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Evaluation of Different DNA İsolation Techniques in Honey, Pollen And Propolis Samples Belonging to Çankırı Province

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Highlights:

- DNA isolation methods
- The efficiency of the DNA extraction methods
- DNA quality in honey production

Keywords:

- Honey
- pollen
- propolis
- DNA isolation

ABSTRACT:

Honey is a natural and complex nutrient produced by collecting and processing secretions obtained from insects that feed on nectar or plant extract by bees. Honey, which is one of the most consumed natural foods, has many benefits in terms of human health. One of the most effective ways to access this information is to isolate the DNA found in honey by analyzing some of the specific gene sequences it contains. However, due to the inhibitors it contains, which are quite viscous in nature, there are a number of problems and limitations in the analysis of DNA in honey. In present study, it was aimed to compare 3 different isolation techniques, CTAB, TRIzol methods and DNeasy® Plant Pro Kit in honey, pollen and propolis samples and to recommend the most appropriate method for these samples. As a result of the spectrophotometric method and gel analyzes to image the genomic DNA, DNA isolation from propolis samples could not be performed with CTAB and DNeasy® Plant Pro Kit methods, and DNA isolation from honey sample could not be performed in TRIzol method. The TRIzol method was successful compared to the other two methods for the propolis sample. Especially for the propolis sample with a very dense chemical structure, the TRIzol method was studied for the first time and yield was obtained. The data obtained as a result of this study support the implementation of such applications for other types of honey and honey-based products produced in our country and thus the raising of the quality standards of honey, which has an important place in the economy of our country.

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INTRODUCTION

Since ancient times, honey has been consumed a lot due to its nourishing and therapeutic properties. In addition to its natural consumption, it is also used as a sweetener in many drinks and foods (Meo et al., 2017). Honey is produced and processed by honey bees (*Apis mellifera* L.) from the nectar of plants and honey extract. Honey is a natural product with a consistency consisting mainly of carbohydrates such as glucose and fructose, as well as important components containing amino acids, organic acids, enzymes, vitamins and minerals. It is also widely used in many foodstuffs because it has significant antioxidant activity (Ferreiro-González et al., 2018). There are also substances such as proteins, aroma compounds, various bioactive substances, volatile compounds, waxes, pollen grains, phytochemicals, phenolic compounds in honey (De-Melo et al., 2018). Phenolic compounds (phenolic acids and flavonoids) in honey have been accepted as the main components with many effects on human health such as antimicrobial, anti-inflammatory, antimutagenic, antitumor, antiviral, anti-oxidant, antibacterial agent, antioxidative activity (Ciucure et al., 2019). The botanical and geographical origin of honey varies depending on the weather at the time of harvest, the climatic conditions of the region and storage. There are about 320 different varieties of honey from various flower sources. The types of honey, the taste, color and smell of honey depend on the different sources of liquid of flowers and plants from which the bee receives honey. Honey varieties are classified in terms of temperature, precipitation, seasonal and climatic changes (De-Melo et al., 2018).

Carbohydrates (fructose, glucose, maltose, sucrose and higher sugars) constitute about 95% of the chemical composition of honey, 15-17% corresponds to water and 0.5% to protein. In addition, it contains enzymes (invertase, glucose oxidase, catalase, phosphatases), amino acids, organic acids (gluconic acid, acetic acid, etc.), lipids, vitamins (ascorbic acid, niacin, pyridoxine, etc.), volatile chemicals, phenolic acids, flavonoids and carotenoid-like substances and minerals.

The chemical composition of honey varies depending on the types of flowers, geographical region, season, storage and method of harvesting (Saxena et al., 2010). The proteins and amino acids in honey can be both animal and vegetable sources, the main of which are pollens. Due to the fact that pollen is the main source of amino acids contained in honey, it is also characteristic of the botanical origin of honey. The difference in protein content in honey varieties may vary depending on the origin of honey and the type of pollen (El Sohaimy et al., 2015). The feathers on the bees that wander the flowers to collect nectar, the raw material of honey, unwittingly hold the pollen. The bodies of honey bees undergo metamorphosis in order to collect pollen. They have feathers on their bodies that allow pollen to adhere when they come into contact with flowers, and pollen sacs on their hind legs that allow pollen to collect. Pollen is the most important and only natural food source for honey bees (Waykar and Alqadhi, 2016). Due to the high nutritional value of pollen and the vitamins, proteins and minerals in its content, it has been supported by researches that it has a protective and healing effect in many diseases. If we list the effects of pollen on human health with the researches conducted; memory strengthening, anti-anemia, metabolism regulator, nervous system sedative, immunity strengthening effects have been proven. It is effective in the rehabilitation of radiation and cancer, wounds and skin problems, beauty and prostate disease. It also stands out with its hypodemic, hypoglycemic, fibrinolytic and antimicrobial properties (Gomes et al., 2010; Silici, 2019; Muradian et al., 2020). Propolis is a natural resinous substance collected by honey bees (*Apis mellifera* L.) from the buds or exudates of plants near the hives and formed by hydrolysis of beeswax, pollen and salivary enzymes (Bankova et al., 2016). The color of propolis is usually brown, depending on the plant source from which it is collected by bees, but can vary up to yellow, red, green or black tones. It has a unique smell

due to the essential oils it contains. The chemical composition of propolis depends on various factors such as vegetation, geographical and ecological region, collection season, collection method and diversity of foraging bee species. Although their compositions differ, most of the propolis samples share important similarities in their general chemical structures. Raw propolis consists of approximately 50% resin and balsam, 30% beeswax, 10% essential and aromatic oils, 5% pollen and 5% organic compounds (Luo et al., 2011; Oryan et al., 2018).

In recent years, in parallel with the developments in molecular biology and bioinformatics, a great deal of research has been carried out in areas such as metagenomics, biotechnology, whole genome sequencing, and genome evolution. Plants, animals, plant/animal products, which are important members of biological diversity, have been the subject of numerous studies based on DNA sequence information, which seek answers to different questions in parallel with these developments. Especially the development of DNA sequencing technology and the relative decrease in sequencing costs have increased the number of such studies (Van Dijk et al., 2014). Recently, studies limited to a single or a few DNA markers have been replaced by studies using larger data sets, thanks to platforms known as next-generation DNA sequencing (NGS), which can generate genome data at a very high capacity (Mardis et al., 2017). Today, using DNA sequences in organisms, researches are carried out in many fields such as kinship relations and evolution, comparative genomics, genetic diversity, population genetics, conservation biology, biogeography, biological control of agricultural pests, combating vector carriers, and molecular identification of species (DNA barcoding) with reference DNA libraries (Blaxter et al., 2010; Furlong et al., 2015; Kress et al., 2015). In order to maintain a successful methodological stage in all these researches, the first and most important step is the use of an effective DNA isolation method. Effective DNA isolation will ensure that stages such as PCR, DNA sequencing (NGS or Sanger), cloning and hybridization can be carried out successfully. In order to ensure successful DNA isolation, all other macro-molecules of the cell must be removed from the environment without damaging the DNA molecule. For this purpose, numerous methods and kits have been developed to perform total genomic DNA isolation (Wingfield and Klein, 2012; Asghar et al., 2015). However, the preferred method directly affects the quality and quantity of the DNA being isolated.

Honey is used for treatment and nutrition purposes from past to present, in every period of life and in various branches of science. In the current period, it is known that the use and importance of honey in the field of alternative medicine is increasing day by day. Therefore, it is quite important to determine the botanical origin of which honey occurs along with its physicochemical and biological properties. Knowing the status of pollen diversity, the presence of microorganisms and GMOs in honey is important both economically and healthily. One of the most effective ways to obtain this information is the detection of biological species by the analysis of honey's DNA. However, DNA analysis of honey is not easy due to the inhibitors it contains, which are quite viscous by nature. When the studies conducted so far are examined, it is seen that there is no reliable and efficient DNA isolation technique for DNA analysis from honey. In this study, it was aimed to isolate DNA from honey, pollen and propolis samples belonging to Çankırı province by using three different techniques. The methods used as standard and the three different DNA isolation methods obtained by modifying these methods were compared in terms of efficiency.

MATERIALS AND METHODS

Sample Preparation

In this study, DNA isolation of honey, pollen and propolis samples belonging to Çankırı province was performed using three different methods. In addition, the vine leaf was used for each method as positive control (PC) to determine the accuracy of DNA isolation methods. Before isolation, each sample was made ready for DNA isolation. For this purpose; **Honey sample:** 35 gram honey samples were taken into three different 50 ml falkon tubes and 35 ml sterile 1x phosphate buffer (PBS; pH 7.2) was added to it. It was then incubated for one hour at 65°C in a shaken oven. After this process, each sample was centrifuged at a rate of 9500 x g for 20 minutes and stored at -20°C until the pellet isolation process was performed by removing the upper phase. P**ollen sample:** After homogenizing the pollen samples in a pestle, they were taken into three different 1.5 mL microcentrifugal tubes of 100 milligrams and 1 mL sterile 1x PBS (pH 7.2) solution was added to it. It was then incubated for one hour at 65 °C in a shaken oven. After this process, each sample was centrifuged at a rate of 12000 x g for 20 minutes and stored at -20°C until the pellet isolation process was performed by removing the upper phase. **Propolis sample:** Since propolis samples are quite sticky and resinous, they are pulverized with the help of liquid nitrogen. After this procedure, the samples were taken into three different 1.5 mL microcentrifugal tubes of 100 milligrams and 1 mL sterile 1x PBS (pH 7.2) solution was added to it (Note: PBS was not added to the propolis sample separated for isolation with TRIzol and subjected to rapid isolation after shredding) Then they were incubated for one hour at 65°C in the shaken oven and after this process, each sample was centrifuged for 20 minutes at a rate of 12000 x g and the upper phase was removed and pellet DNA isolation stored at -20°C until the procedure is carried out. **Vine (*Vitis vinifera* L.) leaf sample (PC):** After the vine leaves are cut to 30 milligrams, weighed on a precision balance, they are taken into a 1.5 mL microcentrifugal tube and homogenized in this tube. These homogenization processes were carried out freshly before each DNA isolation method and then immediately started to be insulated. Each isolation technique was made in the form of three biological replicates.

DNA Extractions

DNA Extraction with CTAB Buffer

Waiblinger et al. (2012) optimized the protocol and performed as follows: On each sample; It was homogenized by adding 700 µl of CTAB buffer [0.1 M Tris (pH:8), 1.4 M NaCl, 0.02 M EDTA and 0.05 M CTAB], 10 µl proteinase K (20 mg ml^{-1}) and β-mercaptoethanol with PVP40 in accordance with the ratios. Then, after 30 minutes of incubation at 55°C, the temperature was increased to 65°C and incubated for a total of 2 hours. The samples taken from the incubation were allowed to cool until they reached room temperature, during which time the samples were turned upside down for about 20 minutes at certain intervals. At the end of this period, an equal volume (700 µl) of chloroform:isoamyl (24:1) was added and it was turned upside down for 5 minutes. It was centrifuged at 13520 x g for 5 minutes and the supernatant (V volume) was transferred to a new tube. The upper phase was incubated at -20 °C for 1 night (overnight) by adding 0.1 times the volume of 3M sodium acetate (pH: 5.2) and 2 times the volume of cold 99% EtOH. Samples taken from -20°C were centrifuged at 28620 x g for 6 minutes and the supernatant was carefully removed. 700 µl of 70% EtOH was added to the upper phase, inverted for 1 minute and centrifuged at 28620 x g for 2 minutes. Then, the supernatant was carefully removed without damaging the pellet, and the samples were left to dry at room temperature for approximately 20-25 minutes. Depending on the density of the dried

pellets, they were dissolved in varying amounts (30-50 µl) of distilled water or 1X TE (10 mM Tris-Cl, 1 mM EDTA, pH: 8.0).

DNA Extraction with TRIzol

The protocol for DNA isolation with TRIzol (Chomczynski, 1993) was optimized and performed as follows: 1 mL of TRIzol was added to each sample. It was incubated at room temperature for 5-10 minutes (not exceeding 10 minutes) and 300 µL of the prepared chloroform:isoamylalcohol mixture in a 24:1 ratio was added to the sample after incubation. Centrifugation was performed at +4°C for 15 minutes at a speed of 12000 x g, and the colorless upper phase was removed from the post-centrifugation phases because it contained RNA. The processes were continued using the intermediate phase and subphase containing the remaining protein and DNA. Then, 0.3 mL 99% ethanol was added per 1 mL TRIzol and centrifugation was applied for 5 minutes at a rate of 2000 x g at 4°C. The upper phase (containing protein) was removed and the pellet was resuspended in 1 mL 0.1 M sodium citrate (containing 10% ethanol; pH 8.5). The samples were incubated for 30 min at -20°C and centrifugation was applied for 5 minutes at a speed of 2000 x g at 4°C. After centrifugation, the supernatant was removed and 1 mL 0.1 M sodium citrate (containing 10% ethanol; pH 8.5) was added to the pellet again and suspended. Then, without incubation, centrifugation was applied at 4°C at a speed of 2000 x g for 5 minutes. 75% 1 mL ethanol was added to the resulting pellet and centrifuged at 4°C at a speed of 2000 x g for 5 minutes. After centrifugation, the ethanol was removed and the pellet was left to dry for 5-10 minutes. After centrifugation, the ethanol was removed and the pellet was left to dry at room temperature for 5-10 minutes. The DNA isolated after drying was dissolved with 50-100 µl of distilled water and stored at -20°C.

DNA Extraction with Dneasy® Plant Pro Kit

With the DNeasy® Plant Pro Kit (Qiagen, Germany), the commercial kit protocol for DNA isolation has been optimized. Honey, pollen, propolis and vine sample were placed in a 1.5 ml ependorf tube. Since phenolic compounds were excessive, 450 µl CD1 and 50 µl PS Solution were added to it and centrifuged for 2 minutes at a rate of 12000 x g. The supernatant was transferred to a 1.5 ml collection tube, 200 µl of CD2 Solution was added and vortexed for 5 seconds. Centrifuged at room temperature at a speed of 12000 x g for 1 minute. The supernatant (400-500 µl) was transferred to a new 1.5 ml collection tube, 500 µl of APP Buffer was added to it and vortexed for 5 seconds. MB was centrifuged for 1 min after loading 600 µl lysate onto the Spin Colon and at a speed of 12000 x g. The liquid phase was removed and the MB Spin Colon was transferred to a clean collection tube of 2 ml. MB Spin Colon 650 µl AW1 Buffer solution was added and centrifuged at a speed of 12000 x g for 1 minute. After centrifugation, the liquid phase was removed and the MB Spin Colon was placed back into the same 2 ml collection tube. 650 µl Buffer AW2 solution has been added to the MB Rotation Column. It was centrifuged at a speed of 12000 x g for 1 minute. The liquid phase is removed and the Spin Colon is placed in the same 2 ml collection tube. It was centrifuged at a speed of 16000 x g for 2 minutes. The MB Spin Colon is carefully placed in a new 1.5 ml collection tube. After adding 50–100 µl EB Buffer to the center of the white filter membrane, it was centrifuged at a speed of 12000 x g for 1 minute. The MB Spin Colon, the center of the white filter membrane, was then removed and dissolved in a 40 µl TE buffer.

The quality and concentrations of the isolated DNA were confirmed by NanoDrop measurement and gel electrophoresis. The NanoDrop (Thermo Scientific, Germany) spectrometer measured at

wavelengths 230, 260 and 280. The integrity of the isolated DNA was imaged by loading 1-2 µl sample in 0.8% agarose gel and executing for 1 hour with 60 V and 55mA energy.

Polymerase Chain Reaction (PCR) Condition

PCR was performed using honey, pollen, propolis, and DNA samples isolated from the vine plant as positive control, primers specific to the *rbcL* gene region (Table 1), which is the chloroplast gene (~500 bç) (de Vere et al., 2017). PCR amplification; Using 5 µl from 10x Buffer, 5 µl from 25mM $MgCl_2$, 5 µl from 2.5mM dNTP, 5 µl from 6% DMSO, 1 µl from 10 pmol primers (forward and reverse) and 0.5 µl from Taq Polymerase, the final volume was completed with ddH_2O with a final volume of 25 µl. The primary sequences used in this context are given in Table 1. PCR operation; 3 min initial denaturation at 95°C, 45 sec at 95°C, 30 sec at 51.5°C, 45 sec at 72°C and 35 min at 72°C and last elongation at 72°C for 5 min. PCR products were executed in 1% agarose gel at 60V and 65 mA to obtain gel images.

Table 1. The primer sequences used in PCR

Gene	Primer Name	Primer Sequence (5'-3')
Ribulose-1,5	*rbcLaF*	ATGTCACCACAAACAGAGACTAAAGC
biphosphatecarboxylase/oxygenase	*rbclr506*	AGGGGACGACCATACTTGTTCA

RESULTS AND DISCUSSION

In this study, three different DNA isolation methods were applied to honey, pollen and propolis samples. The vine leaf sample was used as a positive control for each method. The efficiencies of the applied techniques were compared primarily in terms of DNA quality and purity. For this, absorbance measurements of DNA were made in NanoDrop Spectrophotometer. Furthermore, genomic DNA was analyzed by agarose gel electrophoresis and standard PCR. 5 µL of the obtained genomic DNAs were taken, run in agarose gel electrophoresis and photographed under UV light. The gel image contains a sample of honey, pollen and propolis and a positive control for each isolation technique. In order to determine the concentration and purity levels of the isolated DNAs, measurements were made with the NanoDrop Spectrophotometer device at A230, A260 and A280 nm wavelengths, and the data were recorded and given in Table 2.

Table 2. DNA concentration and purity measurements of samples

Sample	DNA İsolation Method	Concentration (ng/µl)	A260/280	A260/230
Honey (1.1)	CTAB	632.5	1.22	1.17
Honey (1.2)	CTAB	515.9	1.18	1.19
Honey (1.3)	CTAB	601.5	1.24	1.20
Pollen (1.1)	CTAB	1570.3	1.89	2.01
Pollen (1.2)	CTAB	1207.8	1.90	2.00
Pollen (1.3)	CTAB	987.5	1.89	2.01
Propolis (1.1)	CTAB	42.2	1.05	0.78
Propolis (1.2)	CTAB	56.8	1.05	0.89
Propolis (1.3)	CTAB	78.8	1.12	0.94
Vine (PC)	CTAB	748.6	1.82	1.87
Honey (1.1)	TRIzol	36.6	1.14	1.19
Honey (1.2)	TRIzol	46.8	1.12	1.21
Honey (1.3)	TRIzol	51.2	1.14	1.21
Pollen (1.1)	TRIzol	150.1	1.65	2.13
Pollen (1.2)	TRIzol	178.9	1.72	2.11
Pollen (1.3)	TRIzol	166.5	1.68	2.05
Propolis (1.1)	TRIzol	69.8	1.46	1.31

Table 2. DNA concentration and purity measurements of samples (Continued)

Propolis (1.2)	TRIzol	76.2	1.45	1.33
Propolis (1.3)	TRIzol	77.8	1.55	1.39
Vine (PC)	TRIzol	254.2	1.76	1.88
Honey (1.1)	DNeasy® Plant Pro Kit	401.2	2.20	2.11
Honey (1.2)	DNeasy® Plant Pro Kit	456.2	2.10	2.05
Honey (1.3)	DNeasy® Plant Pro Kit	414.2	2.11	2.06
Pollen (1.1)	DNeasy® Plant Pro Kit	141.1	1.91	1.85
Pollen (1.2)	DNeasy® Plant Pro Kit	155.6	1.98	1.87
Pollen (1.3)	DNeasy® Plant Pro Kit	156.4	1.95	1.87
Propolis (1.1)	DNeasy® Plant Pro Kit	55.3	0.78	1.08
Propolis (1.2)	DNeasy® Plant Pro Kit	65.2	0.89	1.02
Propolis (1.3)	DNeasy® Plant Pro Kit	49.8	0.91	1.06
Vine (PC)	DNeasy® Plant Pro Kit	258.2	1.90	1.88

According to NanoDrop Spectrophotometer measurements in DNA isolations, there are problems in the amount and quality of DNA due to the high polysaccharide and polyphenolic structures in the structure of honey and honey-derived products. In this study, DNA isolation from propolis samples could not be performed with CTAB and DNeasy® Plant Pro Kit methods, and DNA isolation from honey sample could not be performed in TRIzol method. The TRIzol method was successful compared to the other two methods for the propolis sample, which has a very dense structure. 0.8% agarose gel image of DNA obtained from all samples by different isolation methods is given in Figure 1.

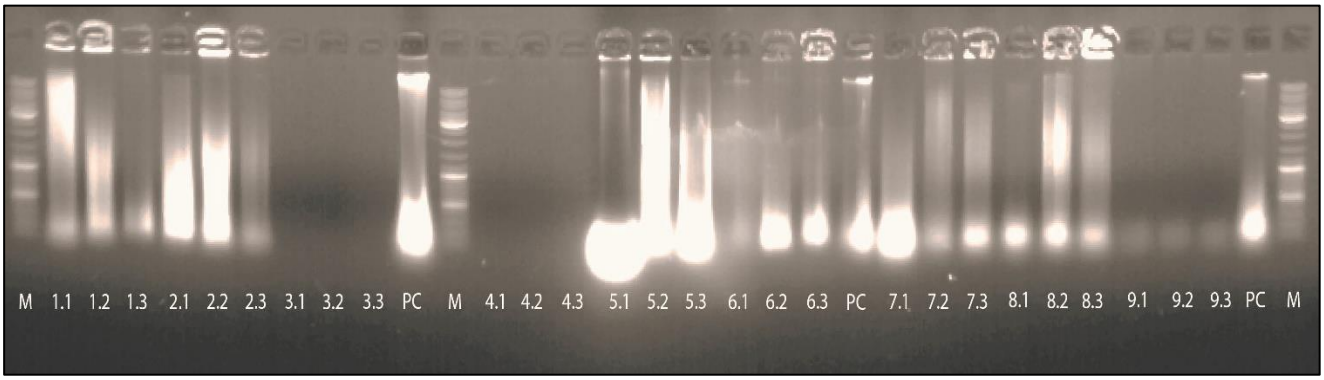

Figure 1. Result from agarose gel electrophoresis analysis of DNA extracted from samples (1.1 CTAB bal, 1.2 CTAB bal, 1.3 CTAB bal, 2.1 CTAB pollen, 2.2 CTAB pollen, 2.3 CTAB pollen, 3.1 CTAB propolis, 3.2 CTAB propolis, 3.3 CTAB propolis, 4.1 TRIzol bal, 4.2 TRIzol bal, 4.3 TRIzol bal, 5.1 TRIzol pollen, 5.2 TRIzol pollen, 5.3 TRIzol pollen, 6.1 TRIzol propolis, 6.2 TRIzol propolis, 6.3 TRIzol propolis, 7.1 DNeasy® Plant Pro Kit bal, 7.2 DNeasy® Plant Pro Kit bal, 7.3 DNeasy® Plant Pro Kit bal, 8.1 DNeasy® Plant Pro Kit pollen, 8.2 DNeasy® Plant Pro Kit pollen, 8.3 DNeasy® Plant Pro Kit pollen, 9.1 DNeasy® Plant Pro Kit propolis, 9.2 DNeasy® Plant Pro Kit propolis, 9.3 DNeasy® Plant Pro Kit propolis, PC: Pozitif control; Vine)

According to the appearance of the gel; in accordance with the NanoDrop results, DNA isolations from propolis sample by CTAB method, honey sample by TRIzol method and propolis sample by DNeasy® Plant Pro Kit method were found to have failed. It is thought that this is due to the density of the honey structure and the high polysaccharide content, as well as the amount of material of vegetable origin from honey, which is below the threshold of these methods and no yield can be obtained. The *rbcL* gene regions of the isolated DNA were amplified in standard PCR. In order to visualize the products after the reproduction process, agarose gel electrophoresis was performed. After isolation, it was observed that the products could not be obtained in PCR made from DNA whose bands could not be imaged in the gel (Figure 2).

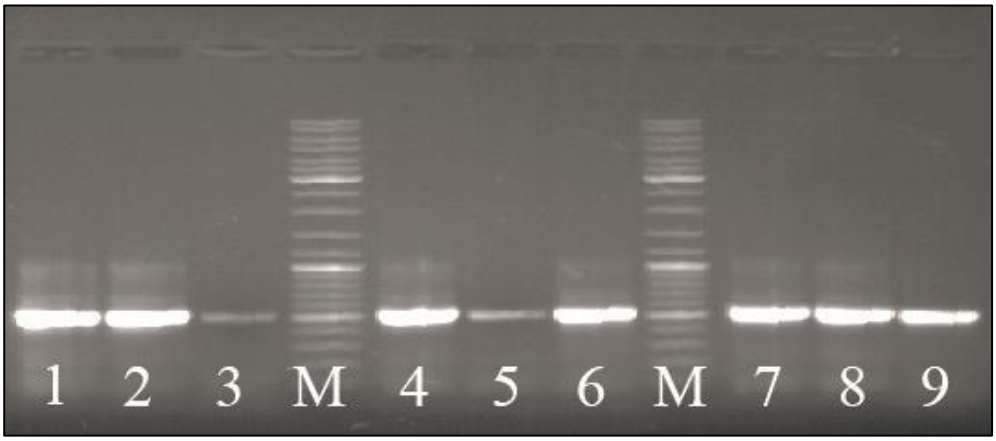

Figure 2. Results of PCR amplification. PCR products obtained with primers *rbcLaF*/*rbclr506* (1. CTAB honey, 2. CTAB pollen, 3. CTAB vine (PC), M. Marker-Gene Ruler 1 kb DNA ladder, 4. TRIzol pollen, 5. TRIzol propolis, 6. TRIzol vine (PC), 7. DNeasy® Plant Pro Kit honey, 8. DNeasy® Plant Pro Kit pollen, 9. DNeasy® Plant Pro Kit vine (PC)

Some of the molecular biology studies are DNA-oriented studies. For this reason, the isolation of DNA, the techniques applied in the process of obtaining it and the chemicals contained in these techniques are very important. Since the beginning of human history, honey, which has an important place in all civilizations, has been used as a food source and for therapeutic purposes. In many studies, the physical and chemical properties of honey have been examined. Chemical and chromatographic techniques were used to determine these properties (Stanimirova et al., 2010). However, the chemical structures in honey can change over time, which affects the reliability of the studies. In recent years, some studies on honey have been shown to be DNA-based. Soares et al. (2014) used 5 different DNA isolation techniques with 3 different pretreatment techniques in their study to determine the botanical origin of honey by molecular methods. Bruni et al. (2015) investigated the effects of the DNA barcode approach in determining the botanical origin of honey. They used rbcL and trnH-psbA barcode genes, which are easily amplified and sequenced, in Real-Time PCR analysis in the detection of plant species. Guertler et al. (2013), modified the CTAB technique in their study of DNA isolation from pollen in honey. In the study conducted by Olivieri et al., (2012) it was shown that honey contains DNA belonging to other groups of living things besides plants. In the study, DNA isolations were made from honey by modifying the CTAB method and these DNAs were propagated in PCR by designing primers suitable for the regions accepted as barcode gene candidates of insects, yeasts, bacteria and plants.

In this study, DNA isolation was performed with 3 different isolation methods from honey, pollen and propolis samples belonging to Çankırı province. When the isolation methods used were compared, it was determined that CTAB and commercial kit methods could be used successfully for honey and pollen samples, and TRIzol method could be preferred with high efficiency for propolis sample. Especially for the propolis sample with a very dense chemical structure, the TRIzol method was studied for the first time and yield was obtained. The data obtained as a result of this study support the implementation of such applications for other types of honey and honey-based products produced in our country and thus the raising of the quality standards of honey, which has an important place in the economy of our country.

CONCLUSION

Honey, which is one of the most consumed natural foods, has many benefits in terms of human health. It is known that honey contains glucose, fructose, carbohydrates, lipids, flavonoids, proteins, enzymes, macro and micronutrients. Honey production in the world and in our country is becoming a trade area whose importance is increasing day by day. Knowing details such as pollen diversity in

honey, the presence of microorganisms and GMO status is of potential importance both economically and healthily. One of the most effective ways to access this information is to isolate the DNA found in honey by analyzing some of the specific gene sequences it contains. Due to the inhibitors that are very viscous in nature and contain them, there are a number of problems and limitations in the analysis of the DNA in honey. In the studies carried out, it has been evaluated that a more effective DNA isolation technique that gives reliable and efficient results is needed for DNA analysis from honey. With this study, it is aimed to propose the most appropriate DNA isolation method of different DNA isolation methods. In this study, DNA isolation was performed from honey, pollen and propolis samples of çankırı province with 3 different methods (CTAB, TRIzol and DNeasy® Plant Pro Kit). While the TRIzol method was not suitable for the honey sample, DNA isolation from the propolis sample with CTAB and commercial kit could not be performed. The TRIzol method has been determined to be suitable for DNA isolation from a propolis sample. It is thought that the inability to detect DNA in some samples may be due to the nature of honey, the presence of contaminations outside the DNA, such as some sugars that can be transported during the isolation process. The data obtained as a result of this study support the implementation of such applications for other types of honey and honey-based products produced in our country and thus the raising of the quality standards of honey, which has an important place in the economy of our country.

Conflict of Interest

The author declared that there is no conflict of interest.

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