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Quality control of pine needles extracts (PNE) by using electroanalytical methods

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Abstract— *pH represents the concentration of free H^+ in pine needles extracts (PNE) and is therefore an important initial parameter in quality control. Electrical conductivity and pH of samples of fresh and stored for 20 days of PNE with black cumin oil and olive oil had values of 0.00 due to the encapsulation of water molecules, pH and electrical conduction was not possible. The pH of the other samples was in a weakly acidic environment because the pH of natural pine needles is 3.8. Electrical conductivity values in all samples except pine needle extract and honey increased during storage. By monitoring the parameters of pH and electrical conductivity in the quality control of PNE, it gives us a significant insight into the physical state of the phases and the way of storage.*

Keywords— ***electrical conductivity, extracts, PNE, pH, quality control***

I. INTRODUCTION

Koutsaviti et al. 2022 reported that pine needles has many physiological, pharmacologial activities and it is a good source of antioxidants and phenolic compounds including phenolic acids, flavonoids, procyanides [1], [2]. Pine trees (*Pinus densiflora*) have shown a wide range of biologically functional activities and many methods have been applied for phenolic compounds extraction from pine trees. Increasing interest has been devoted to naturally occurring compounds polyphenols, because of their beneficial health effects [3].

In this research, natural extraction agents such as: honey, black cumin oil (*Nigella sativa*), ethanol, olive oil and traditional jam (pekmez) were used to extract ions and bioactive components from pine needles. Analyzes of pH and electrical conductivity of fresh extracts and after 20 days of storage in a dark place were performed. There is growing interest in the antioxidant activities of plant extracts such as that obtained from pine needle. To obtain these antioxidant compounds, an extraction process is necessary. Extraction is a procedure performed to obtain metabolites in plants such as alkaloids, phenolics, flavonoids, glycosides, and others using selective solvents [4].

Honey is one of the most appreciated and valued natural products introduced to humankind since ancient times [5]. It is a by-product of flower nectar and the upper aero-digestive tract of the honey bee, which is concentrated through a dehydration process inside the bee hive [6]. The main nutritional and health relevant components are carbohydrates, mainly fructose and glucose but also about 25 different oligosaccharides [7]. Honey plays an important role as an antioxidant, anti-inflammatory, anti-bacterial agent and augments the adherence of skin grafts and wound healing process [8].

Black seed or black cumin (*Nigella sativa*), which belongs to the Ranunculacea family, is an annual herb with many pharmacological properties [9]. Black cumin is native to a

vast region of the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia, and is cultivated in many countries, including Egypt, Iran, Greece, Syria, Albania, Turkey, Saudi Arabia, India, and Pakistan [10]. Black seed and its main active constituent, thymoquinone, to be medicinally very effective against various illnesses including different chronic illness: neurological and mental illness, cardiovascular disorders, cancer, diabetes, inflammatory conditions, and infertility as well as various infectious diseases due to bacterial, fungal, parasitic, and viral infections [11]. The aqueous and oil extracts of the seeds have been shown to possess antioxidant, antiinflammatory, anticancer, analgesic and antimicrobial activities.

Ethanol has been widely applied as a viable solvent [12]. Park et al. 2022 reported that using ethanol at a concentration of 1000 µg/mL showed good electron donating ability among the pine needle extracts [13].

The olive tree is a relatively small evergreen tree, with narrow silvery leaves and small white flowers, known for its longevity [14]. Olive oil is characterized by a high proportion of monounsaturated oleic acid, but the main peculiarity of extra-virgin oil is the presence of remarkable quantities of phenolic compounds, notably hydroxytyrosol and oleuropein [15]. High consumption of extra-virgin olive oils, which are particularly rich in these phenolic antioxidants (as well as squalene and oleic acid), should afford considerable protection against cancer (colon, breast, skin), coronary heart disease, and ageing by inhibiting oxidative stress [16].

Jams are delicious and nutritious spreads typically made from fruit, sugar and pectin that ensure availability of fruits in off-season [17]. The apple jam (traditionally called pekmez) can be defined as a concentrated and shelf-life extended form of apple juice produced by boiling without adding sugar or any other food additives [18]. Pekmezs are especially rich in carbohydrates and minerals, also pekmezs contain vitamins, antioxidants and flavonoids [19]. There are two different kinds of pekmez, either liquid pekmez or solid pekmez [20].

II. EXPERIMENTAL PART

Instrumentation

The following instrumentation was used in this research: pH meter GLP 21 CRISON with a resolution of 0.1, 0.01, 0.001, conductometer GLP 31 CRISON.

Methods

Preparation of PNE

Pine needles extracts (PNE) were prepared by adding 20 g of pine needles accurately weighed on an analytical balance ± 0.0001 g to 100 mL of a natural extraction agent (honey, ethanol, black cumin, olive oil, apple jam/nigella sativa). .

Further electroanalysis of the PNE prepared in this way was performed immediately and after 20 days of storage in a dark place.

Fig. 1: Pine needle extracts (PNE)

Determination of pH

The measurement procedure with a combined glass electrode is simple, and involves carefully immersing the electrode in the extract, whereby the instrument measures the potential difference, i.e. the pH value. Before using the combined glass electrode, it is necessary to calibrate the instrument with standard buffer solutions of exactly known pH value.

Exactly 10 g of pine needle extract with each individual natural extractant was weighed on an analytical balance with an accuracy of 0.0001 g. Then the combined glass electrode was immersed and the pH value was measured. Three parallel measurements were performed for each sample.

Determination of electrical conductivity

The platinum electrode measurement procedure is simple, and involves carefully immersing the electrode in the extract, whereby the instrument measures the electrical conductivity expressed in µS/cm. Before using the platinum electrode, it is necessary to calibrate the instrument with standard buffer solutions of exactly known pH value. As with the measurement of the pH value, the conductivity of all samples was measured in three parallel measurements using the same procedure.

III. RESULTS AND DISCUSSION

Table 1. shows the results of determining the pH and electrical conductivity (κ, μS/cm) of various natural extraction agents.

Table 1. Results of pH and electrical conductivity (κ, μS/cm) of various natural extraction agents

Natural extraction agents	pH	κ, μS/cm
Honey	0,00	2,61
Ethanol	6,99	0,17
Black cumin	0,00	0,00
Olive oil	0,00	0,00
Apple jam/Pekmez	4,79	0,00

Based on the results, it can be seen that pure honey, nigella sativa and olive oil have a pH value of 0.00. The highest pH value was observed for pure ethanol and that value was 6.99, while the slightly lower pH value was for jam and was 4.79. Slightly different results were observed for electrical conductivity, (κ, μS/cm), and honey had a conductivity of 2.61 μS/cm, while ethanol had a conductivity of 0.17 μS/cm. The values of pH and electrical conductivity (κ, μS/cm) for the samples that had values of 0.00 are actually a consequence of their viscosity. Namely, with less viscous samples, it is much more difficult to measure pH and conductivity with electrodes. *Table 2.* shows the results of determining the pH and electrical conductivity (κ, μS/cm) of various natural extracts of pine needles immediately after maceration.

Table 2. Results of pH and electrical conductivity (κ, μS/cm) of various freshly prepared PNE

PNE	pH	κ, μS/cm
Honey	4,44	11,52
Ethanol	5,39	14,25
Black cumin	0,00	0,00
Olive oil	0,00	0,00
Apple jam/Pekmez	3,87	0,00

The pH value of the extract of honey and pine needles was 4.4, while the conductivity of this mixture was 11.52 μS/cm. A slightly higher pH value was 5.39 for extract ethanol and pine needles, and the conductivity of this extract was also slightly higher than extract honey and pine needles and that value was 14.25 μS/cm. The values of pH and electrical conductivity were again 0.00 for samples of macarata *Nigella sativa* and pine needles, as well as olive oil and pine needles. According to Palamutoğluthe et al. 2022 oil is the continuous phase in all emulsion formulations and has no electrical conductivity, indicating that the emulsions are water-in-oil emulsions [21]. Which suggests that the results in this research for the mentioned samples that had a pH and electrical conductivity of 0.00 are in accordance with the mentioned phenomenon that the emulsions are water-in-oil emulsions. The pH value of the jam and honey extracts was slightly lower than the pH value of the jam without the addition of pine needles. ***Table 3.*** shows the results of determining pH and electrical conductivity (κ, μS/cm) of various natural extracts of pine needles after 20 days of maceration.

Table 3. Results of pH and electrical conductivity (κ, μS/cm) of various stored PNE

PNE	pH	κ, μS/cm
Honey	3,50	9,48
Ethanol	5,65	39,2
Black cumin	0,00	0,00
Olive oil	0,00	0,68
Apple jam/Pekmez	4,26	60,3

The results of pH and electrical conductivity of the extracts after 20 days of storage showed that the extract of honey and pine needles had a lower pH value and a lower value of electrical conductivity than the freshly prepared extract. However, the values of pH and electrical conductivity of the extract of ethanol and pine needles had higher values compared to the freshly prepared extract. The jam extract of pine needles had a significantly higher electrical conductivity value of 60.3 μS/cm.

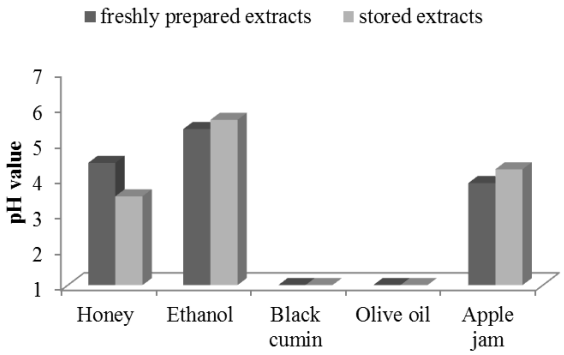

Fig. 2: Results of pH of various freshly prepared and stored PNE

On Figure 1. and Figure 2. the results of the influence of storage time on pH values and electrical conductivity (κ, μS/cm) of pine needle extract are clearly presented.

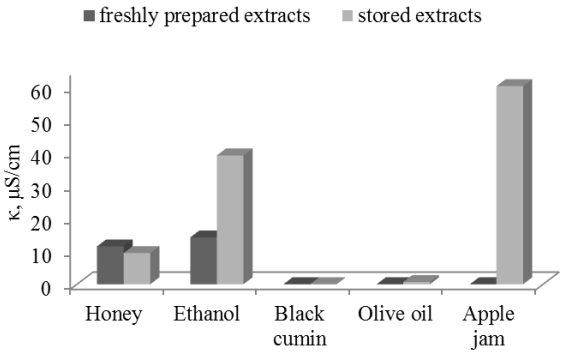

Fig.3: Results of electrical conductivity (κ, μS/cm) of various freshly prepared and stored PNE

IV. CONCLUSION

Pine needle extracts (PNE) can be used as additives in food, pharmaceutical and cosmetic industries. pH and electrical conductivity of black cumin oil was 0,00 in freshly prepared and stored PNE due to the encapsulation of water molecules. In the other samples, an increase in the value of electrical conductivity was observed, which can be concluded that the ions were extracted from the samples during storage.

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