements are important players for honey's acidic pH [52]. Having a pH between 3.2 and 4.5 [53], honey is not an appropriate medium for bacterial growth since most strains need, for optimal growth, a pH between 6.5 and 7.5 [50]. The main organic acid that is responsible for honey's acidity is gluconic acid. By analyzing different types of honey, Pauliuc et al. observed that the highest concentration of gluconic acid is found in raspberry honey. The concentration of gluconic acid, thus the pH of the honey, is influenced by its botanical source [54].

One of the major elements that exhibits inhibitory activity against bacterial growth and proliferation is hydrogen peroxide (H_2O_2). In order for H_2O_2 to be produced, the presence of glucose oxidase (GOx) is required [55–58], but in view of honey's low pH range, the enzyme is inactive until the honey is diluted. There are several scenarios that could negatively influence the level of H_2O_2 in honey. For instance, by exposing to heat, the GOx could be inactivated, but also, a catalase enzyme present in the pollen could deteriorate the

H₂O₂. Additionally, as it was expected, the level of GOx influences the H₂O₂ [59]. Another study also highlighted the strong connection between the presence of H₂O₂ and the strong antibacterial activity of honey, evidencing at the same time that there are some exceptions that exhibit strong bactericidal activity but have low levels of H₂O₂ [60].

It was shown that honeydew honey possesses a very high level of H₂O₂ in addition to a great amount of polyphenols [61,62]. Bucekova et al. illustrated that the polyphenols that are found in honey interact with the H₂O₂. They showed not only that these interactions lead to a high antibacterial activity, but that they are the key elements responsible for the bacteriostatic and bactericidal properties of honeydew honey [63]. There are several studies that demonstrate that the level of phenolic compounds found in honey is influenced by the botanical and the geographical origin [64–67]. Lachman et al. determined the phenolic contents of different types of honey, analyzing their variation according to the date of harvest (May to August). They revealed that in honey, the highest content of phenolic compounds is found in June [68]. The polyphenols are divided into two classes of compounds, namely, flavonoids and phenolic acids. The determination of the phenolic composition represents an important tool for honey authentication and classification [69].

Bees possess several antimicrobial peptides that are involved in the innate immune response. The most outstanding antimicrobial peptide is defensin-1 (Def-1) [70–72]. In order to analyze the efficacy of this peptide against several bacterial species, Sojka et al. obtained recombinant Def-1. They successfully demonstrated that Def-1 inhibits the most common wound pathogens, namely *S. aureus* and *Pseudomonas aeruginosa* (*P. aeruginosa*). On the other hand, Def-1 did not exhibit antibacterial activity against *Enterococcus faecalis* (*E. faecalis*) [73]. Furthermore, it has been shown that Def-1 has an important role in the wound healing process. Specifically, it stimulates the production of matrix metalloproteinase-9 [74], that is, endopeptidase, more specifically, a type IV collagenase, involved in tissue remodeling [75,76].

However, even if H₂O₂ is a major player involved in honey's bactericidal activity, upon dilution, Manuka honey does not display a detectable concentration of this element, but exhibits great antibacterial activity [59]. Nevertheless, Manuka honey contains up to 800 mg/kg of methylglyoxal (MGO) [50]. There are numerous studies that demonstrate that the main element responsible for its antiseptic features is MGO [77–82]. The antibacterial effectiveness of Manuka honey is measured through a ranking system that quantifies the level of MGO present in the natural substance. The ranking system is called the unique Manuka factor (UMF) system [83].

3.1.2. Antioxidant and Anti-Inflammatory Effect

Besides the antibacterial activity, honey possesses another great characteristic when it comes to its biomedical applications, namely, it exhibits antioxidative effects [84]. The antioxidative effect has an important role by counteracting the action of oxidant compounds [85]. Because of honey's complex composition, there are several compounds that are responsible for its antioxidative activity, such as phenolic compounds [86,87]. It has been demonstrated that the presence of these types of compounds is a key player for the antioxidant activity [69]. However, in addition to the main constituents, there are various elements, such as sugars, proteins and carotenes, that are involved in honey's antioxidative capacity [85]. This extraordinary property is influenced by honey's botanical origin [88]. In this direction, it has been shown that heather honey exhibits great antioxidant activity due to its complex composition [89].

Phenolic compounds are not just involved in the antioxidant capacity of honey, but they are involved in its anti-inflammatory effect [90,91]. Even if inflammation represents an important part of the immune response when it comes to cellular injury, there are several scenarios in which it can be extreme and cause severe damages [88]. Along this line, it has been shown that honey is a promising immunomodulatory agent. It exhibits positive results when it comes to suppressing the production of NF- κ B, MAPK (transcription factors), IL-6

and IL-1 β (cytokines). On the other hand, regarding the inflammatory mediators, it has been evidenced that honey stimulates their production [2,92].

3.2. Silk Fibroin as a Biomaterial

As an emerging field, tissue regeneration represents one of the top priorities for the scientific community. Even though there have been advances in this direction, there are numerous drawbacks, such as the biocompatibility or biodegradability of the materials used for treating or replacing damaged tissues [93].

Silk represents one of the most promising natural biopolymers that exhibit a great potential for biomedical applications and beyond. The main source for this biopolymer is *Bombyx mori*, which produces silk that displays numerous and unique features. The silk produced by *Bombyx mori* is composed of two fibers of fibroin and sericin that holds them together by acting like a glue. The fibroin fibers are responsible for the extraordinary features of *Bombyx mori* silk [94]. As for sericin, it is removed by using a degumming process due the fact that it has been shown that the presence of sericin deteriorates the outstanding mechanical strength and the superior level of biocompatibility exhibited by fibroin [93]. In addition, silk fibroin possesses extraordinary features, such as biodegradability, stiffness and thermal stability [10,95]. As for the structure of the fibroin fibers, it includes a complex consisting of a light chain (L-chain) and a heavy chain (H-chain) linked through disulfide bonds [96] and a glycoprotein P25 that is linked to the complex [93]. In the light of this, silk fibroin has received great attention from the scientific community [97]. Its features make silk fibroin the most important candidate for developing hydrogels, sponges, nanofibers, biofilms or nanoparticles in order to make important advances in biomedicine and regenerative medicine [89]. The most important directions of using silk fibroin are wound healing, drug delivery and bone tissue engineering [93,98]. There have been performed numerous studies that successfully used silk fibroin-based biomaterials for biomedical applications (Table 6).

Table 6. Silk fibroin-based biomaterials and their applications.

Biomaterial	Applicability	References
Titanium/Silk fibroin scaffolds	Bone repair	[99]
Silk fibroin nanofibers	Drug delivery system	[100]
Silk fibroin hydrogel	Cancer therapy	[101]
Silk fibroin/xanthan hydrogels	Connective tissue regeneration	[102]
Silk fibroin sheet	Vitrification of oocytes	[103]
Silk fibroin microsphere-nanofiber	Vascular tissue engineering	[104]
Silk fibroin and chitosan scaffolds	Tracheal transplantation	[105]
Silk scaffolds	Craniofacial bone repair	[106]
Silk fibroin/cellulose acetate/gold-silver nanoparticles	Cancer therapy	[107]
Silk fibroin spheres	Controlled drug delivery	[108]
Silk fibroin nanoparticles	Target therapy	[109]
Silk fibroin hydrogel	Drug delivery system for bone defects	[110]
Silk fibroin matrices	Drug delivery and controlled release	[111]
Silk hydrogels	Drug delivery for sustained release	[112]
Silk fibroin scaffolds	Femur defects	[113]
Silk fibroin scaffolds	Mandibular repair	[114]
Silk biofilms	Blood contacting devices	[115]

3.3. Honey-Enhanced Silk Fibroin-Based Biomaterials

As for using biomaterials in the tissue engineering field, there is an important drawback when it comes to its high susceptibility to infections. In this regard, there are several natural compounds that exhibit antimicrobial activity that can be used together with the biomaterials in order to promote healing processes [45]. There are numerous studies that have described honey as a promising, effective agent in the process of tissue healing. It

is well known that honey reduces inflammation, stimulates collagen production, and promotes the growth of fibroblasts [2,116–119].

When it comes to bone defects, the most appropriate method for improving or treating them is represented by bone tissue engineering. In this direction, the tissue engineered scaffolds that exhibit the most promising results. The most important biological requirements for an engineered scaffold are biocompatibility and degradability [120]. Silk fibroin fibers meet both requirements [121].

Furthermore, there was performed a study that aimed to compare and analyze the effects of 5% Manuka honey on two silk fibroin-based biomaterials, namely cryogel and electrospun scaffolds. They pursued to determine the impact of various UMF of Manuka honey on the two biomaterials. In regard to the scaffolds' features, the authors analyzed the pore structure and surface topography with a scanning electron microscope; the porosity was determined by using microcomputed tomography. Moreover, the bacterial clearance and the bacterial adhesion were tested. In addition, the release of glucose and the cytotoxicity were investigated. Their results showed that, regardless of the UMF value of the honey that was incorporated in the scaffolds, there was not an observed significant difference on any of the studied parameters [122].

In this direction, Hixon et al. performed a study and developed scaffolds: specifically, silk fibroin cryogels that contained Manuka honey. They analyzed the effects of Manuka honey on the cryogel scaffold features, namely on the scaffold's porosity, biocompatibility and stability. The maintenance of scaffold's structure and properties has great importance for its applicability in bone engineering. On the other hand, they investigated whether silk fibroin cryogels could represent an ideal delivery and sustained release vehicle for honey. The authors also evaluated if the incorporation of Manuka honey in the cryogels maintained its antimicrobial activity. Their results revealed that by incorporating the Manuka honey, the porosity of the cryogels scaffolds was not affected. The large pores are necessary when it comes to bone repair. Additionally, by adding honey in these biomaterials, the swelling potential and the mechanical properties were optimal. By including 5% Manuka honey in the silk fibroin scaffolds, honey's antibacterial property was functional. Furthermore, the study's outcome revealed that the antibacterial activity was fulfilled by the sustained release [123].

Another study revealed that the incorporation of honey into silk fibroin substrates inhibits cancer development. The authors analyzed the effect of using this type of biomaterial on precancer and normal fibroblasts. By using the honey/fibroin-based substrate, the cell–cell interaction in precancer fibroblasts, but also the cell–matrix interaction, were low. On the other hand, the same interactions in normal fibroblasts were stronger. There the performance of honey on different concentrations was analyzed (1%–6%); by using silk fibroin membranes with 2% honey, the best results were observed. They concluded that the honey-enhanced silk fibroin substrate inhibits the spreading of the precancer cells, illustrating a new direction toward anticancer therapies [124].

Along the same page, Rajput et al., developed honey-blended silk fibroin membranes in order to improve the bio-functionality of silk fibroin biofilms to promote re-epithelization and tissue reconstruction. The results of the study showed that when high concentrations of honey are used together with silk fibroin, there is a great molecular affinity between the two elements. Furthermore, by including honey, the silk fibroin membranes exhibit a higher level of water retention than when silk fibroin is used alone. By analyzing the biocompatibility of honey-enhanced silk fibroin membranes, they observed that honey exhibits several extraordinary advantages for this biomaterial; specifically, honey increases cells proliferation, adherence and its viability [125].

In order to develop ideal antimicrobial wound dressings, Yang et al. used aqueous-electrospinning to obtain Manuka honey-enhanced silk fibroin matrices. Their results showed that by using Manuka honey, the interaction and the affinity between silk fibroin matrices and cells were enhanced. Herein, once again, the extraordinary antibacterial feature of honey is highlighted. The results confirmed that silk fibroin matrices enhanced

with Manuka honey exhibit antibacterial activity against several bacterial strains, such as *Escherichia coli* (*E. coli*) or *P. aeruginosa* [126]. Furthermore, it was reported that by adding honey, the wound-healing process was accelerated. Rajput et al. determined that the optimal concentration of honey to be used when it comes to silk fibroin porous scaffolds is 4%. They also confirmed the abovementioned advantages of using honey-enhanced silk fibroin-based biomaterials. In addition, the authors observed that the inclusion of honey in biomaterials leads to minimum scar formation [127].

Moreover, another research study focused on combining silk fibroin together with honey and with the epidermal growth factor (rhEGF) in order to promote the process of healing of diabetic wounds. The authors demonstrated once more that honey exhibits an extraordinary ability to promote the wound-healing process and great antimicrobial activity against *E. coli* and *Staphylococcus epidermidis* (*S. epidermidis*) [128].

4. Conclusions

As a natural product, honey exhibits a wide range of positive effects for human health and beyond. Honey's most outstanding property is represented by the antibacterial effect against numerous bacterial strains. Additionally, it has been demonstrated that honey exhibits antibacterial effects on antibiotic-resistant strains [46]. As for the applicability of honey on *Bombyx mori*'s economic traits, it has a positive influence. By including honey in the silkworms' diet, the digestive capacity of *Bombyx mori* was enhanced [34]. Furthermore, higher weight gain was observed after adding honey into the silkworms' diet. When it comes to cocoon parameters, by dipping the mulberry leaves in an aqueous honey solution, the cocoon is formed earlier, is also heavier and its shape is more uniform [30]. The positive effects of honey on *Bombyx mori* do not stop here, as it has been revealed that by supplementing the mulberry leaves with honey, the rate of deposited eggs is increased. Moreover, this natural product has a protective effect for silkworms against several diseases [36]. When it comes to future prospects, this review may also lead to new research on the impact of honey on the silkworm gut microbiome, keeping in mind honey's impact on the mouse gut microbiome.

With its extraordinary features, such as the antibacterial, antioxidative and anti-inflammatory effects [62,84], honey is currently used to enhance the silk fibroin-based biomaterials that are used in medical practice. There have been several silk fibroin honey-enhanced biomaterials developed, such as Manuka honey-enhanced silk fibroin matrices, silk fibroin cryogels that contain Manuka honey and honey-blended silk fibroin membranes [123,125]. As for tissue healing, the advantage of adding honey on the biomaterials used in this regard is the fact that re-epithelization and tissue reconstruction are promoted. In addition, it has been shown that honey amplifies the interaction and the affinity between silk fibroin matrices and cells [126].

This review brings forth an overview of the applicability of honey on *Bombyx mori*'s economic traits and on silk fibroin-based biomaterials. Even if this is a limited subject, it is of great importance and brings together two extraordinary natural products that exhibit significant value for the life science fields: honey and silk. Through its remarkable properties, honey opens new doors for the use of silk fibroin-based biomaterials. This review represents a starting point for future research, for instance, to improve the biological traits of silkworms in order to obtain vast amounts of recombinant proteins or to increase the potential of *Bombyx mori* as an experimental model for life science fields. To the best of our knowledge, this is the first paper that brings together the main research studies on this topic.

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