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APPROPRIATE MACERATION DURATION FOR THE EXTRACTION OF PROPOLIS

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ABSTRACT

The importance of the use of natural products as nutritional supplements in order to increase the quality of life and prevent diseases has increased in recent years. Among these products, propolis produced by honey bees is collected from plants and has a very complex structure in terms of chemical content. Propolis and its extracts have been used by humans for centuries because of its various bioactive properties. Although different methods can be used in the extraction stage, the most commonly used technique is extraction by maceration. However, there is no standard time period for extraction of propolis by maceration. We aimed to determine the changes in the concentrations of flavonoid group compounds pinocembrin, chrysin, tectochrysin, pinostrobin chalcone, gengwanin, naringenin and galangin from propolis samples exposed to seven different periods (1, 2, 5 10, 15, 20, 30 days) of maceration using GC-MS. The flavonoids pinocembrin, chrysin, tectochrysin, and naringenin reached their maximum concentrations at the end of 15 days of maceration and a decrease in their concentrations was observed after this period. The concentrations of other flavonoids did not show a steady increase or decrease with different maceration times. As a result, it can be said that the appropriate duration of maceration to extract the flavonoids responsible for propolis' numerous activities varies depending on the origin of the propolis and the nature of the compounds to be extracted.

KEYWORDS:
Propolis, maceration, flavonoid, extraction, chemical composition

INTRODUCTION

Propolis having hundreds of polyphenols, is a mixture produced by the honeybee [1]. Honeybees enrich propolis from different plant sources by mixing it with active enzymes and plant pollen secreted from the glands between their head and the thorax, and the final product is known as crude propolis [2,3]. In addition to pharmacological properties of clinical interest (antimicrobial, antiinflammatory, antioxidant, antiproliferative), propolis in general is also considered a functional food, as the biologically active constituents in its extract have been documented to provide health benefits [4, 5]. Crude propolis is hard and wax-like when cool, but soft and very sticky when warm. The material has a pleasant aromatic smell; its color varies from yellow, green, or red to dark brown, depending on its plant origin and age [6]. It cannot be consumed directly owing to the presence of a mixture of ash, wax, bioactive compounds, and pollen. Therefore, various extraction procedures are used to separate the bioactive components of propolis [7]. The purpose of the extraction is to provide the maximum amount of material at the highest possible quality. Factors such as pretreatment of the sample, solvent/sample ratio, solvent type, extraction time, and temperature are important in the extraction stage [8]. The propolis extracts are used in various fields, such as food, pharmaceuticals, and cosmetics, and therefore the chemical composition and the quality vary according to the procedure applied [9].

For propolis extraction, different methods, such as traditional maceration extraction, ultrasound extraction, soxhlet extraction, supercritical fluid extraction, and microwave assisted extraction, are used, with method traditional maceration extraction being the most commonly applied [3]. Ethanol is the best solvent for propolis preparation, and other solvents such as ethyl ether, water, methanol, and chloroform may be used for extraction and identification of propolis compounds. Despite being used for the preparation of propolis extracts in solvents and cosmetics industries, solvents other than ethanol does not extract as many bioactive compounds as ethanol does [10, 11]. Woisky and Antonio Salatino [12] found in their study on propolis that the use of 70% aqueous ethanol in the extraction stage produced approximately 20% more total phenolic material than extraction using absolute ethanol. Therefore, in recent years the commercially available propolis extract has been prepared using 70% ethanol solution, which extracts more bioactive compound compared to other solvents [13, 3, 10].

The compounds found in the chemical structure of propolis are an indication of its quality. Its biological activities are often associated with flavonoids

from polyphenols. Today, owing to the growing interest in natural products, many studies have been carried out to determine the biological activities of propolis, which are linked to the propolis flavonoid composition. However, in these studies conducted by different research groups, different maceration times were applied for the preparation of the propolis extract [14, 15, 16, 17]. Propolis found in Turkey has a very rich content in terms flavonoids, as supported by research [18, 19, 20]. Methods that will allow the extraction of flavonoids in propolis produced in Turkey are of great importance to establish a standard in this regard. Therefore, we aimed to establish a relationship between the propolis flavonoid concentration and the duration of maceration in extracts prepared for human consumption using ethanol, and the chemical content of which changes depending on many factors.

MATERIALS AND METHODS

Origin of Propolis. The propolis sample used in this study was collected from Turkey's Eastern Black Sea region from an apiary in the province of Bayburt in June 2017. The collected propolis sample was left in the refrigerator for 24 hours at -18ºC, powdered with a grinder, and then prepared for extraction.

Maceration Extraction of Propolis. Extraction of propolis was carried out using a method described by Bayram et al. [17] with minor modifications. For the extraction, 70 g of the powdered propolis sample was placed in a dark bottle containing 210 ml of 70% ethyl alcohol and left to rest at room temperature on a stirrer. This mixture was filtered twice using Whatman papers 1 and 4, and 30-ml aliquots were removed at the end of the 1st, 2nd, 5th, 10th, 15th, 20th, and 30th days. The extracts obtained were diluted with 70% ethyl alcohol in a ratio of 1:10 (w / v) and evaporated to dryness.

Preparation for Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of propolis extracts. Each of the samples obtained as a result of the above-mentioned extraction process were subjected to heating at 80–100 ° C for 20 minutes with 75 µl of dry pyridine and 50 µl of bis(trimethylsilyl)trifluoroacetamide (BSTFA). Finally, 1.0 µl was taken from this last supernatant and injected into the GC-MS.

The gas chromatography-mass spectrophotometry (GC-MS) analyses conducted at the Environment and Instrumental Laboratory of Istanbul University using an Agilent brand GC (model 7890A) and MS (model 5975C) equipped with a mass selection detector (MSD). The GC was equipped with a DB-5MS column (30 m × 0.25 mm and 0.25 µm of film thickness) and an Agilent automatic injection system. The chromatogram was produced by holding the oven temperature at 35 °C for 8 min initially and then increasing the temperature to 60 °C at a rate of 6 °C/min followed by an increase at a rate of 4 °C/min to 160 °C and 20 °C/min to 200°C/min and kept at 200 C for 1 min at which it was held for 1 min. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. Split ratio 1:80, injector temperature 280 °C, ionization voltage 70 eV. Identification of components in propolis extracts were carried out with the WILEY-NIST MS data library.

Statistical Data Analysis. Repeated measures analysis of variance (ANOVA) was used to test the significance of the effects of maceration time. All statistical analyses were conducted with the software package Graphpad Prism 5.01(GraphPad Software Inc, San Diego). Each data point represents values from three independent experiments (n=3).

RESULTS AND DISCUSSION

Propolis resin is mainly composed of flavonoids, phenolic acids and their esters, which often form up to 50% of the total ingredients [21]. Phenolic compounds constitute the most numerous group of propolis components with respect to the quantity and type. Among them there are phenolic acids, phenolic aldehydes, phenols and their esters, ketophenols, coumarins and others compounds, including eugenol, anethole, hydroquinone, pterostilbene, naphthalene, *etc*. [22]. There are no standard extraction procedures or compositions for propolis extracts, with such products having many bioactive properties [3]. In the process of applying maceration for propolis extraction, ethyl alcohol retention is applied to propolis for periods ranging from 1 day [23] to 1 year [24]. We aimed to test the relationship between the flavonoid concentration and the maceration time for propolis collected from Turkey, Bayburt. According to the GC-MS analyses, we found that there was a statistically significant difference in the concentrations of pinocembrin, chrysin, tectochrysin, pinostrobin chalcone, gengwanin, naringenin and galangin after different periods of maceration (Table 1). As a result of 1, 2, 5, 10, 15, 20, and 30 days of maceration, 12.52%, 14.89%, 14.46%, 14.89%, 17.66%, 16.43%, and 16.07% concentrations of pinocembrin, respectively, were found in the propolis extracts, while the concentrations of tectochrysin were 4.82%, 6.07%, 6.19%, 6.10%, 6.86%, 6.75%, and 5.70%, respectively; the chrysin concentrations were 9.12%, 10.62%, 10.56%, 10.58%, 12.04%, 11.53%, and 11.58%, respectively; the pinostrobin chalcone concentrations were 7.38%, 9.62%, 9.76%, 9.54%, 10.07%, 10.11%, and 9.87%, respectively; the gengwanin concentrations were 1.64%; 1,39%, 1.73%, 1.30%, 0.93%,0.89%, and 0.74%, respectively; the naringenin concentrations were 5.06%, 8.72%, 8.53%, 8.77%, 10.13%, 9.65%, and 9.90%,.

TABLE 1
Changes in flavonoid concentration of propolis depending on time (% of total ion current)

Compounds	1 day	2 days	5 days	10 days	15 days	20 days	30 days
Pinocembrin	12.52	14.89	14.46	14.89	17.66	16.43	16.07
Tectochriysin	4.82	6.07	6.19	6.10	6.86	6.75	5.70
Chrysin	9.12	10.62	10.56	10.58	12.04	11.53	11.58
Pinostrobin chalcone	7.38	9.62	9.76	9.54	10.07	10.11	9.87
Gengwanin	1.64	1.39	1.73	1.30	0.93	0.89	0.74
Naringenin	5.06	8.72	8.53	8.77	10.13	9.65	9.90
Galangin	4.43	5.05	5.23	4.81	4.11	4.11	4.00

*P values less than 0.05 or equal were considered statistically significant.

respectively, and the galangin concentrations were 4.43%, 5.05%, 5.23%, 4.81%, 4.11%, 4.11%, and 4.00%, respectively. The results obtained indicate that there was no steady increase or decrease in the amount of any compound over the 30-day extraction period. However, a statistically significant increase in the concentrations of flavonoids pinocembrin, chrysin, tectochrysin and naringenin was observed up to 15 days of maceration. It was determined that there was a statistically significant decrease in the concentration of these compounds at the end of 20 and 30 days of maceration.

The concentration of pinostrobin chalcone was determined to be significantly increased at 1, 2, 5, 10, 15, and 20 days of maceration, and it was determined that there was a significant decrease in its concentration at the end of 30 days of maceration. It was observed that the value of genkwanin decreased steadily after 5 days of maceration, although there was no clear change during 1, 2, and 5 days of maceration. Galangin reached its highest level at 5 days of maceration. A steady decrease was observed in the galangin concentration after this period. A limited number of studies have investigated the effect of maceration on the chemical content of propolis extracts. In one of these studies, Cunha et al. [3] reported that the propolis composition appeared qualitatively the same after 10, 20, and 30 days of maceration, but there was a slight increase in yield with time. In another study, Cunha et al. [24] examined the effect of 20 days, 30 days, 6 months, and 1 year of maceration on the chemical composition of the Brazilian propolis. These researchers reported that the duration of maceration applied for crude propolis had an effect on the yield and that the yield of the components obtained increased to 60.1% (m/m) after 20 days of maceration and 67.0% (m/m) after 1 year of maceration. Similarly, Trusheva et al. [13] determined that the yield of biologically active substances in propolis extracts obtained by ultrasound extraction increased over time. Unlike the results reported by these researchers, we did not observe a time-dependent increase in the concentration of the components in our study. Fluctuations were seen in the concentrations of the compounds depending on the maceration time. This may be owing to the characteristics of the propolis sample or the flavonoids. Other studies have been conducted on the importance of maceration time in the extraction of bioactive compounds during the processing of different products consumed as food [25, 26]. Gambuti et al. [25] aimed to establish a relationship between the appropriate maceration duration and the concentrations of antioxidant compounds transresveratrol, quercetin, (+)-catechin, and (-)-epicatechin during the wine-making process from three different grape cultivars (Aglianico, Piedirosso, Nerello Mascalese). The researchers reported that different periods of maceration applied to grape varieties could affect the concentrations of (+)-catechin and quercetin Similarly, Merida et al. [26] found a positive correlation between duration of maceration and quercetin extraction. In one study, a significant reduction in the amount of phenolic compounds was observed after 20 hours of extraction of grape pulp [27]. These results, reported in different products, indicate that the appropriate maceration time for propolis, which has a highly variable and complex structure, may differ according to the origin of the propolis.

These results show that there is no optimum period of maceration that can be commonly applied for all of the compounds pinocembrin, chrysin, tectochrysin, pinostrobin chalcone, gengwanin, naringenin, and galangin and that the compounds are extracted with varying concentrations with different maceration times. However, according to the characteristics of the compound to be extracted, maceration times of 15 days for compounds pinocembrin, tectochrysin, chrysin, and naringenin, 20 days for pinostrobin chalcone, 5 days for gengwanin, and galangin yielded the highest concentrations. This indicates that the appropriate maceration period can be applied according to the desired compound so that the maximum concentration can be extracted from the propolis in accordance with the application area

CONCLUSIONS

Modern plant specialists recommend the use of propolis owing to its antibacterial, antifungal, antiviral, antiinflammatory, antidiabetic, and antiulcer properties [28, 29]. Despite the fact that propolis has gained so much popularity as a functional food in recent years because of its wide bioavailability, many countries are still unable to establish a clear standard

for the chemical content and extraction procedure. However, studies in this regard, as in many countries, have been carried out by numerous researchers in Turkey [30, 21, 19]. Although the researchers have recently started to use methods that allow more yield than traditional maceration extraction methods, the traditional maceration method is applied intensively by commercial propolis-selling beekeepers because it is easy to apply and does not require expensive equipment. Although new techniques have been developed, it is a disadvantage for beekeepers that the equipment in these techniques is expensive and not easily accessible. Therefore, it is essential to establish a standard procedure for the extraction of propolis by traditional maceration method. However, the fact that the complex chemical structure differs according to the region of origin is one of the factors that restricts the formation of a propolis standard. For these reasons, the duration of maceration to be applied to different propolis samples may also vary. In this respect, establishing a standard by determining the appropriate duration of maceration for the propolis sample exhibiting a characteristic structure for each region in this area may lead to higher yields in the extracts. This preliminary research is expected to contribute to the creation of standards for the extraction of these flavonoids observed at relatively high levels in propolis in Turkey. However, further, more detailed studies need to be carried out with more variable parameters and more samples than applied in this research to develop a standard process. We continue our detailed research in this regard.

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