# PHYSICO-CHEMICAL AND ANALYTICAL CHARACTERIZATION OF SOME EXTRACTS, MACERATES AND FRESH FRUITS FROM *ROSACEAE* FAMILY♦

**Viorica Popescu[1], Alina Soceanu*[1], Simona Dobrinas[1], Semaghiul Birghila[1], Vasile Magearu[2]**

*[1]Department of Chemistry, University "Ovidius" of Constanța, 124 Mamaia Blvd, 900527, Constanța, Romania*
*[2]Department of Analytical Chemistry, Bucharest University, 4-12 Elisabeta Blvd., 030018, Bucharest, Romania*

*Corresponding author: asoceanu@univ-ovidius.ro

**Abstract:** The aim of this work was to study the content of Fe, Mn, Zn and Cu in the mixtures which contain volatile oils among other components, obtained after the Soxhlet extraction, maceration or ultrasonic technique of the samples and in the fresh fruits (*Malus domestica* and *Cydonia oblonga*). Both fruits belong to the same family named *Rosaceae*. subfamily Maloideae (or Pomoideae) whose fruits consist of five capsules (called "cores") in a fleshy endocarp, surrounded by the ripened stem tissue. Flame atomic absorption spectrometry (FAAS) was used for the quantitative determination of metals in these matrixes and fruits (dry weight). Also, the refraction indices and conductivity were measured using Abbe refractometer and LF 340-A conductometer, for the characterization of the obtained mixtures, respectively for correlation with the mineral content. It was observed that iron concentrations are in higher quantities than Mn, Zn and Cu in both studied fruits.

**Keywords:** *heavy metals, FAAS, Malus domestica, Cydonia oblonga*

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## INTRODUCTION

*Malus domestica* (apple) and *Cydonia oblongan* (quince) belong to the same family named *Rosaceae*, subfamily Maloideae (or Pomoideae) whose fruits consist of five capsules (called "cores") in a fleshy endocarp, surrounded by the ripened stem tissue. Apples have long been considered healthy, as indicated by the proverb *"an apple a day keeps the doctor away"*. Apples contain Vitamin C as well as a host of other antioxidant compounds, which explains the reduced risk of cancer (with the free radical explanation of reduced cancer risk to due prevented DNA damage). They may also help with heart disease, weight loss and controlling cholesterol.

The quince *Cydonia oblonga* is the sole member of the genus *Cydonia* but in the same family (*Rosaceae*) as well as apple and native to warm-temperate southwest Asia in the Caucasus region. The immature fruit are green, with dense grey-white pubescence, which mostly rubs off before maturity in late autumn when the fruit changes color to yellow with hard flesh that is strongly perfumed. A syrup prepared from the fruit may be used as a grateful addition to drinks in sickness, especially in looseness of the bowels, which it is said to restrain by its astringency [1].

Essential oils include any of a class of volatile oils composed of a mixture of complex hydrocarbons (terpenoids, alkaloids and other large molecular weight compounds) extracted from a plant. Sucrose (the major end product of photosynthesis in most plants) and many other compounds including amino acids, lipids and secondary metabolites (such as terpenoids) are synthesized from plants [2]. Sample pretreatment is often one of the most time consuming steps of the analytical process, particularly when solid samples are involved. Solvent extraction of solid samples, which is commonly known as solid-liquid extraction, but which should be referred to, in a more correct use of the physicochemical terminology, as leaching or lixiviation, is one of the oldest ways of solid sample pretreatment. Essential oils that derive from aromatic plants are typically obtained by steam distillation a simple and relatively inexpensive process in which the essential oils are removed from the plant by a stream of water vapor, and then both phases are separated easily [3 – 6]. During distillation, part of the essential oil components become dissolved in and remain in the distillation water and the 'product' is called hydrosol, which is also known as the distillate water [7].

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination, high ambient air concentrations near emission sources, or intake via the food chain. Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment.

In humans exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Even though zinc is an essential requirement for a healthy body, too much zinc can be harmful. Excessive absorption of zinc can also suppress copper and iron absorption. On the other hand, the free zinc ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish. Copper is an essential

substance to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation [8].
The aim of this work was to study the content of Fe, Mn, Zn and Cu in the mixtures, which contains volatile oil, obtained after the Soxhlet extraction, maceration or ultrasonic technique of apples and quinces and also the content of these metals in these fresh fruits. Flame atomic absorption spectrometry (FAAS) was used for the quantitative determination of metals in these matrixes and fruits. Also, the refraction indices, relative density and conductivity were measured for the characterization of the obtained mixtures, respectively for correlation with the mineral content.

## EXPERIMENTAL

### Reagents and solutions

All metal stock solutions (1000 mg/L) were prepared by dissolving the appropriate amounts of the spectral pure metals in dilute acids (1:1) and then diluting them with deionized water. The working solutions were prepared by diluting the stock solutions to appropriate volumes. All reagents were of analytical-reagent grade and all solutions were prepared using deionized water. Merck supplied the organic solvents: methanol and carbon tetrachloride and the nitric acid 65 % and hydrogen peroxide 25 % solutions used were of ultra pure grade.

### Sample preparation

Fruits (apples and quinces) were gently washed with deionized water (to remove surface dust). 100 grams of each sample (the pulps) was submitted to conventional Soxhlet extraction respectively, to maceration (for three weeks) and ultrasonic technique. In order to determine metals concentrations from fresh fruits, samples were washed with deionized water, dried and homogenized. 0.5-0.9 grams of each dry sample was submitted digestion with 8 mL $HNO_3$ 65 % and 10 mL $H_2O_2$ 25 % at 150 $^oC$ in a Digesdhal device provided by Hach Company [9]. After the complete digestion the sample solution was filtered, made up to 50 mL in volumetric flask with deionized water. Analyses were made in triplicate and the mean values are reported.

### Sample analysis

For the Soxhlet extraction, methanol has been used, as from other studies it has been proved that the extraction capacity of this solvent is higher than other ($CCl_4$, $CHCl_3$, $C_6H_6$) [10]. After complete extraction the solvent was separated by the extract using vacuum distillation. The solvent used to achieve the maceration of samples was carbon tetrachloride, because is less toxic. Finally was obtained approx. 50 mL of mixtures containing volatile oil. These mixtures were stored in Teflon vessels until analysis. Another extraction of the samples was made using the ultrasonic technique in an extraction installation type IUS – 150.
A Shimadzu atomic absorption spectrometer (Model AA 6200) equipped with air-acetylene flame was used for the determination of four heavy metals (Fe, Mn, Zn, Cu) in extractions, macerations and fresh fruits. Acetylene of 99.99 % purity at a flow rate of 1.8 – 2.0 L/min was utilized as a fuel gas and also as a carrier gas for introducing

aerosols. Fe, Mn, Pb and Cu were measured using monoelement hollow cathode lamps. The characteristics of metal calibration are presented in Table 1. The refraction indices and conductivity were measured using an Abbe refractometer and LF 340-A conductometer.

***Table 1.*** *Characteristics of metal calibration curves*

| Metal | λ, nm | Concentration range (ppm) | Correlation coefficient |
|---|---|---|---|
| Fe | 248.3 | 0.020 - 4.000 | 0.9976 |
| Mn | 279.5 | 0.008 - 1.600 | 0.9984 |
| Cu | 324.7 | 0.010 - 1.200 | 0.9990 |
| Zn | 213.9 | 0.016 - 0.510 | 0.9932 |

## RESULTS AND DISCUSSION

For the characterization of the studied samples obtained by extraction, maceration and ultrasonic technique, refraction indices and relative density were measured (Table 2). The values of refraction indices from literature correspond with those measured in mixtures with volatile oil obtained from apples and quinces [11]. It can be observed that the highest value of the refraction index is for quinces obtained by ultrasonic technique. All these values are very close to that from the Romanian standards [12, 13].
Volatile oils are volatile substances mixtures, liquids (very rarely solids), with aromatic odor, without color, or weakly yellow, blue, red or green, with density between 0.850 – 1.070 g/cm$^3$ and refraction indices between 1.41 – 1.61 [14].

***Table 2.*** *Values of refraction indices and relative density in the mixtures studied*

| Sample | Sampling method | Refraction indices | Relative density |
|---|---|---|---|
| Apple *Malus domestica* | Soxhlet extraction | 1.3994 | 0.852 |
| | Maceration | 1.4074 | 0.871 |
| | Ultrasonic technique | 1.4292 | 0.968 |
| Quince *Cydonia oblongan* | Soxhlet extraction | 1.3892 | 0.879 |
| | Maceration | 1.3835 | 0.905 |
| | Ultrasonic technique | 1.4319 | 0.923 |

In table 3 are presented the values of conductivity for the mixtures studied. The highest value of conductivity was measured in the mixture obtained from apple (960 µS/cm) by ultrasonic technique and the lowest value was found in Soxhlet extract of quince (95 µS/cm). The above obtained conductivity values indicate that apple extract has a powerful electrolytic character than quince extract and quince macerates are strong electrolytes than apple macerates.
On the other hand the conductivity was measured for correlation with the mineral content. So, the electrolytic character was demonstrated by the presence of iron in higher or lower concentrations in mixtures of studied fruits. It was observed that there is a good correlation between the iron concentrations and conductivity values (Table 4).

***Table 3.*** *Conductivity values of obtained mixtures with volatile oil from apple and quince*

| Sample | Sampling method | Conductivity (µS/cm) |
|---|---|---|
| Apple *Malus domestica* | Soxhlet extraction | 137 |
| | Maceration | 167 |
| | Ultrasonic technique | 960 |
| Quince *Cydonia oblongan* | Soxhlet extraction | 95 |
| | Maceration | 246 |
| | Ultrasonic technique | 783 |

***Table 4.*** *Metal concentrations in mixtures with volatile oil obtained from studied sample and in fresh fruits by FAAS*

| Sample | Sampling method | Concentrations (mg/kg) ± SD | | | |
|---|---|---|---|---|---|
| | | Fe | Mn | Zn | Cu |
| Apple *Malus domestica* | SE | 13.8743±0.0064 | 0.3728±0.0015 | 1.2340±0.0004 | 0.098±0.001 |
| | M | 15.9726±0.0002 | 0.2136± 0.0029 | < DL | < DL |
| | US | 48.413±0.0031 | 0.3796±0.0012 | 0.3316±0.004 | < DL |
| | FF | 20.46±0.0021 | < DL | 0.61±0.0013 | 1.35±0.0002 |
| Quince *Cydonia oblongan* | SE | 11.7901±0.0689 | 0.0546±0.0019 | < DL | < DL |
| | M | 29.9532±0.0065 | 0.0541±0.001 | < DL | < DL |
| | US | 42.0542±0.0004 | 0.5423±0.0007 | < DL | < DL |
| | FF | 55.023±0.0007 | 1.4339±0.0013 | 0.2374±0.0005 | 1.0653±0.0476 |

SD – standard deviation; DL – detection limit; SE – Soxhlet extraction; M – Maceration; US – Ultrasonic technique; FF – Fresh fruits

The obtained results (Table 4) show that iron was found in higher quantities than Mn, Zn and Cu in both studied fruits. This result means that in time, Fe is suffering some changes, being transferred from leaves, soil and water sources.

The highest zinc concentration was observed in apple Soxhlet extract (1.2340 mg/kg). So, these extracts can be use like tincture, knowing the healing effect of zinc [15].

Ellen et al. [16] found the median contents for fruits (in mg/kg fresh mass): Cd 0.002; Pb 0.017; Hg 0.002; Cu 0.61; Mn 0.52 and Zn 0.99.It can be notice that our results are comparable with those showed in literature.

Silva et al. [17] found in apple leaves 82 mg/kg Mn, 12.5 mg/kg Zn, 5.64 mg/kg Cu and 80 mg/kg Fe, and Ivanova et al. [18] also found in apple leaves 15.6 mg/kg Zn and 5.09 mg/kg Cu. These results show that in the samples studied the metals content is lower that those showed in literature and data suggest that each part of plant accumulate heavy metals differently.

Matei and al. [19] show in their study that while peach (fresh fruits) accumulated high concentrations of heavy metals (Cu, Fe, Mn Zn), nectarine (fresh fruits) accumulated low concentrations. Their results are comparable with ours.

## CONCLUSIONS

The content of Fe, Mn, Zn and Cu in the mixtures, which contains volatile oil, obtained after the Soxhlet extraction, maceration or the ultrasonic technique and in fresh fruits of

apples and quinces have been studied. For all mixtures the refraction indices, the relative density and the conductivity values were measured. The highest zinc concentrations were observed in apple extract and iron concentrations are in higher quantities than Mn, Zn and Cu in both studied fruits.
All these determinations were made because these mixtures contain volatile oils that can be use like tinctures in medical purposes, in perfumery industry and in aromatherapy treatments.

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