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Editor's Note

The second 'International Congress on Bee Sciences' was organized online and free of charge. We are very happy and proud that various Bee science-related fields attended the congress. During this event, distinguished and respected scientists came together to exchange ideas, develop and implement new researches and joint projects. There were 33 invited speakers from 19 different countries. The scientific committee of the congress consisted of 274 scientists from more than 160 universities. Almost 500 participants participated in the congress. We would like to thank all participants and supporters. Hope to see you at our next congress.

Best wishes from Turkey

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Hydroxymethylfurfural in Honey Bee Diet

Aleš Gregorc, Caio Eduardo Da Costa Domingues, Leticia Salvioni Ansaloni

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Abstract:
Hydroxymethylfurfural (HMF) concentrations in commercially available High-fructose corn syrup (HFCS) from different brands range between 3.1 and 28.7 ppm HMF. HMF is a chemical compound formed from carbohydrates, particularly fructose, under thermal and/or acid-catalyzed degradation conditions. Fructose is only stable to a limited extent in solution and gradually decomposes, forming HMF in a temperature-dependent manner. A temperature of 49°C can result in over 200 ppm HMF formation in 36 days, while exposure of HFCS samples to 69°C for the same duration can lead to HMF values exceeding 30,000 ppm. Honey exposed to 75°C for 24 hours resulted in HMF concentrations ranging from 43.4 to 226 ppm. In our laboratory experiment, we investigated the effects of different concentrations of HMF (100, 500, 1,000, and 1,500 ppm) on the longevity and midgut integrity of worker *Apis mellifera carnica*. We provided bees with standard diets containing these HMF concentrations and examined the impact of HMF on *Nosema ceranae* spore counts in infected honey bees. We observed changes at the cellular level through immunohistochemical analysis of the honey bee midgut. No correlation was found between the concentration of HMF and *N. ceranae* spore counts. Adverse effects of HMF on bees were not observed within the first 15 days of exposure. However, after 15 to 30 days of exposure, HMF caused the death of midgut cells and increased worker honey bee mortality across all treatment groups. Currently, there is no standardized limit for HMF in bee nourishment. Approximately 250 ppm HMF in the honey bee diet is considered toxic. High concentrations of HMF in stored honey could contribute to early bee deaths and the decline of honey bee colonies. Therefore, it is crucial to comprehend the potential adverse effects of elevated HMF doses on honey bees.

Keywords: honey bee, carbohydrates, nutrition, intoxication, toxicology.

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1. Introduction

Honey bees (*Apis mellifera*) collect nectar, pollen, water, and propolis to meet all their needs for survival and reproduction in honey bee colonies. Pollen is the primary source of proteins and contains up to 41% of sugars, which are mainly hard to digest (Carroll et al., 2017). Bees need an additional source of energy, mainly nectar. The foraging bees need a continuous supply of sugar because of their high metabolic rate compared to younger bees in the hive. Carbohydrates are also essential for colony thermoregulation (Wheeler and Robinson, 2014). Honey bees can collect and store nectar in the comb cells, which are transformed into honey by bees containing less than 20% water for long-term storage.

Honey is a highly concentrated mixture of mainly two dissolved sugars, fructose, and glucose, plus at least 22 other composite sugars (White and Doner, 1980) and 70 other compounds, including proteins, vitamins, minerals, organic acids, aromatic compounds, and various derivatives of chlorophyll (Hermersdörfer, 1995). Many more honey components may remain undiscovered. Therefore, identifying potential fraudulent honey by component analyses may be difficult unless specific breakdown products (metabolites) can be identified, such as substances like hydroxymethylfurfural or 5-hydroxymethyl-2-furaldehyde ($C_6H_6O_3$) (HMF). HMF can be selectively produced from keto-hexose, notably D-fructose (Shalumova and Tanski, 2010) and other acidic media containing dissolved monosaccharides (Teixidó et al., 2006). Usually, HMF is present in honey in trace amounts (Basumallick and Rohrer, 2001). The rate of HMF formation in foods depends on environmental temperature, the type of sugar, pH, and the concentration of divalent cations in the medium (Lee and Nagy, 1990; Gökmen et al., 2007). Excessive heating or inappropriate storage conditions can increase HMF levels, which are recognized as a marker of quality deterioration for a wide range of foods containing carbohydrates (Morales, 2008). Inappropriate heat processing of honey affects honey fermentation and reduces honey quality (Tosi et al., 2002). In fresh honey, HMF can occur at concentrations as high as 15 mg HMF/kg honey, but it normally occurs at levels between 0.06 – 0.2 mg HMF/kg (Basumallick and Rohrer, 2001). For the most part, HMF is naturally present in honey and at low concentrations (e.g. ~100 – 500 ppm), it does not reduce honey quality, and thus could be used as an identifier of a honey's origin and quality.

The Codex Alimentarius of the World Health Organization (WHO) and the European Union (EU Directive 110/2001) have defined a maximum HMF quality level in heat-treated honey (40 mg HMF/kg), above which honey quality begins to deteriorate. HMF concentration increases above 20°C. Temperatures inside a hive usually exceed 20°C (~28 – 30°C) and in summer can reach as high as 40°C or more, where the concentration of HMF can reach 10 mg/kg of honey (Ribeiro et al., 2012), a level one-third that of HMF concentration known to be harmless to bees 30 mg/kg, (Jachimowicz and Sherbiny, 1975). Although these summer levels of HMF are considered nontoxic to bees, few studies confirm a safe level of HMF in honey bee colonies (Bailey, 1966). Low concentrations of HMF < 10 - 15 mg/kg in honey does pose little risk to honey bees, but toxic concentrations of HMF seem to induce lethal intestinal tract ulceration (Bailey, 1966). About 150 mg HMF/kg of commercially acid-hydrolyzed inverted

sugar syrup can cause 50% bee mortality within 16 days (Jachimowicz and Sherbiny, 1975). HMF concentration in inverted syrup for feeding bees may not exceed 20 mg/kg as it is in most honey (Kammerer, 1989).

There is no standard limit value of HMF in bee nourishment. Approximately 250 ppm HMF in a honey bee diet is considered toxic (LeBlanc et al., 2009). High concentrations of HMF in honey stores could represent a factor in the early deaths of bees and the extinction of bee colonies (Van der Zee and Pisa, 2010). It is, therefore, essential to understand the potential adverse effects of high HMF doses on honey bees. Thus, this study aimed to determine the toxic effects and mode of action of HMF on caged bees fed in a laboratory assay. We also use an immunohistochemical assay to examine the impact of HMF toxicity on the cellular death of epithelial cells lining the worker midgut.

2. Materials and Methods

2.1. Toxicological test

In plastic cages, worker bees were maintained in incubators at a near-constant 28°C and at ~ 65% relative humidity (RH). Bees were fed with Apifonda sugar candy (Südzucker, Germany) as control feed and water, respectively. To simulate colony habitat, each containment unit contained a 4 cm x 5 cm piece of wax foundation. Bees were divided into five treatment groups. Each group of 20 bees was placed into its own individual containment unit. We provided thoroughly homogenized control feed as bee nourishment, into which was added HMF (5-hydroxymethyl-2-furaldehyde, Sigma Aldrich) at concentrations of 0 mg HMF/kg candy (control feed), 100 mg/kg, 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg. We took the capped brood from three healthy honey bee colonies a day before starting the experiment and placed combs into the incubator at 34.5°C and 65% RH. The next day, newly emerged 0 - 24 h-old bees were randomly placed into the containment units. Each HMF treatment group was replicated five times in parallel. We followed the confined worker bees' daily food consumption and mortality rates.

Mortality rates of bees exposed to the control and HMF concentrations were calculated as the number of dead bees post-treatment divided by the initial bee population (n = 20) multiplied by 100. Data analyses were performed using ANOVA (analysis of variance). Mean bee mortality and survival rates were compared among the treatment groups with one-way ANOVA and mean separation accomplished with Tukey tests.

2.2. Immunohistochemical analyses

For immunohistological tests, 5 groups of 50 bees were established. The first group received no HMF added to the control feed. The second, third, fourth and fifth groups received different concentrations of HMF in candy. Group 2 received 100 mg HMF / kg of candy. Group 3 received 500 mg HMF/kg. Group 4 received 1,000 mg HMF/kg. Group 5 received 1,500 mg HMF/kg. Three bees from each treatment group were randomly sampled at 5-day intervals: on days 5, 10, 15, 20, 25, and 30. Sampled bees were dissected, and their midgut was removed. Midguts were fixed in 10% neutral buffered formalin, dehydrated in ethanol, and embedded in paraffin wax and sliced into 5-µm sections, which were then de-paraffinized and processed

following the instructions provided with the *in situ* cell death detection kit, AP' (ISCDDK) (Roche). TUNEL-positive cells possessed red nuclei, which are the reaction products indicating cell death. TUNEL-negative nuclei of healthy intact cells appeared blue. A control labeling of midgut tissues was accomplished by substituting the deoxynucleotidyl transferase (TdT) enzyme with phosphate-buffered saline (PBS) during the TUNEL reaction.

3. Results

About 90% of caged bees in each treatment group survived the first 15 days. After day 30, less than 30% of bees were left alive in the five treatment groups, which included the control feed. The steepest declines in bee survival occurred between days 15 - 30 post-treatment. Treatment-differences were detected among the treatment groups ($F = 2.968$; $df = 4$; $P = 0.028$). The longevity of bees receiving the highest HMF concentration (1,500 mg HMF/kg) was reduced when compared with other HMF treatment groups and untreated control bees.

Varieties of apoptotic midgut cell deletions were observed in bees exposed for 10 days to different HMF concentrations. The amount of immunohistochemically positive cells was not dependent on HMF concentration between 5 and 30 days. However, in bees exposed to the lowest dose of HMF, cell death was observed in the epithelium of the midgut (sporadic positive cells) but at a level below that of bees fed higher concentrations of HMF. In contrast, the level of cell death in the midgut epithelium of bees exposed to 500 mg HMF remained high throughout the bioassay. The proportion of midgut digestive cell death of the bees treated with 100 and 500 mg HMF was much lower than in the groups exposed to 1,000 or 1,500 mg HMF. In bees exposed to 100 mg HMF, affected midgut epithelial cells were vacuolated with apical cell fragments released into the lumen. Similar vacuolization was observed in bees exposed to 500 mg HMF. Midgut cells with positive red reaction products in the apical epithelium region were also seen in bees exposed for 10 days to 100 or 1,500 mg HMF and also in bees exposed for 15 days to 1,500 mg HMF. Midgut epithelial cells were still intact and attached to the basal membrane even at the highest HMF concentration over the course of the experiment. In control, untreated bees (without added HMF), sporadic midgut cells with signs of apoptosis were found. In live bees exposed to any HMF dose for 25 or 30 days, no morphological alterations or increased levels of positive ISCDDK cells were detected. Bees exposed to the two highest HMF concentrations, 1,000 or 1,500 mg, surviving to 25 – 30 days displayed a few midgut cells with positive reaction, an observation similar to the reaction levels displayed by untreated control bees. Taken together, the highest levels of ISCDDK positive midgut epithelial cells were found in bees exposed for 15 to 20 days to 500, 1,000 or 1,500 mg HMF. After that time, there was a notable decrease in the proportion of midgut cells with specific red reaction products. The lowest HMF dose effect at the first and second sampling dates (5 and 10 days) is characterized by red reaction products in midgut epithelial cells (Gregorc et al., 2020).

4. Discussion

Caged bee investigations, showed no significant increase in bee longevity over using High-fructose corn syrup (HFCS) compared to honey (Barker and Lehner, 1978). When sucrose syrup was hydrolyzed to fructose and glucose (inverted sugars) and fed to bees, the inverted mixture was found to be toxic to bees when mineral acids or organic acids were used to hydrolyze the sucrose. In comparison, invertase-hydrolyzed sucrose syrup was found to be

nontoxic to bees. Hydroxymethylfurfural (HMF) and its hydrolysis products levulinic and formic acids were all approximately equally toxic to bees (Bailey, 1966).

In one case report of HMF in sugar candies purchased in SLO containing HMF were studied. 'MedoPip Standard' containing 914.6 mg HMF/kg; 'Medopip Plus' containing 437.0 mg HMF/kg; 'Apimel' containing 58.3 mg HMF/kg; 'home-made sugar candy' containing < 10.0 mg HMF/kg; 'Stimulans' candy < 10.0 mg HMF/kg. The longest worker survival was found in those fed 'Apimel', 'home-made' and 'Stimulans' candy (27 days), where HMF was the lowest using HPLC analyses. The bees fed 'Medopip Standard' and 'Medopip Plus', the candies with the highest HMF, had a shorter life span (24 and 20 days) respectively (Smodiš Škerl and Gregorc, 2014). It was found that HMF has a dosage-dependent cytotoxic effect on honey bee digestion; both sublethal and subclinical changes to the midgut occur at the cellular level before bees eventually die from high doses (Gregorc et al., 2020).

Sucrose, or starch, hydrolysed with mineral or organic acids is toxic for honey bees (Bailey, 1966). Concentrations of HMF below 30 mg/kg honey is harmless to bees (Jachimowicz and Sherbiny, 1975). It is suggested that the HMF concentration in inverted syrup for feeding bees should not exceed 20 mg/kg, as in most honeys (Kammerer, 1989). In experiments it is demonstrated that on day 7 the adult worker control group and the 2.000 ppm HMF test group both showed a mortality of 0.7 % (SD ± 0.470). The 4.000 ppm HMF test group evinced a mortality of 3 % (± 0.985) and the treatment group receiving 8.000 ppm HMF showed a mortality of 5 % (± 1.26). At d20 the mortality of the untreated control group was 6.7 % (± 1.44), whereas all bees (100 %) feed with 4.000 ppm HMF in sucrose solution were dead. At day 15, bees fed with 8.000 ppm HMF showed a mortality of 100 %; at day 22 bees fed with 2.000 ppm HMF, a mortality was of 67 % (± 2.71) (Krainer et al., 2016). In other experiment 50 % mortality of adult caged bees was recorded when fed 20 days with 150 ppm HMF (Jachimowicz and Sherbiny, 1975).

The tests made with mixtures of HMF, levulinic acid and formic acid do not suggest that these compounds have a synergic effect in sucrose solution; and they seem unlikely to be more toxic in glucose + fructose solution, which itself is harmless (Bailey, 1966). HMF +levulinic acid + formic acid cumulatively, as toxins found in acid-hydrolyzed sucrose, but did not attempt to estimate the toxicity of HMF only. Bees fed on honey 8 years old (absorption of U.V. at 284 *mμ)* became markedly dysenteric compared with those fed on fresh honey or syrup (Bailey, 1966).

It was also found that the toxicity of 30 ppm HMF, fed to caged bee was not found (Jachimowicz and Sherbiny, 1975). The amount of HMF that killed 50% of the bees (LD-50) was near 100 ppm, and 150 ppm HMF in foraging formulation resulted in 50% mortality in 16 days (Jachimowicz and Sherbiny, 1975). Very similar results were found that 50 % caged bee died 19 days after feeding the HFCS-55, containing 150 ppm HMF. The 250 ppm HMF concentration induced higher mortality (LeBlanc et al., 2009). Greater longevity for bees was establish when fed sucrose compared to HFCS (Barker and Lehner, 1978). Tests conducted on adult bees, concentrations of 2.000 mg HMF/kg sucrose syrup induced a higher number of deaths from day 22, in comparison to the control feeding bees (Krainer et al., 2016).

The syrup HFCS A-55, which contained 57 ppm HMF was spiked with a HMF standard to produce 100, 150, 200, and 250 ppm HMF. The syrup consumption during the first 3 days was estimated at levels between 50 to 70 mg of HFCS per bee. Drinking water was supplied *ad*

libitum and after the water supply expired without being replenished, more syrup was consumed and the mortality increased dramatically (LeBlanc et al., 2009). It is evident that the toxicity of HFCS depend also on water supply. In the commercial setting bees would be required to forage pollen. Forager bees would likely consume more syrup before foraging and consequently consume more HMF from stored food reserve before they forage for pollen.

Honey stored under natural conditions within the hive will probably less likely develop an HMF concentration toxic to worker bees in colonies as found in study of Krainer et al. (2016). However, there are potential sublethal HMF intoxication in combination with other stressors, that can reduce resistance against environmental influences, e.g. pesticides, parasites and diseases (Zirbes et al., 2013). Adult bees in colonies usually consume food including HMF *ad libitum* during long period of time; e.g. continual feeding. It means that bees can be exposed to HMF in diet for longer period. High concentrations of HMF in stored honey could represent a factor in the early death of bees and in the extinction of honey bee colonies (Van der Zee and Pisa, 2010).

It is supposed that there is no standard limit value of HMF in bee nourishment. Approximately 250 ppm HMF in the honey bee diet is considered toxic (LeBlanc et al., 2009). No statistical difference was found in colony performance between HFCS with HMF fed colonies (HFCS-42 and HFCS-55 from one manufacturer), comparatively to sucrose solution fed colonies in open bee colony studies. Even more, the HFCS-55 produced the highest seasonal honey production. Finally, significantly higher brood cluster size in the spring was recorded in colonies fed with the sucrose syrup (Severson and Erickson Jr, 1984)

Bees fed acid-hydrolysed carbohydrates developed 'dysentery' before they died, possibly because essential solutes and water were lost from the body into the rectum (Bailey, 1966). Carbohydrates or decomposition products soon developed dysentery and bees die. Part of the gain in weight of the rectum may have been unassimilated carbohydrate, which might be indigestible or be passed through a poisoned mid-gut. The toxins in the midgut may upset the normal excretory or resorption processes, or cause other damage leading to a loss of both water and essential solutes (Bailey, 1966). Acid-inverted sugars may have no obvious effect when fed to a colony already provided with a considerable amount of food; when they were diluted to one-eighth, their toxicity became undetectable (Bailey, 1966). An HMF concentration from 30 mg/kg to 48 mg/kg is supposed to be harmless to honey bee workers, whether or not bees are overwintering (LeBlanc et al., 2009).

Carniolan honey bees were exposed to HMF (100, 500, 1000, and 1500 ppm). Negative effects of HMF on bees were not observed in the first 15 days of exposure; increased death was recorded after feeding on it for 15 to 30 days (Gregorc et al., 2020). The effects of HMF in the first two weeks of feeding are sublethal for bees, demonstrated on a midgut cellular level. It appeared hypertrophic enlargement of the digestive cells in the first 5 days after HMF treatment. Later, ~10 days post-HMF treatment, numerous affected cells were released into the midgut lumen, as evidenced by observable apoptotic cell death in the apical region of midgut. Between the 10th and 15th day of feeding, 1000 mg/kg of HMF and 1500 mg/kg of HMF increased the cell death rate, resulting in the shedding of dead cells from the epithelium into the midgut lumen. There were also observed some variations in pathological cell death when bees were fed with 500 mg/kg of HMF (Gregorc et al., 2020).

When caged bees were exposed to high doses of HMF (e.g. 1500 mg/kg), apoptotic cell death was followed by typical of necrotic deletion. Caged bees were sensitive to changes found in the midgut tissue level at sublethal HMF doses and its potential detrimental effect can result in higher bee mortality which was monitored 14 days after caged bees were exposed to HMF concentrations (Gregorc et al., 2020).

Crystallized Honey Can cause dysentery. Bees discard large crystals and consume only the diluted fluid surrounding them. Crystallization is induced due to a high content of glucose (38%) combined with a relatively low content of fructose (22.1%) (Rybak-Chmielewska et al., 2006). It is indicative that winter food will not crystallize, when fructose predominate over glucose in the composition of sugars, and their quantitative ratio (F/G) should be higher than 1. The most beneficial ratio exceeds 1.26. When there is maltose in the honey, bee enzyme action induce transformed of maltose into two molecules of glucose and subsequently F/G ratio of the winter supply, thus increasing the risk of their crystallization (Konopacka, 2007). Winter supply crystallization had no significant effect in the condition of bee colonies before overwintering, after overwintering, and during spring development. It was also found that in delaying the time of autumn feeding had no influence on the condition of overwintering colonies, nor their spring development (strength of colonies, bees mortality, brood area) in comparison to colonies fed earlier in the autumn (Semkiw and Skubida, 2016).

Honeydew as a raw material contains melezitose - a trisaccharide made from glucose and sucrose, which can be traced back to plant louse activity. Excessive concentrations of melezitose lead to crystallization in the honeycomb (Deifel, 1989). The beekeeping experiences in the field confirm that bees cannot dissolve crystallized saccharides from bee comb. Bees in winter cannot collect water to dissolve crystals and possibilities for mechanical agitation are limited. Therefore the bees starve to death despite having full honeycombs of crystalized honey. Pure sucrose for over wintering stores made by bees from pure sucrose are less likely to crystallize than honey and probably contain less of the decomposition products of sugar, because much of the sucrose remains unhydrolyzed (Simpson et al., 1968). Therefore, sucrose syrup is more suitable than honey as carbohydrate for overwintering honey bee colonies.

5. Conclusion

HMF has a dosage-dependent cytotoxic effect on honey bee digestion; both sublethal and subclinical changes to the midgut occur at the cellular level before bees eventually die from high doses. Adverse effects of HMF on bees were not observed in the first 15 days of exposure; increased death was recorded after feeding on it for 15 to 30 days (Gregorc et al., 2020). The effects of HMF in the first two weeks of feeding are sublethal for bees, demonstrated on a midgut cellular level. High HMF concentrations, 1000 mg/kg of HMF and 1500 mg/kg of HMF, increased the cell death rate, resulting in the shedding of dead cells from the epithelium into the midgut lumen. When bees are fed with crystallized Honey, which can cause dysentery, bees discard large crystals and consume only the diluted fluid surrounding them. Winter supply crystallization had no significant effect on the condition of bee colonies before overwintering, after overwin-tering, and during spring development. It was also found that delaying the time of autumn feeding had no influence on the condition of overwintering colonies or their spring development.

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Determination of Hydroxymethylfurfural and Proline Amounts of Ardahan Raw Flower Honey

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Abstract:
The present study focused on the moisture content hydroxymethylfurfural (HMF) values and proline content of raw flower honey from Ardahan. According to the study, the raw floral honey from Ardahan demonstrated specific features that contribute to its quality and potential uses in the food and pharmaceutical industries. In 2020, 20 flower raw flower honeys collected and sold during the honey harvest season were purchased from local vendors. HMF and prolin were analyzed in honey. Honey samples were analyzed for the detection of HMF and proline. In addition, it has been evaluated whether the results are in accordance with the Turkish Food Codex Honey Communiqué. The TSE 3036:2002 method was used to for the HMF analysis of the honey samples. The IHC technique was used to analyze the proline content of honey samples. The average HMF value of 7.1±1.4 mg/kg demonstrated freshness and a minimal level of honey deterioration. The honey's legitimacy and purity were suggested by its proline concentration, which was 424.6±36 mg/kg. The average HMF and proline values detected in honey samples were found to be in compliance with the Turkish Food Codex Honey Communiqué. These results provide valuable information about the raw flower honey from Ardahan, highlighting its quality, authenticity and potential applications. Further research can be done on these findings to explore the unique properties and potential benefits of honey from this region, which adds to the value of honey and its use in a variety of industries.

Keywords: Raw honey, HMF, proline, Ardahan

1. Introduction

Honey is a naturally sweet substance that honey bees produce from flower nectar. Humans have been using it as a food source, sweetener, and folk cure for ages. In addition to its distinct flavor, honey is appreciated for its plethora of health benefits (Bogdanov 2017). A number of variables, including beekeeping practices, processing techniques, and storage conditions, can affect the quality of honey. The quality and authenticity of the honey are crucial factors (CAC 2001). Honey as well as other food products contain the naturally occurring substance hydroxymethylfurfural (HMF). When honey is heated and stored, the Maillard reaction and caramelization processes take place, resulting in its formation (White and Doner, 1980). The presence of HMF in honey is a significant sign of its quality, freshness, and storage conditions. Due to things like extended storage, exposure to high temperatures, and enzymatic activities, the content of HMF in honey may rise with time (Gomes et al., 2010) Higher amounts of HMF can be used to determine the age or storage history of honey as well as to indicate the degree of

honey degradation. On the other hand, less processed, fresh honey often has lower HMF levels. The potential effects of HMF in honey on health have drawn increasing attention. High concentrations of HMF in honey have been linked in certain studies to genotoxic and cancer-causing effects (Gökmen et al. 2008). It is crucial to remember that more research is required to draw better conclusions from the current evidence regarding the health concerns of HMF in honey. HMF levels in honey can be precisely measured using analytical techniques like chromatography (Bogdanov et al. 2008). These techniques make it possible to precisely quantify HMF, which facilitates quality control and compliance with rules and standards for honey. The purpose of this study was to examine the significance of HMF in honey, including its mechanisms of synthesis, variables affecting its concentrations, and potential health effects related to high quantities. We will also go through the analytical techniques used to identify HMF in honey as well as the suggested HMF level thresholds for Turkish honey standards and laws. Beekeepers, honey producers, and consumers can make knowledgeable judgments about the quality of honey, ideal storage conditions, and potential health concerns by being aware of the function of HMF in honey. The current understanding of HMF in honey can be examined to get important insights into its effects on honey quality and more general consequences for the honey industry and consumers.

Proline is an amino acid that occurs naturally in many foods, including raw flower honeys. It is important for determining the quality of honey and is a key sign of its authenticity, floral origin, and processing conditions. Proline concentration in honey can offer important clues about its make-up, enzymatic functions, and potential health advantages. Proline is an amino acid used in the synthesis of proteins that helps to maintain the stability and structure of proteins. Proline is largely obtained from floral nectar and is found in honey in varying concentrations depending on the flowers that the bees visit to gather nectar (Beretta et al. 2005). Proline levels from various floral sources vary, making it possible to distinguish between different kinds of honey and their botanical sources. The enzymatic processes used in the manufacture and processing of honey can also be reflected in the proline content of the honey. Proline is metabolized by enzymes found in bees, such as proline dehydrogenase, which affects the amount of proline in honey (White and Subers 1963). As a result, the proline content of honey can be used to determine its freshness and enzymatic activity during manufacture. Proline has also been acknowledged for its possible health advantages. It acts as a precursor for the creation of collagen, a protein that is crucial for the health of connective tissues, the skin, and wound healing (Kang et al. 2017). Proline also functions as an antioxidant, assisting in the removal of free radicals and lowering oxidative stress (Vallianou et al. 2014). Proline can be found in honey, which increases its total nutritional and medicinal value. Proline levels in honey can be precisely determined using analytical techniques such high-performance liquid chromatography (HPLC) (White and Subers 1978). This makes proline quantification possible and makes regulating, standardizing, and evaluating honey quality easier. The purpose of this study is also to examine the significance of proline in raw flower honeys, including its function as a marker of floral origin, enzymatic activity, and potential health advantages. Beekeepers, honey producers, and consumers can learn more about the quality, authenticity, and possible medicinal value of honey by knowing the proline content in raw flower honeys.

Turkey has a long history of beekeeping and honey production, and different locations are renowned for their distinctive honey tastes and properties. Northeastern Turkey's Ardahan is a location renowned for its varied flora, which helps to produce many kinds of honey. The present study focused on the moisture content hydroxymethylfurfural (HMF) values and proline content of raw flower honey from Ardahan.

2. Materials and Methods

Collection of honey samples

Following the 2020 honey harvest, 20 honey samples were bought from beekeepers in the Turkish city of Ardahan who produce and sell honey**.**

Hydroxymethylfurfural (HMF) analysis

The TSE 3036:2002 method was used to handle HPLC-UV detector (Shimadzu UV-1800, Japan) for the HMF analysis of the honey samples. With the help of a solution of 5-hydroxymethylfuran-2-carbaldehyde (HMF), HPLC-UV was calibrated. By adding 90% distilled water and 10% methanol at a flow rate of 1 ml/min and handling the C18 reversed-phase column below the isocratic mobile phase parameters, analyses were carried out (Küplülü and Kahraman 2017).

Proline analysis

The IHC technique was used to analyze the proline content of honey samples

3. Results

The average HMF value of 7.1±1.4 mg/kg demonstrated freshness and a minimal level of honey deterioration. The honey's legitimacy and purity were suggested by its proline concentration, which was 424.6±36 mg/kg.

The amounts of HMF and proline detected in honey samples are given in Table 1.

Table 1. Amounts of HMF and proline detected in honey samples

Content	Unit	Mean±SD
HMF	**mg/kg**	**7.1±1.4**
Proline	**mg/kg**	**424.6±36**

4. Discussion

The average HMF (Hydroxymethylfurfural) value in raw flower honey is an indicator of its quality and freshness. In this study, the average HMF value was 7.1±1.4 mg/kg in the raw flower honeyfrom Ardahan region. Depending on the link between the sugars and amino acids in honey, improper temperature storage or heat treatment can produce HMF compound (Gökmen 2007) According to the Turkish Food Codex Honey Communiqué, there can be up to 40 mg/kg of HMF in honey. None of the raw flower honey examined for this study had HMF

values higher than 40 mg/kg. The HMF results in a different study on honey samples from various parts of Greece ranged from 2.7 mg/kg to 32.1 mg/kg (Theodorou et al. 2017). Once more, the Ardahan honey's average HMF value of 7.1±1.4 mg/kg is within the specified range, indicating a comparable level of freshness. Therefore, the average HMF value of 7.1±1.4 mg/kg in the raw flower honey from Ardahan suggests a relatively fresh and high-quality honey product. As sugars in honey break down over time, particularly when exposed to heat and kept in storage for an extended period of time, HMF is created. Higher HMF levels may be a sign of poorer quality honey, honey that has been overheated, or honey that has been stored for an extended period of time. It is crucial to keep in mind that allowable HMF levels in honey can change based on local laws and norms. Lower HMF values, on the other hand, typically suggest fresher, better honey.

Proline, one of the amino acids present in honey, should be present in amounts greater than 300 mg/kg (TFC 2020). Because proline value in raw flower honey is an important indicator of its quality and authenticity. It was found that all of the honey samples evaluated in this study complied with the Turkish Food Codex Honey Communiqué, with an average proline content of 424.6±36 mg/kg. A study on honey samples from different regions of Iran reported proline values ranging from 124 mg/kg to 732 mg/kg (Jamshidi et al. 2016). The average proline value of 424.6±36 mg/kg in the raw flower honey from Ardahan falls within this range, indicating a comparable level of proline content. In another study on honey samples from different floral sources in India, the proline values ranged from 154 mg/kg to 663 mg/kg (Gupta et al. 2017). The average proline value of 424.6±36 mg/kg in the Ardahan honey falls within the reported range, suggesting a similar level of proline content. Higher proline values typically signify pure, high-quality honey. It is important to keep in mind that proline levels can change based on things like the source of the flowers, the area, and the beekeeping methods. However, the raw floral honey from Ardahan with an average proline value of 424.6 mg/kg reveals a significant amount of this amino acid, indicating a high-quality honey.

5. Conclusion

The average HMF and proline values detected in honey samples were found to be in compliance with the Turkish Food Codex Honey Communiqué. These results provide valuable information about the raw flower honey from Ardahan, highlighting its quality, authenticity and potential applications. Further research can be done on these findings to explore the unique properties and potential benefits of honey from this region, which adds to the value of honey and its use in a variety of industries.

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Apitherapeutic Potentials High Turkish Honeys

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Abstract

Turkey is one of the leading countries in the world in terms of honey production and honey diversity in terms of its geographical location and climate characteristics. Turkey is a bridge between Asia, Europe and Africa and has a rich flora. It also has a rich variety of bee products for reasons such as rich plant diversity, bee breeds and 9 months of the year being suitable for beekeeping activities. In this study, I will give you about apitherapeutic value high Turkish honeys. Honey is a functional food, and most of the bioactive components in its structure come from polyphenols. Our studies show that the higher the number of polyphenols in honey, the higher the antioxidant, antimicrobial and many other biologically active properties of honey. For this reason, in the studies we carried out in honeys belonging to different flora of Turkey, it was determined that the total phenolic substance amounts varied from 10 to 110 mg GAE/100 g. For this reason, it showed that the honeys with high TPC value in Turkey are chestnut honey, oak honey, pine honeys and black cumin honeys.

Keywords: Anatolia, honey, apitherapeutic,

Introduction

Honey is the most valuable natural product that has been used as a medicine since ancient times. Although it is 96% sugar by dry weight, it is the only natural product that can remain intact for many years (1,2). Complementary medicine applications of honey and other bee products are called apitherapy. Apitherapy have been widely used for centuries because of their nutritional and healing values Functional beekeeping products include honey, bee pollen, propolis, bee bread, royal jelly, beeswax and bee venom. Worldwide reports on these bee products have largely focused on their therapeutic effects, such as antioxidant, anti-inflammatory, antibacterial and anticarcinogenic activities (3,4). Honey has been the most frequently used agent of apitherapy from past to present. Honey has been used as a high antioxidant, antimicrobial, anti-inflammatory and antidepressant, especially due to its viscous structure and its secondary metabolites (5,6). Honey is an important antimicrobial agent due to its viscous structure due to high sugar concentration, hydrogen peroxide from glucose oxidase, polyphenols, and pH value. Polyphenols, which are found as secondary metabolites in the structure of honey, affect the apitherapeutic properties of honey (7,8).

Located at the crossroads of three major continents (Asia, Africa and Europe), Turkey has an important place in the world in beekeeping in terms of rich vegetation, suitable ecology and colony existence. Experiencing floristic climate transitions in many regions of Anatolia causes our country to be rich in terms of endemic plant resources. In addition, in parallel with the plant biodiversity in our country, nectar resources are also abundant in terms of the area where it is spread (9). Among bee products, the product with the highest awareness is honey, followed by pollen, royal jelly, bee venom and propolis. Commercial products such as honey, pollen, royal jelly and propolis produced as an important result of beekeeping activities can find markets both in the domestic and foreign markets and make significant contributions to the country's economy. (10).

Honey is a natural sweet substance that is collected by honeybees, mixed with specific substances, and left to the honeycomb to mature (4,7). Consume directly as it is taken from the hive, honey is called raw honey. Although the chemical composition of honey varies according to its geographical and botanical source, it consists of macro and micro components such as 75% carbohydrates, 20% water, 0.7% minerals, 0.3% protein, vitamins, organic acids, phenolic compounds and free amino acids (1,12). According to their floral sources, honeys are divided into two classes as flower honey and secretory honey (5,10). While honey produced from the extracts collected by honeybees from flower nectars is called flower honey, approximately 50 kinds of monofloral and heterofloral flower honeys are produced in Turkey. Honey produced from extracts collected from the secretions of trees and plants is called secretory honey, and there are many different types of secretory honey such as pine honey, oak honey, and cedar honey in Turkey. More important than the nutritional value of honey is its biologically active value. The biological active value of honey consists of polyphenols and vitamins in its structure (11).

In this study, our studies with honeys with high apitherapeutic value in Turkey will be explained.

Results and Discussion

There are studies that we have done in characterizing honeys belonging to the flora of Turkey and revealing their biological benefits. In a first study published in 2007, about chestnut, rhododendron and multifloral blossom honey (12). We had found that chestnut honey high phenolic substances and antioxidant capacity nearly two times higher than other honeys. Then we did many comparative studies on Turkish monofloral and heterofloral honeys (13). In a study with 20 different Turkish honeys, heather honeys were distinguished from others with significantly high vitamin B2 and iron contents. Considerably higher antioxidant capacities and Mn contents were observed for oak and chestnut honeys (2). In a later study, a positive correlation was found between the pollen percentage of chestnut honey and the amount of phenolic substances (14, 15). Kırklareli region is the geography where the most oak honey is produced in Turkey, and in our study, it was determined that the honey of this region is always

raw honey in dark color and has high antioxidant properties (15). Turkey's largest secretion honey is pine honey. All the properties of pine honey in the Aegean and Mediterranean regions were investigated with a large project (16). There are industrial honeys produced in Turkey, and buckwheat honey, black cumin honey and sunflower honey are among them (16).

Our studies have also shown that dark colored honeys have higher polyphenol content. The honeys with the highest polyphenol content in Turkey are chestnut honey, oak honey, followed by heather honey, black cumin honey and pine honey (12-16). In addition to its high antioxidant activity, chestnut honey is the most commonly used honey especially in colds, colds and flu cases, upper respiratory tract infections and gastritis, due to its high antimicrobial properties.

In our studies with Turkish honey, it was determined that chestnut, rhododendron and some flower honeys stopped the proliferation of Helicobacter pylori bacteria. It is thought that the polyphenols found in honey inhibit the urease enzyme secreted by *Helico bacter pylory* into the extacellular environment and stop the growth of the bacteria. As a matter of fact, our studies revealed that polyphenols of honey inhibit *Helicobacter* pyro-derived urease (8). As it is known, *H.pylori* is the only bacterium that can live in the acidic stomach environment, and ammonia from the urea can remain in the ureteral stomach thanks to the urease enzyme. With the inhibition of the urease enzyme, the bacteria cannot survive in the stomach (8,17). As a matter of fact, it has been found in many of our studies that dark colored honeys have higher polyphenols and inhibit urease (18). In the previous study, the rats were pretreated with rhododendron, chestnut, and oak honey orally with doses of 1.25 and 2.5 g/kg, bw (body weight) for three consecutive days. On fourth day, nothing was applied, and after the administration of anesthesia on the fifth day, their stomachs were surgically removed to investigate the histopathological examinations. Besides analyses of some blood serum profiles and antioxidant parameters of gastric tissue, some biochemical properties of honeys were investigated to support the histopathological results. The results were showed that all honey samples were found effective, but rhododendron honey was the most (19).

The characteristics of oak honeys produced towards autumn in Turkey, especially in the Thrace region, were also studied. We determined that oak honeys are secretory honeys, dark colored honeys and honeys with high polyphenols and antioxidant capacity (16). Black cumin honey produced from nigella sativa fields produced industrially in the Burdur region of Turkiye was also studied. It was found that this honey was in the category of dark colored honeys (16). The honey contained high amount of ellagic acid and pinocembrin as the major components. The honey showed high antimicrobial activities against Escherichia coli, *Enterococcus faecalis, Staphylococcus aureus, Salmonella enteric* subsp. enterica, *Bacillus subtilis, Bacillus cereus, Listeria monocytogenes* and *Candida albicans*. And also, the honeys were especially effective against *L. monocytogenes, S. aureus, and E. faecalis*. The results show that *N. sativa* honey possesses high apitherapeutic potential, although further research is now needed (16).

Another type of honey that is rarely produced in the world is Mad honey. This honey is produced from rhododendron flowers grown in forest areas, especially in the Black Sea region. It is known as blood pressure lowering due to the grayanotoxanes it contains. Rhododendron

honey has the characteristic of mad honey due to the grayanotoxanes it contains, it is known that this honey is traditionally used as a blood pressure reducer (19,21). We have investigated the anti-hypertensive effect of mad honey and *Rhododendron luteum* Sweet extracts containing grayanotoxane (GTX)-III in a rat model of hypertension induced by N-ω-nitro L-arginine methyl ester (L-NAME). The results are showed that the honey reduced systolic and diastolic blood pressure (22). We investigated the role of some honeys produced in Anatolia in preventing liver damage. It was determined that chestnut honey has a high hepatoprotective. Effect (23).

In conclusion

Turkey is one of the rare countries with rich vegetation, rich bee breeds and a wide variety of honey biodiversity. It has apitherapeutic honeys with rich honey biodiversity from light to very dark colors. Chestnut honey is one of the honeys with the highest apitherapeutic value in the world, and it is produced mostly in the Black Sea region, in Turkey.

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Conservation of the gene pool of local honey bee populations

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Abstract:

Wild and feral honey bee populations receive little attention despite being in danger. Introduced honey bee subspecies have the potential to have a severe negative impact on native honey bee wild population. Introgressive hybridization and exposure to new pests and pathogens are both favored when wild and feral bee populations coexist. Because of the spread of the ectoparasitic mite Varroa destructor, wild and feral populations of A. mellifera in Europe saw a rapid decline that brought them dangerously close to extinction. Through time, populations of honey bees in wild and feral honey bees developed resistance to the V. destructor mite but drastically lost their genetic diversity. Because of their higher genetic variety than other bee populations in their natural habitat, wild and feral honey bee populations are more resilient to changing climatic circumstances and new diseases. By means of hybridization, the interaction of a bee population with a wild or feral population can alter the genetic diversity of both groups. According to previous studies, honey bee populations in the wild and feral states are genetically distinct from honey bee populations and serve as a source of genetic variety and advantageous adaptations for bee populations. Nowadays, populations in hives and the wild are mainly mixed but not identical. Different selection pressures are applied to these populations. In apiary populations, artificial selection is used to maintain pure subspecies, but in wild populations, natural selection is used to favor features that improve fitness and survival. In our research, we discovered genetic diversity of the Bashkir bee population on the territory of the Toratau Geopark", Ural, Russia.

Keywords: *Apis mellifera*, honey bee, gene pool, population, conservation.

Introduction

Bees are the main pollinators of over 80% of flowering plant species (Ollerton et al., 2014; López-Uribe et al., 2017). The close interaction between bees and plants was formed as a result of long coevolution (Dellicour et al., 2016; López-Uribe et al., 2017). Each honey bee subspecies is adapted to its unique local environment and has its own preferences for flowering plants (Ollerton et al., 2014; Tandon et al., 2016). In addition to breeding bees for crop pollination, bees are used by humans to produce honey (López-Uribe et al., 2017). It has been shown that as a result of habitat disturbance and a number of additional potential causes, the decline in the bee population has increased over the past decade in all countries of the world (Cameron et al., 2011; Bartomeus et al., 2013; Lopez-Uribe et al., 2017). A decline in the bee population results in both environmental and economic problems (Zayed, 2009), as it can jeopardize human food security (Potts et al., 2016; López-Uribe et al., 2017). Thus, the decline in bee populations poses a serious threat to humans and requires the development of effective

strategies for the conservation and restoration of bee populations in various regions (Brown and Paxton, 2009; López-Uribe et al., 2017). The study of polymorphism, gene diversity, and gene diversity in bee subspecies populations is crucial for developing strategies for the conservation of their gene pools (Duennes et al., 2012; López-Uribe et al., 2017; Dellicour et al., 2016).

Hybridization and the threat of loss of local gene pools

Historically, human-mediated hybridization has been considered a key conservation issue. Until the middle of the 19th century, beekeeping consisted only of catching swarms and collecting wild honey, while modern beekeeping is based on the use of standard hives, which make it possible to keep a large number of bee colonies per beekeeper all year round, move hives over long distances, carry out sanitary and veterinary measures, and also exercise partial control over the biology of the bee colony. Both queen purchase and large-scale movements have resulted in human-mediated hybridization of bee subspecies worldwide (Ruttner, 1988; Moritz et al., 2005).

Gene flow between subspecies of honey bees is currently common in European bee populations due to the introduction of subspecies and hybrids and the impossibility of subsequent control of their reproduction (Oleksa et al., 2013; Soland-Reckeweg et al., 2009). As a result of mass hybridization due to the importation of queen bees and the export of bee colonies to remote regions, the natural structure of the geographical distribution of bee subspecies throughout the range in Europe was disturbed (Jensen et al., 2005). In Germany, as a result of the mass introduction of *A. m. carnica*, intensive hybridization occurred with the subspecies *A. m. mellifera*. In Russia, the subspecies *A. m. mellifera* in a significant part of the territory also undergoes hybridization with the bee subspecies *A. m. caucasia* and *A. m. carpatica* (Ilyasov et al., 2007).

As a result of the wide distribution of the subspecies *A. m. carnica*, *A. m.* caucasia, and *A. m. ligustica* in Eurasia, they hybridized with the subspecies *A. m. mellifera* in northern Europe and were replaced by bees of hybrid origin (Jensen et al., 2005). Modern molecular genetic data have shown that between populations of *A. m. carnica*, *A. m. macedonica*, and *A. m. cecropia* of the Balkan countries and Eastern Europe Thus, in Bulgaria, the native subspecies *A. m. macedonica* has been almost completely replaced since 1980 by the introduced subspecies *A. m. ligustica*, *A. m. carnica*, and *A. m.* caucasia (Ivanova et al., 2010). The population of bees of the subspecies *A. m. iberiensis* of the Balearic Islands also undergoes intensive hybridization with the introduced subspecies *A. m. carnica* and *A. m. ligustica* (De la Rua et al., 2006). Native bees of the subspecies *A. m. mellifera* on the island of Sardinia and *A. m. intermissa* on the island of Sicily were relatively recently completely replaced by introduced bees of the subspecies *A. m. ligustica* from continental Italy (Franck et al., 2000).

The consequences of hybridization may be to reduce the effective population size and genetic diversity. The gene pools of local subspecies of bees may be lost in the near future as a result of a decrease in the effective abundance and mass introgression of alien genes (Uzunov et al., 2014). The genetic diversity of honey bees is constantly decreasing under the influence of pesticides, intraspecific hybridization, and infectious and parasitic diseases (Genersch et al., 2010; Cornman et al., 2012). Decreased genetic diversity in bee populations is a major concern as it can lead to reduced immunity, adaptability, and productivity, as well as reduce the effective size of the honey bee population (Page et al., 1995; Palmer et al., 2000). Genetic studies aimed

at identifying subspecies will make it possible to control bee movements and reduce the process of hybridization between subspecies (Jensen et al., 2005; Ilyasov et al., 2016). Genetic studies aimed at characterizing the population will make it possible to track the genetic processes in the population, timely identify critical moments, and develop new promising bee breeding strategies based on the analysis of genetic indicators (Soland-Reckeweg et al., 2003; Muoz et al., 2014; Wallberg et al., 2014; Ilyasov et al., 2016).

Wild and feral honey bee populations

Wild and feral honey bee populations are endangered but receive little attention (Jaffé et al., 2010). Introduced honey bee subspecies can have a significant impact on wild populations of native honey bees (De la Rúa et al., 2009). Coexistence with wild and wild bee populations promotes introgressive hybridization (Jaffé et al., 2010; Whitfield et al., 2007) as well as exposure to new pests and pathogens (Fries et al., 2006; Graystock et al., 2013). The spread of the ectoparasitic mite *Varroa destructor* caused a sharp decline in wild and feral populations of *A. mellifera* in Europe (Pirk et al., 2017) to almost complete extinction in Europe (De la Rúa et al., 2009; Ilyasov et al., 2015). However, later on, wild and feral populations of honey bees acquired resistance to the V. *destructor* mite, but drastically lost their genetic diversity (Le Conte et al., 2007; Seeley, 2007).

In their natural range, wild and feral honey bee populations have significantly higher genetic diversity than bee populations (Whitfield et al., 2006; Wallberg et al., 2014), which provides them with increased resilience to changing climatic conditions (Pirk et al. , 2017) and emerging pathogens (Moritz et al., 2005; Moritz et al., 2007; Dietemann et al., 2009). The interaction between a bee population and a wild or feral population can change the genetic diversity of both populations through hybridization (Harpur et al., 2012).

Wild and wild populations are genetically distinct from honey bee populations (Sheppard, 1988; Lodesani and Costa, 2003) and represent a source of genetic diversity and adaptations that can also benefit bee populations (Chapman et al., 2016). Currently, both wild and apiary populations are largely mixed but not identical (Chapman et al., 2008; Oxley and Oldroyd, 2009; Chapman et al., 2016). These populations experience different selection pressures. The genetic diversity in commercial populations of honey bees is influenced by artificial selection to maintain pure subspecies, whereas in wild populations it is influenced by natural selection that provide better survival and fitness (Harpur et al., 2014).

Honey bee gut microbiome

The bee gut microbiome plays an important role in maintaining the bee population and is involved in the degradation and detoxification of xenobiotics and pesticides (thiacloprid, imidacloprid, and fluvalinate) by increasing the expression of detoxification enzymes in the gut (Wu et al., 2020). Lactic acid bacteria of the genus Lactobacillus are represented in the intestines of bees by the greatest species diversity, are characterized by an increased antioxidant potential, and allow bees to survive a long winter period without emergence (Nowak et al., 2021). The gut microbiome exerts a protective effect on bees in infectious diseases by altering the gut environment, inhibiting pathogen development, and eliciting a host immune response. The bee microbiome also produces antimicrobial peptides that play a key role in pathogen defense (Carina Audisio et al., 2011; Ilyasov et al., 2012; Salman and Saleh, 2018; Kacaniova

et al., 2019). The bee gut microbiome promotes accelerated growth, a longer lifespan, improved food absorption, and increased body weight by modulating the insulin-like signaling pathway involved in growth, reproduction, aging, and homeostasis (Zheng et al., 2017).

It has been shown that disruption of the functioning of the intestinal microbiome of bees leads to a decrease in the fitness of bee populations, an increase in morbidity, and a decrease in the number of bee populations (Salman and Saleh, 2018; Kacaniova et al., 2019). Disorders of the gut microbiome can be caused by environmental degradation as a result of the use of pesticides in agriculture as well as the spread of new pathogens as a result of the mass transportation of bee colonies (Carina Audisio et al., 2011; Ilyasov et al., 2012). It has been shown that CCD colony collapse syndrome is explained by a disruption in the functioning of the intestinal microbiome since undigested pollen was found in the feces of honey bees that died from CCD as a result of infection of symbiotic bacteria with viruses and antibiotics (Smaghe et al., 2017).

Conclusion

As can be seen from our study, it is necessary to protect the native dark forest bee from crossing as soon as possible, and without human help, the local bee is not able to restore its purebred. For thousands of years, bees have adapted to a cold climate with long winters, and hybrid colonies come out of the winter weakened, do not work well for pollination, and show low productivity. The aggressiveness of colonies often sharply increases. So far, no one can predict the consequences of the destructive influence of southern bees on aboriginal northern ones. Bees are indispensable components of terrestrial ecosystems, and their conservation is of great ecological and economic importance. The use of genetic approaches in the conservation of bees can be of great importance for the conservation of bees and the identification of genetic threats to the adaptation of bee populations. Bees in their natural habitat have increased genetic diversity and, through natural selection, improved survival, fitness, and immunity. The conservation of wild and feral populations of honey bees leads to an increase in the resilience of bee populations due to the constant exchange of genes between them. Population genetic studies can be used to estimate population structure, gene flow, effective population size, and identify subspecies. Genetic methods provide a reliable and fast estimate of population size.

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An effective product in the treatment of obesity: Perga

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Abstract:
Adequate and balanced nutrition constitutes one of the most important prevention strategies in the prevention of non-communicable diseases, which are among the leading causes of death all over the world. In addition, obesity is one of the protective factors that play a role in minimizing nutritional and health problems such as malnutrition, vitamin and mineral deficiencies. However, nutrition, which is necessary for the protection and development of the health of individuals and societies and for increasing the quality of life, is an action that must be done consciously. In order to provide adequate and balanced nutrition, the energy and nutrients needed; It should be met in the required amounts according to the age, gender, physiological characteristics, physical activity status and genetic structure of the individual. Therefore, nutrition is unique to the individual. In addition, a healthy diet should be based on food variety. While meeting the energy and nutritional needs, it is recommended to consume foods with different colors, types and contents instead of a monotonous diet. This study aims to summarize current scientific knowledge on bee bread and its effect on obesity.

Keywords: bee bread, perga, obesity, leptin, ghrelin

1. Introduction

Diseases such as obesity, dyslipidemia, glucose intolerance and hypertension, which are important health problems in the world, are associated with increased cardiovascular risk factors. The main cause of obesity is the imbalance between energy intake and energy expenditure. Accumulation of adipose tissue, particularly visceral adipose tissue, is also the primary cause of the “metabolic syndrome,” a combination of multiple cardiovascular risk factors such as dyslipidemia, glucose intolerance, and hypertension. Dyslipidemia and oxidative stress, which are closely related to diabetes, hypertension, obesity and cancer, can be the consequences and causes of high mortality from cardiovascular diseases in humans and animals. Dyslipidemia is a physiological condition associated with high plasma triglyceride, LDL, and VLDL levels and low levels of high-density lipoprotein (HDL) cholesterol.
Obesity and overweight are multifactorial chronic disorders characterized by an elevated oxidative state and chronic activation of macrophages in peripheral tissues. Activated macrophages cause inflammation in peripheral organs including adipose tissue and liver. Effective therapeutic approaches to lower inflammation and oxidative stress are of interest.Leptin and ghrelin are fasting and fullness hormones regulating the body weight by affecting the total calorie intake of the fasting-fullness mechanism (Cooper et al., 2010). Ghrelin/leptin concentrations are controlled with a “feedback” mechanism through Y neurons

in the hypothalamus, and body weight is controlled in this way (Ozcan, 2009). While leptin is an appetite-suppressant peptide, ghrelin is an appetite-stimulant. They show opposite effects to each other in the regulation of energy balance (Yang et al., 2010), and they influence eating behavior and appetite by directly signaling to hypothalamic neuropeptide Y neurons (Zhang et al., 2016). Neuropeptide Y (NPY)-positive cells, located in the hypothalamic arcuate nucleus (ARC), are effect sites for both ghrelin and leptin. While ghrelin stimulates synthesis and secretion of NPY, leptin inhibits it (Parker and Bloom, 2012).

Diet comes to the fore in the treatment of obesity. Foods that are low in calories but have high nutritive value are gaining importance in the diet. For this reason, the desire for a natural and healthy diet is increasing every day. Bee products draw attention as an option for a natural and healthy diet. Bee bread mainly includes pollen, honey, and secretions of bees' salivary glands (Vasquez and Olofsson, 2009), and bees pack the components in the cells of the honeycomb, then secure the mixture with wax and honey (Barene et al., 2015). After this, pollen is subject to lactic fermentation in the environment of a bee nest. Fermented bee pollen is called bee bread (De Grandi-Hoffman et al., 2013; Fuenmayor et al., 2014; Kieliszek et al., 2018).

It is known that bee bread contains approximately 20% proteins, 3% lipids, 24%–35% carbohydrates, 3% minerals, and vitamins. Bee bread is composed of well-balanced proteins containing all essential amino acids, the full spectrum of vitamins (C, B1, B2, E, H, P, nicotinic acid, folic acid), pantothenic acid, pigments, and other biologically active compounds, like enzymes such as saccharase, amylase, phosphatases, flavonoids, carotenoids, and hormones. Bee bread also contains over 25 different micro- and macroelements such as iron, calcium, phosphorus, potassium, copper, zinc, selenium, and magnesium (Nagai et al., 2005; Khalifa et al., 2019). It was reported that bee bread involves more reduced sugar than the pollen of the same plant, as well as vitamin K and microorganisms' digestive enzymes (Haydak, 1958). Bee bread is considered to be a beneficial food supplement. Therefore, in recent years, there has been significant attention paid to the use of bee bread to treat many illnesses. By means of the antimicrobial activity of bee bread, mold and fungus development are inhibited and thus the bee bread is protected better (Nagai et al., 2004). Although there are studies about the biological activities and chemical content of bee pollen having different botanic origins, studies on bee bread are in the early stages. In recent years, there has been significant interest in the use of bee bread to treat many illnesses (Khalifa et al., 2019) and it has been shown to exhibit antimicrobial (Veiga et al., 2017), antioxidant (Nakajima et al., 2009), anticancer (Liu et al., 2016), and antiinflammatory (Rimbach et al., 2017) activities. In addition, fatty acid contents of bee bread (Kaplan et al., 2016) and its antibacterial (Abouda et al., 2011) and antioxidant (Haydak, 1958; Bakour et al., 2017) activities are topics of studies in recent years. The antibacterial activity of samples of bee bread and bee pollen collected from Morocco was shown against bacteria including *E. coli*, *Staphylococcus aureus*, and *Bacillus cereus*, and it was reported that fresh bee pollen and bee bread showed higher antibacterial activity than dried ones (Abouda et al., 2011). It was also indicated that antioxidant activity was high in the samples in which water was used as a solvent from among hot water, water, and ethanol extracts of bee bread (Haydak, 1958). Bakour et al. (2017) indicated that bee bread has an important protective effect on aluminum-induced toxicity in rats with its antioxidant activity.

In addition to the expense of traditional pharmaceutical drugs used in obesity treatment, the toxicity caused as a result of their long-term use and their side effects have increased the need

for exploring new alternatives. In this study, it was aimed to determine the effects of bee bread, known for being natural, rich in nutrients, and healthy, on the expression of leptin and ghrelin hormones in the hypothalamus tissue of obese experimental animals at histological, immunohistochemical, biochemical, and molecular levels.

Obesity is one of the most important health problems of the modern age. Many of the methods developed to treat this health problem are related to dietary substances. Bee products are accordingly becoming increasingly important as a natural and healthy diet option. Since ancient times, bee bread has been used in different cultures for several nutritional and therapeutic purposes. Different biological effects and dietary properties have been attributed to bee bread, including antimicrobial, antioxidant, anticancer, and antiinflammatory activities (Khalifa et al., 2019). The chemical content of bee bread has been revealed in various studies (Nagai et al., 2005; Kaplan et al., 2016). Bee bread has a much better composition than many animal protein products due to the presence of all the essential amino acids and significant amounts of proteins, vitamins, and phenolic compounds such as natural antioxidants (Nagai et al., 2005; Abouda et al., 2011). Bee bread is reported to help regulate lipid metabolism (Nagai et al., 2004). Unsaturated fatty acids (FAs) have many beneficial health effects, such as the reduction of triglyceride (Von Schacky and Harris, 2007) and cholesterol levels in the blood (Simopoulos, 2004). Polyunsaturated FAs are required for the body to function. The ratio of unsaturated/saturated FAs in bee bread varies between 1.38% and 2.39%, suggesting that bee bread can be used as a good source of unsaturated FAs (Kaplan et al., 2016). Because of all these features, we investigated the effects of bee bread on obesity based on the hypothesis that bee bread can play a therapeutic role in the treatment of obesity.

According to the Doganyigit et al. (***) , bee bread supplementation did not lead to weight gain in rats. A dose of 200 mg/kg/day showed a weight-reducing effect, though it was not as effective as metformin. Bee bread, which is the fermented state of bee pollen, contains important nutrients such as proteins, amino acids, lipids, minerals, carbohydrates, and vitamins (Campos et al., 2003). It was indicated that bee bread increased growth performance and feed intake in rabbits, and it could be beneficial for feed conversion rates (Attia et al., 2011). In another study, it was determined that a fresh bee pollen formula had useful biological activities such as the recovery of muscle protein and energy metabolism in rats that suffered from severe food restrictions. Results obtained from prior studies revealed that fresh bee pollen has good anaboli and metabolic activity, and it may be beneficial in the prevention or recovery of malnutrition as it has good anabolic and metabolic activity (Salles et al., 2014).

Obesity, which is a significant risk factor for the development of diabetes mellitus, cardiovascular diseases, and some cancer types, generally originates from a chronic positive energy balance due to an increasingly static lifestyle with genetic and epigenetic background and unlimited access to food (Cui et al., 2017). The ability of the hypothalamus to control the energy balance is compromised and degraded in many obese individuals (Williams, 2012). There is a mechanical connection between overeating, especially for long-chain FAs with inflammatory responses, which can reduce the running of the hypothalamus in obese individuals, and systemic formation (Williams, 2012; de Git and Adan, 2015). Ghrelin is produced by gastric mucosa, which mainly stimulates the appetite, leading to body weight increase and causing a positive energy balance (Kirsz and Zieba, 2011). Leptin is basically secreted by adipocytes of white adipose tissue and it may diminish the appetite, and both of

them can also decrease fat formulation and deposition, which are closely related to the formation of type 2 diabetes mellitus (Zhang et al., 2013). Furthermore, NPY-positive cells in the hypothalamic ARC are effective sites both for ghrelin and leptin. While ghrelin stimulates synthesis and secretion of NPY, leptin inhibits it (Parker and Bloom, 2012). However, changes in hypothalamic NPY content and its relationship with insulin, leptin, and ghrelin levels has not yet been reported, along with its correlation to the potential mechanism underlying their interactions during the development of the type 2 diabetes mellitus rat model. The NPY system is accepted as the last mutual pathway for expression of appetite in the hypothalamus. Leptin is an appetite-suppressant peptide while ghrelin is an appetite-stimulant. They show opposite effects in the regulation of energy balance (Yang et al., 2010), and they regulate appetite and eating behavior by giving signals directly to hypothalamic NPY neurons (Zhang et al., 2013). In the study by Zhang et al. (2013), they investigated the change of NPY in the hypothalamus and its correlations with insulin, leptin, and ghrelin in the development of a type 2 diabetes serum (ng/mL) significantly increased and fasting plasma ghrelin concentration considerably reduced its content of hypothalamic NPY (pg/mg) during the development of the type 2 diabetes mellitus rat model. Hypothalamic NPY concentration showed a positive correlation with changes in serum insulin and leptin, and a negative correlation with plasma ghrelin. The same researchers also indicated in another study that serum leptin concentrations and adipocyte leptin production increased significantly with the gain in body weight of animals while their serum and gastric ghrelin levels decreased significantly with obesity (Zhang et al., 2013). As has been stated before, many studies reported that the average serum ghrelin level is generally lower in obese patients compared to thin individuals (Abdemur et al., 2014). Metformin is an agent taken orally as an adjuvant of insulin sensitivity. It not only inhibits the production of hepatic glucose; it also increases the effects of insulin on glucose uptake in the skeletal muscle and adipocytes, and it diminishes the absorption of glucose from the intestines. Although its mechanism of action is still not fully explained, it is known that metformin decreases body weight (Campos et al., 2003; Kim, 2006; Malin and Kashyap, 2014). In addition, instead of using drugs for the treatment of obesity, healthy eating and physical activity are recommended. Therefore, metformin was chosen as a positive control since it is not a conventional drug for treatment. Our study, in line with the related literature, revealed that the amount of ghrelin was diminished in both serum and hypothalamus tissue in the obesity group, and the levels of tissue and serum leptin increased. It was observed that metformin and 200 mg/kg bee bread relieved these impacts.

A high-fat diet triggers reactions including an increase in oxidative stress reagents, inflammation, endoplasmic reticulum (ER) stress, and autophagy defects and changes in apoptosis and neuronal regeneration rate in the hypothalamus (de Git and Adan, 2015). In many of the studies conducted on obese patients, it has been reported that oxidative stress reagents increased in the body, and antioxidant defense enzymes decreased, and as a result, obesity led to inflammation and chronic oxidative stress in the body (Vincent and Taylor, 2006). Increasing oxidative stress in obesity was put forward as the primary reason for tissue and function disorders (endothelial dysfunction, increased platelet aggregation, atherogenesis, etc.) that appeared in those patients (Uzun et al., 2004). It was indicated that induction of hypertriglyceridemia in rats increased with mitochondrial respiration in the hypothalamus in parallel with the rise in ROS production (Benani et al., 2007). It was also specified that ROS is

significant in both glucose and lipid detection by the hypothalamus (Leloup et al., 2006), and ROS, increased in the hypothalamus of rats, had a correlation with abnormal glucose sensitivity (Colombani et al., 2009). Regulation of the melanocortin system in the hypothalamus requires ROS as an acute effect of its stimulation by POMC (proopiomelanocortin) neurons, and it also causes reduction of food intake, while suppression of ROS leads to activation of AgRP (agouti-related peptide)/NPY neurons and increased nutrition (Schrader and Fahimi, 2006). It was also reported that diabetic and high-fat diets evoke central leptin resistance and an inflammatory reaction in the hypothalamus that promotes the development of obesity (de Git and Adan, 2015).

Obesity is associated with increased lipid peroxidation; MDA is a biological reagent reflecting the level of lipid peroxidation. It was stated that MDA levels seemed to have a positive correlation in obese and nonobese healthy individuals with body mass index (Yilmaz et al., 2007). Increased lipid peroxidation in obesity may compromise permeability as it affects the structure and integrity of cell membranes. Proinflammatory cytokines, secreted from increased fat tissue in obesity, cause lipid peroxidation by producing high amounts of free oxygen radicals (Trayhurn and Wood, 2004). Growing epidemiological evidence indicates that metformin, which is the most appropriate first-step antidiabetic drug, reduces the incidence and severity of stroke. In addition, a clinical study found that metformin decreased oxidative stress by diminishing ROS production and developing the antioxidant reserve (Esteghamati et al., 2013). Al-Osaimi et al. (2018) aimed to assess the therapeutic and protective effects of pollen in improving the toxic effects of MeHg by measuring biochemical parameters selected due to oxidative stress in the brain homogenates of newborn male offspring, energy metabolism, and neurotransmission. They found that while MeHg administration increased lipid peroxidation and catalase activity, it decreased the levels of glutathione in an insignificant manner. Bee pollen administration was quite effective in both normalization of Mg^{2+}, $K+$, lipid peroxidation, and glutathione levels and the recovery of the activities of catalase, lactic dehydrogenase, and creatine kinase. Moreover, it was concluded that the oxidative stress of bee pollen could be used safely to improve metal ion defects and neuronal death along with weak detoxification, and it is a critical mechanism in the etiology of multiple neurological disorders. In another study Mohamed et al. (2018) indicated that both bee pollen and palm pollen had antioxidant and antihyperglycemic effects. Bakour et al. (2017) specified that bee bread has an important protective effect in rats against aluminum-induced toxicity by exerting antioxidant activity. In recent years, the potential for antioxidant usage, especially natural antioxidants, has drawn much interest. Antioxidants may have a protective effect in the prevention of aging and heart and liver diseases, or in reducing their severity. This protective effect is attributed to their ability of combatting the ROS produced during oxidative stress. Therefore, it is well known that antioxidants are beneficial in protecting cellular components against oxidative damage.

It is also known that obesity causes apoptosis (Li et al., 2018). In an investigation carried out by Sa-nguanmoo et al. (2018), it was detected that obesity insulin resistance developed with an increase in systemic inflammation, brain mitochondrial dysfunction, rise in brain apoptosis, and disruption and cognitive reduction in hippocampal plasticity in rats fed a high-fat diet. Chunchai et al. (2018) indicated that long-term high-fat diet consumption induced metabolic disruption, cognitive disruption, glial morphological changes, increased hippocampal oxidative stress, and cell apoptosis in both the hippocampus and cortex, and obesity. When assessing the apoptosis results of our study, it was witnessed that it was effective in the groups to which bee bread was

given upon statistically increasing apoptosis in the obese groups compared to both the control and metformin groups. Kolesarova et al. (2013) reported the positive effects of bee pollen on ovarian cell apoptosis and proliferation. According to the study carried out by Huang et al. (Huang et al., 2017), it was put forward that *Schisandra chinensis* bee pollen extract decreased oxidative stress levels, and it may diminish liver and kidney injury caused by cisplatin by increasing the antioxidant, antiinflammatory, and antiapoptotic capacity of the body.

Othman et al. (2020) aimed to determine the phenolic compounds and the anti-atherogenic effect of bee bread (0.5 g/kg/day) in obese rats fed a high-fat diet. Obesity index, total cholesterol (TC), low-density lipoprotein (LDL), fatty acid synthase (FAS), atherogenic index, oxidized index of bee bread (caffeic acid, ferulic acid, chemferol, apigenin, and isorhamnetin) of which chemical composition was determined, after 6 weeks of trial. It significantly increased the aortic antioxidant enzyme activities such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) while significantly decreasing the levels of -LDL (oxLDL) and malondialdehyde (MDA). While the size of adipocyte was found to be smaller in the bee bread group than in the obese group, no atherosclerotic plaque was observed on the aortic surface. According to the results obtained, the researchers reported that bee bread showed antiatherogenic activity in obese rats and this effect was due to the antioxidant and hypocholesterolemic properties of bee bread.

Schizandra bee pollen has been used in China for centuries in the field of health; Cheng et al. (2019) determined the phenolic compounds of Schizandra bee pollen and investigated its protective role in non-alcoholic fatty liver disease and its effects on modulation of gut microbiota in mice fed a high-fat diet. While 12 phenolic compounds were identified in the pollen extract, the main components were found to be naringenin (1.89 mg/g), rutin and chrysin. While the total amount of phenolic substance was 101.83 mgGA/g, it was determined that giving pollen to obese mice for 8 weeks reduced the weight of the mice by 18.23% and 19.37%, lowered blood sugar and decreased lipid accumulation in the serum and liver. In addition, pollen application was able to reduce oxidative damage and inflammation in obese mice. Pollen extract was able to modulate the constitutive change in the gut microbiota, effectively inhibiting NAFLD formation by inhibiting the expression of LXR-, SREBP-1c and FAS genes in obese mice. Researchers have suggested that pollen extract can be used in the treatment of obesity as it can improve metabolic syndromes in obese mice.

In addition to this, antiproliferative and antiapoptotic properties of metformin, which we used as a positive control in our study, were indicated in various studies (Jia et al., 2015; Lai et al., 2018). It is known today that ROS and oxidative stress emerging as a result of ROS play a significant role in apoptosis, while antioxidants can delay or prevent this process (Kannan and Jain, 2004).

3. Discussion and Conclusion

For this reason, the activity of bee products, which have strong antioxidant activity, should be taken into consideration. The role of bee products, which provide versatile support with their natural and beneficial biological effects to promote treatment of important diseases such as obesity, must be revealed. It is thought that bee bread will have advantages compared to

traditional pharmaceuticals or complementary components for nanoparticle production since it has therapeutic potential in obesity and other metabolic processes associated with it. In addition, rationally designed bee products can be utilized for the treatment of many diseases, including cancer, with future research and developing technology

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The Effect of Apilarnil on Infertility

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Abstract:
For centuries, honeybee products such as honey, bee pollen, royal jelly, bee bread (Perga), and bee venom have been utilized in natural medicine due to their beneficial properties. A great deal of scientific research has been dedicated to exploring their physico-chemical properties and therapeutic effects. Despite this, drone larvae have not received as much attention from the scientific community. Within a honeybee colony, drones are responsible solely for fertilizing queen bee eggs and consuming food reserves collected by worker honeybees. As a result, beekeepers commonly remove excess drone brood from the hive, which is crucial for preventing and treating varroasis. Lyophilization is the most effective method for preserving drone larvae, and the physicochemical properties of fresh and lyophilized drone larvae were compared. The therapeutic effects of drone larvae, such as androgenic, hepatoprotective, immunostimulatory, and hypolipidemic effects in humans and experimental animals, were summarized. This study aims to summarize current scientific knowledge on drone larvae (apilarnil) and its effect on infertility.

Keywords: drone larvae, apilarnil, drone brood, infertility,

1. Introduction

Apitherapy, which has existed since the beginning of humanity and whose importance has increased with scientific studies in recent years, draws attention in terms of preventing many diseases and supporting treatment. Natural bee products such as honey, pollen, Perga, propolis, royal jelly and apilarnil have high antioxidant capacity as well as rich nutritional content (Kumazawa et al. 2004; Leja et al. 2007; Özkök and Silici 2017; Eraslan et al. 2017). These valuable products, which honeybees process by collecting nectar and pollen from plants in nature, consist of many bioactive compounds such as protein, carbohydrates, vitamins, enzymes, phenolic compounds, aroma compounds, phytosterols, terpene and terpenoids, fatty acids and aliphatic compounds. Many different biologically active components of honeybee products have been held responsible for different effects such as antioxidant, antimicrobial, antifungal, immunostimulant and anticarcinogenic activities (Eraslan et al. 2008; Koc et al. 2009; Nassar et al. 2012; Miyata & Sakai 2018). The number of scientific studies on Apilarnil a recently discovered bee among the bee products, is very limited.

Drone brood production depends on pollen supply to meet protein needs. Colonies regulate drone production according to season and food availability (Hrassnigg & Crailsheim 2005). Optimal drone production depends on climate, colony conditions (size and queen age) and food availability (Boes 2010). Regular removal of drones from a colony helps to regulate drone

production. In the Northern Hemisphere, drone production is typically concentrated between May and August. However, the production of drones is ultimately determined by the number of drone honeycomb cells in the colony (Boes 2010). During the drone breeding season, beekeepers can place empty frames in the colony to stimulate the production of drone broods. Removing drone larvae from these combs where Varroa spp. mites laying their eggs can help control their population in the hive. This method has been found to be effective in managing *Varroa* spp. infestations, which can be detrimental to the colony's health (Calderone 2005).

A drone larva (Apilarnil) is obtained by collecting drone larvae 3 to 11 days after hatching. This valuable bee product was discovered by the Romanian scientist Nicolae V. Iliesiu. This name consists of “api” for bee, “lar” for larva and “nil” which is a shortened form of explorer name. However, while drone larvae are collected from the honeycomb cell, they can be collected together with the larval food (drone milk) or it can be obtained by eliminating the larval food.

The content of main components and physicochemical properties of drone larvae are presented in Table 1 (Balkanska et al. 2014; Silici 2019; Koşum et al. 2022; Margaoan et al. 2017; Barnutiu et al. 2013; Prikhodko et al. 2020; Isidorov et al. 2016; Finke 2005; Bogdanov 2016; Sawczuk et al. 2019). The greatest differences in the physicochemical composition were reported between fresh and lyophilized homogenate in terms of water content. While the water content of fresh apilarnil is between 70.30-76.8%, it decreases to 3.0-5.0% when apilarnil is lyophilized.

Apilarnil is a rich source of protein and amino acids as they are the most abundant nutrients in its composition. The protein content of drone larvae is very important for the evaluation of their nutritive properties. Comprehensive analysis proved that the total content of amino acids in brood larvae is 37.57-40.57% (Lazaryan et al. 2002). The amino acid composition was characterized by high levels of glutamic acid, valine, aspartic acid, lysine and leucine (Lazaryan et al. 2002; Isidorov 2016). These amino acids are absorbed and utilized for various metabolic processes, including the synthesis of tissue proteins for growth and repair. Amino acids can also be used to synthesize other nitrogen-containing compounds such as neurotransmitters and nucleotides or catabolized for energy production when needed (Atherton et al. 2010). Among analyzed carbohydrates in apilarnil, fructose and sucrose are at very low levels compared to glucose. Fructose, glucose, sucrose, turanose, maltose, trehalose and isolmaltose contents of fresh apilarnil were determined as 0.6, 3.61,0.14, 0.05, 0.33, 0.44, and 0.11, respectively (Barnitiu et al. 2013). Apilarnil is a rich source of carbohydrates (Balkanska et al. 2014) and is composed of glucose trehalose and glycogen (Lipinski et al. 2008). The carbohydrate level is related to the developmental stage of the larvae. According to the analysis results obtained from the research; the lipid level of apilarnil is approximately 3 times higher in lyophilized form than in fresh one (Table 1). It is an important group of nutrients consisting of lipids, free fatty acids, tri-di- and monoacylglycerols, sterols, phospholipids, and vitamins. Especially the composition and content of fatty acids have an important effect on the functional properties of foods. Alpha linoleic acid (omega 3) and linoleic acid (omega 6) are known as essential fatty acids. The analysis results provided show that drone larvae are a rich source of lipids and fatty acids. (Calder 2015) and lipid contents include free fatty acids, sterols, triacylglycerols, and phospholipids (Isidorov et al. 2016). Fatty acids are energy sources and cell membrane components. It can affect numerous cell characteristics such as metabolism, gene expression,

hormone sensitivity and production of biologically active substances. Therefore, they can affect people's health, physiological function, well-being, and disease risk.

Vitamins are needed for a normal and healthy metabolism, their deficiency can lead to serious diseases and even death. Apilarnil is rich in fat and water-soluble vitamins. The analyzes have reported that the vitamin A content of drone larvae is 0.01-0.05 mg/100 g. Vitamin A (retinol) is a vitamin that is needed in small amounts. Beta carotene is the main source of provitamin A in the diet. It is necessary for the maintenance of vision and immune function, growth and development, epithelial cell integrity and reproduction. The vitamin D content of apilarnil has been reported to be between 0.03-0.9 mg/kg. Vitamin D is needed in all cells of the body for muscle contraction, mineralization of bone, nerve conduction, and maintenance of blood calcium and phosphate levels (Nordin 1976). Fat-soluble vitamin E, which we can only get through diet, is the main antioxidant in the cell (Food and Nutrition Board 2000). Vitamin C is a water-soluble vitamin that plays an important role in the antioxidant system (Sies 1993). Vitamin E content of apilarnil has been reported as 0.4-1.6 mg/kg. Among the B complex vitamins, the highest amount of B vitamin detected is choline (44.3-68.1 mg/100 g). It was followed by nicotinic acid (Vit B3), riboflavin (Vit B2), pantothenic acid (Vit B5), thiamin (Vit B1) and pyridoxine (Vit B6) and biotin.

Scientific research shows that reactive free radicals play a role in many diseases such as heart disease, diabetes and cancer. The cell contains potentially oxidizable substrates such as proteins, fatty acids and DNA (Sies 1993). Therefore, the antioxidant defense system protects it from the harmful effects of free radicals, which are normally produced endogenously in the cell, as well as pollutants and exogenous species such as cigarette smoke. If exposure to free radicals, called oxidative stress, exceeds the protective capacity of the antioxidant defense system, damage to biological molecules may occur. Consuming foods with antioxidant activity that can potentially eliminate or neutralize free radicals may play an important role in disease prevention. Lipophilic molecules such as alpha-tocopherol, retinol and coenzyme Q10 are antioxidants with basic regulatory and metabolic functions in living organism cells. Vitamin E, an endogenous antioxidant, protects lipids in the cell membrane against peroxidation. Along with vitamin E, vitamin A and beta-carotene also protect against oxidation. Another cellular antioxidant is Coenzyme Q10 which acts as an electron carrier in the mitochondrial respiratory chain (Sies 1993). Hryniewicka et al. (2016) determined the content of alpha-tocopherol and coenzyme Q10 in honey bee-derived animal products such as royal jelly, bee bread and drone larvae homogenates by liquid chromatography-tandem mass spectrometry (LC/MS/MS). They found that apilarnil is a rich source of coenzyme Q10. The drone homogenate contained only 8±1 µg/g α-tocopherol and 20±2 µg/g coenzyme Q10.

The effect of apilarnil on infertility

Apilarnil is a natural substance produced by honeybee larvae, which contains a range of hormones. These hormones include testosterone, which is a male sex hormone, as well as female sex hormones such as estradiol, progesterone, and prolactin. These hormones are important for the development and maintenance of reproductive functions in both males and females, and they have been used in traditional medicine for their potential health benefits (Budnikowa 2009; Burmistrova 1999; Bolatovna et al. 2015). It is known that sex hormones such as testosterone, progesterone and estrogen play important roles in various physiological

processes besides reproductive functions and the formation of secondary sex characteristics (Rider & Abdou 2001; Roof & Hall 2000). Testosterone is produced in the ovaries and testicles. Androgens have important roles in muscle development, bone density, production of red blood cells, maturation during puberty, libido and sexual function in both men and women. It also has roles such as regulating menstruation and preventing osteoporosis in women. The other sex hormone, progesterone, receptors have been identified in the brain, cortical and subcortical regions (Woolley & McEwen 1993). This hormone has neuroprotective effects and is effective in promoting nerve regeneration and myelination (Schumacher et al. 2014). Estradiol is an effective hormone in the modulation of neurotransmitter synthesis, release and metabolism, while prolactin is a hormone responsible for breast tissue development and milk production (Barth et al. 2015; Glasier et al. 1984). The content of the hormone of drone larvae is presented in Table 5. Fresh drone homogenate was found to contain 0.31 nmol/100g testosterone, 51.3 nmol/100g progesterone, 410 nmol/100g prolactin and 677.6 nmol/100g estradiol Budnikowa (2009) revealed the dynamics of sex hormones from larva to pupa, while five-day-old larvae contained 8.2 nmol/l testosterone and 2745 nmol/l, 15-17-day-old pupae contained 15.6 nmol/l testosterone and 343.5 nmol/l estradiol. Drone larvae were found to have more pronounced gonadotropic activity than royal jelly. It has the highest amounts of estradiol and prolactin while the lowest levels of testosterone. The testosterone content of apilarnil is about 0.03 nmol/ml has been reported. Studies conducted to date on the therapeutic activities of apilarnil; estrogenic and androgenic effects, antioxidant capacity, protecting testicular damage, reducing sexual dysfunction, protecting testicular toxicity and liver injury, neurprotective effect, stimulating immune system, antiatherosclreotic activity, etc.

Recent studies have shown that drone larvae have both estrogenic and androgenic effects. Seres et al. (2014) found that DM exhibits significant sexual hormonal effects in rats. Drone milk displayed marked androgenic activity in castrated male rats. They identified the compounds methyl oleate and methyl palmitate which are responsible for its androgenic effect. In a study of the estrogenic effect of apilarnil, this effect was attributed to E-dec-2-enedioic acid (Seres et al. 2013). Another study on drone milk by Seres et al. (2014b) showed that the combination of drone milk and spironolactone has a potent gestagenic effect.

Anabolic substances change the metabolism in the direction of increasing the formation of muscle mass and bone tissue, and in the direction of consumption of fat stores. It has been determined that the administration of drone brood homogenate to pigs affects the hormonal status and increases the growth rate (Zdorovyeva et al. 2018). The addition of drone brood homogenate (25 mg/kg feed) to the pig diet showed an anabolic effect and significantly stimulated the growth rate of the animals (Boryayev et al. 2017). In another study, drone homogenate improved the characteristics of the ejaculate, and had a stimulating effect on the reproductive function of rams (Shoinbayeva 2017). Kosum et al. (2022) showed that drone larvae provide sexual function restoration for Saanen male goat kids. In addition, it was reported that testicular growth and an increase in the production of androgen hormone were obtained in the study. In a study on pigs, it was shown that the supplementation with drone brood homogenate stimulates the early stages of folliculogenesis in gilts but provokes atresia of follicular development (Kistanova et al. 2020). In male and female broilers, apilarnil did not have a positive effect on growth performance, but it reduced blood glucose and cholesterol

levels (Altan et al. 2013). An increase in testicular weight, and testosterone level has been shown to stimulate sexual maturation in the early stages. It was found that dietary apilarnil did not have a positive effect on growth performance in male and female chickens, and apilarnil did not show an anabolic effect.

It is reported that eighty million people worldwide are affected by the inability to have children. It has been reported that the high amount of free oxygen products (ROS), such as hydrogen peroxide (H_2O_2), nitric oxide (NO), and peroxynitrite, in spermatozoa, is associated with male infertility. In scientific studies, it has been determined that many antioxidants improve sperm quality and prevent sperm damage; such as vitamins E and C, coenzyme Q10, glutathione, folic acid, zinc and selenium, (Agarwal & Sekhon, 2010). Coenzyme Q10 has been shown to protect the cell membrane against oxidative stress (Bentinger et al. 2010), folic acid plays a role in DNA synthesis and scavenges free radicals (Joshi et al. 2001). It has been reported that lycopene, vitamins A, C and E are antioxidants used to improve sperm quality, and vitamin E, for example, significantly reduces DNA fragmentation rates and improves sperm quality (Ebisch et al. 2007; Rolf et al. 2009). In recent studies, it has been determined that n-3 PUFA deficiency in the diet affects spermatids (Roqueta-Rivera et al. 2011). Nutrition affects sperm quantity, semen quality, and fertility status (Stevermer et al. 1961). Protein quality is highly dependent on amino acid content and amino acid bioavailability (Kim et al. 2009). Different amino acid ratios have significant effects on reproductive performance (Ren et al. 2015). In studies on different experimental and farm animals; dietary lysine (Lys) has been shown to improve semen quality from 0.86% to 1.03% (Rupanova 2007). The amino acid composition of seminal plasma significantly affects sperm motility. Li et al. (2003) determined that the addition of amino acids proline, glutamine and glycine) improved sperm membrane and acrosome integrity as well as sperm motility in monkey semen. Research findings suggest that apilarnil has the potential to function as a natural stimulant in the animal endocrine system, which could be beneficial for restoring and improving male sexual desire (Vakina et al. 2020). A preparation containing drone larvae homogenate has been utilized to regulate androgenic activity in females (Elistratov et al. 2017). Androgen deficiency syndrome causes a decrease in the development of the penis and testicles at an early age and prevents puberty. In young people, gynecomastia causes weakness in facial, body or pubic hair and voice development, while in adults, it causes problems such as mood changes, decreased muscle strength, increase in body fat, decreased libido, difficulty in erection, low sperm volume and gynecomastia. Doganyigit et al. (2019) in their study, which tested the protective effect of apilarnil on endotoxic shock, reported that apilarnil reduced testicular damage caused by LPS and this effect was due to the antioxidant capacity of apilarnil.

2. Results

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Table 1. Chemical composition of drone larvae (fresh and lyophilized)

Characteristics*	Drone larvae	
	Fresh Range (Min-Max)	Lyophilized Range (Min-Max)
Water, %	65.0-78.5	3.0-5.0
Protein, %	4.6-13.2	32.0-52.4
Lipid, %	1.2-8.38	4.8-24.2
Carbohydrates, %	6.22-12.2	9.30-38.9
Fructose	0-0.38	-
Glucose	3.55-7.88	-
Sucrose	0-0.18	-
Ash, %	0.7-4.1	2.7-4.1
pH	5.8-6.63	7.0
Acidity, ml 0.1 NaOH g^{-1}	0.74-2.61	-
Energy value, kJ100g^{-1}	111.9-503.3	501.4-2097.9

3. Conclusion

Drone larvae (Apilarnil), is a little-known honey bee product rich in nutrients and exhibits many healing and therapeutic properties. It has been used as a cheap, safe and effective natural food against different diseases. Its high protein and essential amino acid content, fatty acid composition, vitamin and mineral richness, and hormonal content differentiate it from among other bee products with apilarnil. The biological and therapeutic activities of drone larvae have been confirmed by performing laboratory and animal/human in vivo experiments. In addition to the many biological activities of this product, it is thought that it may lead to important developments in the future, especially in the field of infertility.

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Green Extraction of Propolis with Different Oils and Their Chemical and Oxidative Properties

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Abstract:

Propolis is one of the natural and functional beekeeping products produced by bees and used in different areas such as food, pharmaceuticals, and cosmetics because of its antimicrobial, antioxidant, antitumor, anti-inflammatory, and antimutagenic effects. It contains chemical compounds mainly apolar characteristics like plant resin, wax, and essential and aromatic oils. Since raw propolis is difficult to use, propolis extracts are produced, and its extraction is mostly performed with alcoholic solutions. Besides, propolis extraction with olive, hazelnut, canola, coconut, and soybean oils has been reported in previous studies as alternative natural extraction solvents. Herein, we focus on the extraction of propolis with commercially available vegetable oils such as corn, olive, and sunflower, and the determination of phenolic, flavonoid, antioxidant, and oxidative properties of obtained extracts. The phenolic contents of propolis extracts with corn, olive, and sunflower oils were determined as 93.56, 93.40, and 74.71 mg GAE/100 g, respectively. The phenolic and flavonoid extraction yields were between 1.92-2.49% and 1.12-1.39%, respectively. The corn oil-propolis extract showed the highest yield results than the olive and sunflower oil extracts. According to Schaal oven storage stability test results, peroxide and conjugated diene values of oils and propolis-oil extracts increased during 10 days of storage. The peroxide results for corn, olive, and sunflower oils were 95.34, 20.05, and 135.21 meq O_2/kg, whereas they were detected as 21.53, 12.51, and 115.90 meq O_2/kg in propolis oil extracts, respectively. Regarding conjugated diene results, the corn oil-propolis extracts were more stable against oxidation than the other samples. Overall, natural vegetable oils also have promising potential used in propolis extraction in addition to common solvents, and corn oil-propolis extracts could also be produced in addition to the existing commercial olive oil-propolis extracts as it has both extraction efficiency of phenolic compounds and oxidation stability.

Keywords: propolis phenolic, inhibition, oxidation, conjugated diene

1. Introduction

Propolis is one of the value-added beekeeping products and it is produced from plant resins by bees. It is not only used for sealing cracks in the hive, smoothing inside of the hive by bees but also ensuring inhibition of bacteria, fungi, and viruses to maintain aseptic conditions of the hive (Bankova et al. 2021). It has been identified over 800 compounds in the different propolis types from various geographical locations. Propolis mainly contains plant resin and balsam, beeswax, volatile compounds, aliphatic and aromatic acids and their esters, phenolic acids, flavonoids, terpenoids, amino acids, B group, C and E vitamins and minerals such as aluminum, calcium, zinc, cesium, iron, manganese, and copper (Kasote et al. 2022). Thanks to these rich chemical compounds, the antimicrobial, antioxidant, anti-inflammatory, immunomodulating, anticancer, and cytotoxic effects of propolis have been noted in various studies (Bankova et al. 2021).

Propolis contains these components in a water-insoluble apolar matrix, and these components must be extracted to show their stated activities and ensure beneficial effects on humans (Bankova et al. 2021). There are different extraction methods such as maceration, ultrasonication, Soxhlet, microwave-assisted, supercritical CO_2 extractions, and extraction solvents like ethanol, methanol, ethyl acetate, chloroform, ether, acetone, benzene, dimethylsulfoxide, dichloromethane and propylene glycol (Bankova et al. 2021; Irigoiti et al. 2021). However, these chemicals produce waste and that can be hazardous to the environment.

Green extraction techniques aim to minimize sample treatment processes, reagent consumption, and waste formation (Armenta et al. 2019). The vegetable oils can be considered green solvents and extraction of propolis with these oils can be an eco-friendlier method compared with other solvents (Chutia and Mahanta 2021). Extraction of different bioactive components such as phenolic compounds (Damechki et al. 2001; Jabri Karoui et al. 2016), carotenoids (Chutia and Mahanta 2021; Sharma and Bhat 2021), and aromatic compounds (Gambacorta et al. 2007) with vegetable oils have been reported in some previous studies. Besides, bioactive components of propolis were extracted with different vegetable oils such as canola, soybean, coconut, and olive oils (Bankova et al. 2021). Based on these studies, it was aimed in the presented study to extract propolis with commercially available vegetable oils such as corn, olive, and sunflower, and the determination of phenolic, flavonoid, antioxidant, and oxidative properties of obtained extracts.

2. Materials and Methods

2.1. Materials

The propolis sample was obtained from Bingöl, Türkiye, and corn, olive, and sunflower oils were purchased from the local market. Chemicals used in the analyses were selected as analytical purity and purchased from Sigma (Taufkirchen, Germany) firm.

2.2. Methods

2.2.1. Extraction of propolis

The extraction of propolis was performed with corn (CO), olive (OO), and sunflower (SO) oils. Oil:propolis (20:1) was mixed at 25 °C, 150 rpm, for 24 h. After that, the mixtures were centrifuged at 6000 rpm for 15 min, and upper phase was removed and used for the analyses. Besides, propolis was extracted with %80 methanol under the same conditions, and this sample was used as a control. The corn oil:propolis, olive oil:propolis, and sunflower oil:propolis extracts were coded as COPE, OOPE, and SOPE, respectively.

Oil:propolis extracts and oils were also prepared for the total phenolic, flavonoid and antioxidant analyses with hexane and 80% methanol extraction as described by Silici and Baysa (2020).

2.2.2. Total phenolic and flavonoid content analyses

The 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin-Ciocalteu and 2 mL of 7.5% sodium carbonate solution. That mixture was kept at 50 °C for 5 min and waited for cooling

until room temperature in a dark place. The sample absorbance was determined at 760 nm with a spectrophotometer (Shimadzu, UV-1280, Japan). The results were calculated by using gallic acid standard curve and expressed as mg GAE/100 g (Škerget et al. 2005).

The 2 mL of the extract was mixed with 2 mL of 2% aluminum chloride methanolic solution and left in the dark for 30 min. The absorbance was determined at 415 nm with the spectrophotometer. The results were calculated by using quercetin standard curve and expressed as mg QE/100 g (Bueno-Costa et al. 2016).

The extraction yields (%) for total phenolic and flavonoid contents were calculated by the following equation.

$$Yield = \frac{(Total\ phenolic\ or\ flavonoid\ content\ of\ extract) - (Total\ phenolic\ or\ flavonoid\ content\ of\ oil)}{Total\ phenolic\ or\ flavonoid\ content\ of\ control\ sample}\ x100$$

2.2.3. Antioxidant activity analysis

The 100 µL of the extract was mixed with 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution at 0.039 g/L concentration and left in the dark for 30 min. The absorbance was determined at 515 nm with the spectrophotometer. The results were calculated by using Trolox standard curve and expressed as g TE/kg (Yildiz et al. 2021).

2.2.4. Schaal oven test of oils and oil:propolis extracts

The 3 g of the oil:propolis extracts and oils were weighted in open glass bottles and left 10 days at 60 °C in an oven. The peroxide and conjugated diene values as oxidative stability indicators of samples were determined at 0., 1., 2., 3., 6., 8., and 10. days of storage. These analyses were carried out according to the reported method by Yildiz et al. (2021). The peroxide analysis results were expressed as meq O_2/kg.

2.2.5. Statistical analysis

The extraction of propolis and all analyses were performed in duplicate, and the statistical evaluations were made with the SAS statistical program (SAS Statistical Software, v.9.00, USA). The results were given as mean ± standard error, and statistical significance was evaluated according to $p < 0.05$.

3. Results

The results of the total phenolic and flavonoid content analyses, and peroxide, and conjugated diene values of oils used in propolis extraction were given in Table 1. The oils had statistically significant ($p < 0.01$) differences in terms of these parameters. The total phenolic, flavonoid and DPPH values were high in olive oil. As for oxidation-related results, while the peroxide value of olive oil was high, corn oil had a higher conjugated diene value than other oils.

Table 1. Properties of oils used in propolis extraction

Sample	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/100 g)	DPPH antioxidant activity (g TE/kg)	Peroxide values (meq O_2/kg)	Conjugated diene values
Corn oil	0.45^{b} ± 0.02	0.21^{c} ± 0.00	0.15^{b} ± 0.00	6.88^{c} ± 0.16	11.34^{a} ± 0.08
Olive oil	21.61^{a} ± 0.55	0.93^{a} ± 0.00	0.17^{a} ± 0.00	14.88^{a} ± 0.88	2.12^{c} ± 0.01
Sunflower oil	0.62^{b} ± 0.01	0.34^{b} ± 0.01	0.15^{b} ± 0.00	9.97^{b} ± 0.67	4.46^{b} ± 0.01
Significance	**	**	**	**	**

Superscript letters beside the mean values and ** symbol show differences at $p \leqslant 0.01$ level.

The results of the total phenolic and flavonoid content analyses oil:propolis extracts were shown in Table 2. The oil type had astatistically significant ($p < 0.01$) effect on propolis extraction in terms of these parameters. The total phenolic, flavonoid, and DPPH values were high in corn oil:propolis and olive oil:propolis extracts. Moreover, extraction yields for phenolic and flavonoids were determined between 1.92-2.49% and 1.12-1.39%, respectively. The corn oil:propolis extracts also had the highest extraction yield results.

Table 2. Properties of propolis and oil:propolis extracts

Sample	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/100 g)	DPPH antioxidant activity (g TE/kg)
Control	3746.64 ± 21.53	2478.77 ± 4.71	72.64 ± 1.55
COPE	93.56^{a} ± 0.16	34.54^{a} ± 0.06	2.80^{a} ± 0.05
OOPE	93.40^{a} ± 0.09	31.71^{b} ± 0.17	2.83^{a} ± 0.04
SOPE	74.71^{b} ± 0.43	27.75^{c} ± 0.09	2.31^{b} ± 0.01
Significance	**	**	**

Superscript letters beside the mean values and ** symbol show differences at $p \leqslant 0.01$ level.

The results of the oxidative stability analyses were presented in Figure 1. It was detected that the oxidation properties of oils and oil:propolis extracts showed different characteristics. The propolis extracts with corn and olive oil ensured good oxidation stability, while sunflower oil and sunflower oil:propolis extracts rapidly oxidized after the 3. days and significant increases in peroxide and conjugate values were observed. Besides, it can be evaluated that propolis addition to corn oil ensured significant protection against oxidation. However, this protective effect could not occur in sunflower oil.

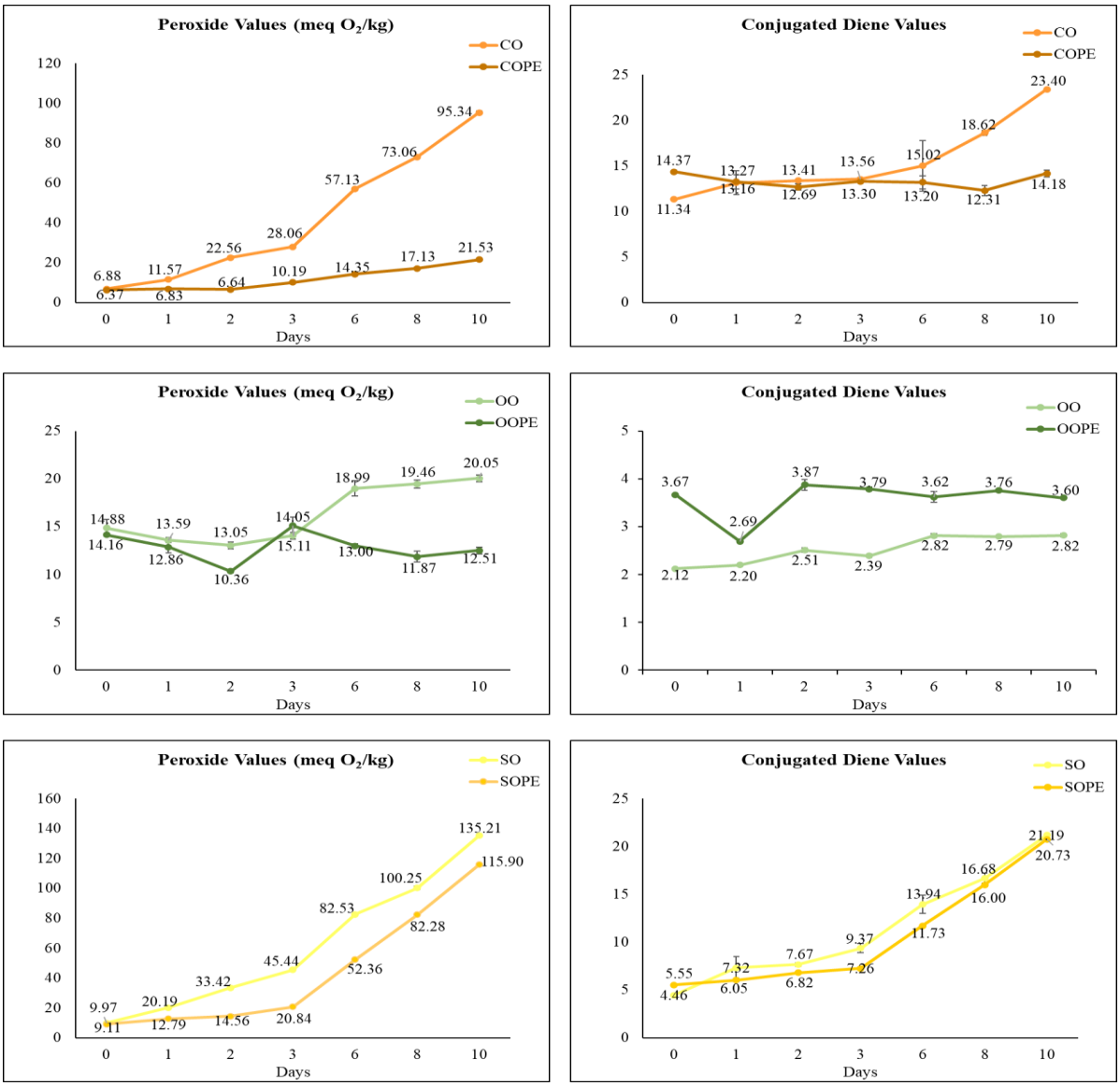

Figure 1. Schaal oven storage stability test results of oils and oil:propolis extracts (CO: corn oil, COPE: corn oil:propolis extract, OO: olive oil, OOPE: olive oil:propolis extract, SO: sunflower oil, SOPE: sunflower oil:propolis extract)

4. Discussion

The total phenolic and flavonoid contents of oils are naturally low because of the refining process carried out for removing undesirable constituents. It has been noted that chemical refining processes such as degumming, neutralizing, washing, bleaching, winterizing, and deodorizing cause the loss of bioactive components (Laulloo et al. 2015; Szydłowska-Czerniak and Łaszewska 2015). In addition, the antioxidant activity of oils considerably decreases depending on loss in polyphenols, tocopherols, sterols, and carotenoid amounts (Szydłowska-Czerniak and Łaszewska 2015).

After propolis extraction the total phenolic, flavonoid, and antioxidant activity of all samples substantially increased because of phenolic compounds coming from propolis. It has been reported that propolis contains lots of phenolic acids such as benzoic acid, phenyl-methyl ester of benzoic acid, salicylic acid, phenyl-methyl ester of salicylic acid, gallic acid, and protocatechuic acid and their derivatives, and flavonoids such as apigenin, catechin, galangin,

pinobanksin, kaempferol, luteolin, naringenin, pinocembrin, pinobanksin, rhamnetin, rutin, and quercetin (Anjum at al. 2019). Besides, the antioxidant activity of propolis is mainly associated with its phenolic content and composition (Forma and Bryś 2021). The total phenolic content of propolis is reported to be 2748.60- 19969.90 mg GAE/100 g (Özdal et al. 2019), and 3453-25940 mg GAE/100 g (Özkök et al. 2021), total flavonoid content was indicated as 388.00-5831.00 mg QE/100g (Degirmencioglu et al. 2019), and 3073.90-29175.00 mg QE/100 g (Özdal et al. 2019) in some previous studies. In a study, it was indicated that the total phenolic content of olive oil increased after propolis extraction and, the total phenolic content of olive oil:propolis extracts at 10-40% propolis concentrations were stated to be 633.76-2064.74 mg GAE/100 g (Silici and Baysa 2020). These values are much higher than the results of the presented study and the difference might be associated with high propolis concentration, and extraction procedure which were applied <40°C for a week by Silici and Baysa (2020).

The phenolic and flavonoid extraction yields were different in each oil type and these differences might cause by the amount and type of the compounds found in oils interacting or binding with phenolic compounds. Different propolis extraction yield results were declared between soybean (5.58%) and canola oils (2.72%) (Schmidt et al. 2014), and olive and virgin coconut oils (Pujirahayu et al. 2014).

The difference in oxidation stabilities of extracts might have resulted from the different fatty acid profiles of oils, and extraction yields of phenolic compounds ensuring protection against oxidation thanks to their antioxidant activity. It was evaluated that extracted phenolic compounds had good protection although low extraction yields in propolis corn and olive oil extracts. Besides, unlike corn and sunflower oil, olive oil mostly contains oleic acid, which is a monounsaturated fatty acid (Jabeur et al. 2014). Therefore, that gains more stability against oxidation comparing other oils. Sunflower oil:propolis extract was vulnerable to oxidation depending on the lowest phenolic compounds extraction yield. Furthermore, sunflower oil is a rapidly oxidizable vegetable oil due to its high linoleic acid content which is a polyunsaturated fatty acid (Javidipour et al. 2017).

5. Conclusion

In conclusion, the corn oil-propolis extract showed the highest phenolic and flavonoid extraction yield results than other extracts. Moreover, regarding peroxide and conjugated diene analysis results, propolis addition gained more oxidative stability to the corn oil. Olive oil also showed good stability against oxidation depending on both the olive oil nature and the addition of propolis. Based on these results, natural vegetable oils have promising potential for propolis extraction in addition to common solvents after the making extraction optimization for reaching higher extraction yields, and corn oil-propolis extract can also be produced in addition to the existing commercial olive oil-propolis extract because it has both extraction efficiency of phenolic compounds and oxidation stability.

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