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Original Article

Determination of Fluoride Concentration in Rosemary (Rosmarinus officinalis)using Selectiv- electrod technique

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Abstract

Background: Fluoride is characterized by high toxicity, especially in prolonged exposures. Some plants including Rosemary can higher of accumulate up to 20 000 times this element concentration in the environment. Consumption of highly fluoridated rosemary can contribute fluorosis and other body dysfunctions.

Aims: This study aims to evaluate and optimize the extraction procedure of fluorides from the dried leaves of the plant, and compare the fluoride levels in collected samples. Methodology: nine of Rosemary samples from different countries including Libya, southern Serbia, Vojvodina and Istria were investigated in this study. Fluoride levels was determined potentiometrically using the calibration curve, via fluoride-selective electrode method based on lanthanum fluoride monocrystal. The sensitivity of the method was defined on the basis of 3SD criteria 3 using excel software.

Results: fluoride concentration in investigated samples was varied. Libyan samples contained (1.8ppm) which is higher than other samples that ranged between 0.07 to0.49 ppm. However, all samples found to have fluoride concentration ranged with the recommended levels and less than the toxic or lethal doses. Conclusion: The modified potentiometric method using fluoride-selective electrode techniques for fluoride determination is highly effective with detection limit of 10-9 mg/100g and is recommended in quantification of fluoride in Rosemary and other plant or spice samples. However,

Results obtained by this study indicated that all samples contained different levels of in which all do not exceed the daily intake. Recommendations. Rosemary may offer an essential levels of required fluoride for daily intake. Further studies are recommended especially in areas with high fluoride content either in soil or via air pollution especially where rosemary is highly consumed to avoid fluorosis.

Keywords: Rosemary, fluoride, fluoride-selective electrode

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Introduction.

Fluoride (F-) is an important anion, present in the environment, clinical and food samples. Small amounts of fluoride are vital for the human organism, but this element is toxic in larger amounts. For adults it is estimated that the lethal dose is 16mg and 32mg F− per kg of body weight for children and adult respectively (Ullah et al, 2017). This element is widely used in various branches of industry and some fluoride compounds are formed as by-products in certain processes. Excessive amounts of fluoride in the form of different compounds can enter the human body by means of polluted air, water and through the food chain (Lubojanski et al., 2023). Additional sources of fluoride for humans are toothpastes containing approximately 0.1% of fluoride in one of the forms: NaF, SnF2, Na2PO3F. Toothpastes are intentionally fortified with fluoride because a small amount of fluoride is beneficial in the prevention of dental caries and the treatment of osteoporosis (Wang et al., 2022). Fluoride prevents tooth decay at about 1 mg L-1, but causes mottled teeth and bone damage at around 5 mg L-1 content in chronic exposures through drinking water (McDonagh et al., 2000) . Fluorosis, a pathological condition of bone or teeth damage, is caused by elevated intake of fluoride over prolonged periods of time (McDonagh et al., 2000) .). In dental fluorosis, the structural integrity of enamel is affected and small pits are left in teeth as it breaks away, exposing dentine underneath (DenBesten and Li 2011). Skeletal fluorosis is the accumulation of fluoride in skeletal tissues associated with pathological bone deformities (Choubisa, 2022).

Excessive exposure to high doses of fluoride can contribute to different health problems. Exposure of individuals to extreme concentrations of fluoride has been reported to cause an acute fluoride toxicity, with symptoms ranged from headaches, nausea, gastric pain and diarrhea at concentrations of 5–8 mg/kg bodyweight (Ullah et al. 2017). In addition, a damage in gastrointestinal tract nerves, inflammatory bowel disease and tissue lining disruption has been reported after Chronic exposure to high fluoride doases (Das et al. 1994; Follin-Arbelet and Moum 2016; de Oliveira et al. 2017; Melo et al. 2017). However, low pH thought to play an important role in releasing Protonated fluoride (HF) which is able to spread into different tissues and caus cellular toxicity in different parts of the body (Johnston and Strobel., 2020).

The common fluoride bearing minerals found in soil are fluorospar (CaF2), cryolite (Na3AlF6) and chiolite (Na5Al3F14) (Dyachenko et al., 2016). The mobility of fluoride in soil is determined by the amount of present clay minerals, the soil pH, the adsorption of positively charged complexes, and the concentration of Ca, Fe, Al and P in soil (Wong et al., 2003). Due to the essential character of fluoride, as well as

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its toxicity, especially in prolonged exposures, regular control of the element intake is of high importance. Rosemary is a spice used since ancient times to preserve food and to improve its sensory properties. In European cuisine, particularly in Mediterranean region, it gained a great popularity due to its distinctive and pleasant flavor (Stefanaki & van Andel, 2021). Some plants are able to accumulate fluoride. For example, tea plant (Camellia sinensis) is very rich in fluoride and accumulates it in its leaves (Luo et al., 2021) Fluoride Ion-selective electrodes (ISEs) have been used for the purposes of fluoride determination in plant samples, replacing the expensive and time-consuming chromatographic methods (Miya et al., 2020). ISEs are easy to use and thus are suitable for continuous monitoring. They are cost-effective, as well as sufficiently sensitive, selective and accurate (Miya et al., 2020. The fluoride selective electrodes are commonly used for the determination of fluoride in water, industrial effluents, air, flue gases, soils and minerals, as well as biological materials such as urine and blood serum (Pandey et al., 2014). In addition, fluoride selective electrodes have been widely used for determination of fluoride in tea and other beverages (López et al., 2009). Not many papers on fluoride accumulation in rosemary have been published, so data on the element content in this important spice plant are not readily available especially using potentiometric method . Therefore, fluoride concentrations was determined in rosemary plant grown from different regions worldwid (South Serbia, Vojvodina, Libya, Istria) using potentiometric method in trials to evaluate this technique in detecting this element. Potentiometric determinations were performed by using fluoride-selective electrode based on lanthan-fluoride monocrystal membrane (is this adopted technique). Samples were prepared by using modified AOAC methods for fluoride determination in feed (Mendes et al., 2020).

Material and methods

Apparatus

For fluoride determination performed in this study, the "Jenway" fluoride selective electrode,
made in England, was used. This electrode consists of a hard crystal membrane made from lanthanum (III) - fluoride, doped with a small amount of europium (III) – fluoride, whose purpose was to create holes in the crystal structure and allow the migration of fluoride ions, as was stated in the theoretical part. The body of the electrode consists of two concentric cylinders, and the annular space contains the reference electrode. The inner cylinder hosts the electrode membrane, as well as the internal reference electrode.

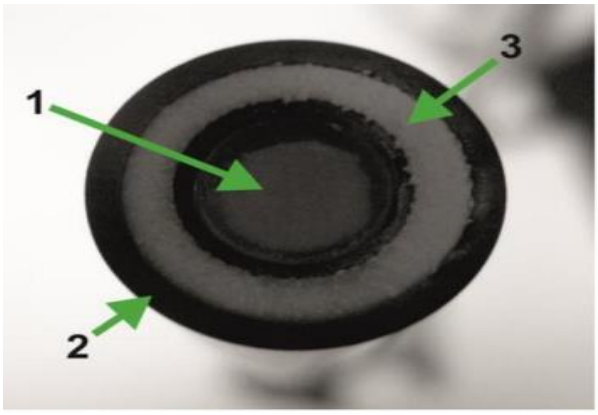

Figure 1: 1- Membrane of the "Jenway" fluoride-selective electrode, 2— the body of the electrode, 3-annular space with the reference electrode.

Figure 2: Combined "Jenway" fluoride-selective electrode and "Hanna" electronic voltmeter.

the detection and analysis of free fluoride ions in water solutions, good for fieldwork and laboratory use. The apparatus, for potentiometric measurements, is attached to "Hanna" electronic voltmeter (figure 2).

Before each use, the electrode needs to be activated. According to specifications, the activation is done by submerging the electrode in a fluoride solution (concentration 10 ppm) for a period of 5 minutes. After that it is ready for use. Upon use, the electrode needs to be washed by de-ionized water, carefully wiped, protective cap needs to be put on and the electrode put back in the box.

Chemicals

Hydrochloric acid, 36.5%. 2) Sodium acetate solution, 3M, was prepared by dissolving 480 g in H2O in 1 L volumetric flask. At room temperature, diluted with H2O to volume. Adjusted to pH 7.0 with a few drops of CH3COOH. 3) Sodium citrate solution, in the same fashion, 1.32 M, 222 g dissolved in H2O in 1 L volumetric flask. Added 28 mL HClO, diluted to volume, obtained by solving of 1.105g NaF in 1 L volumetric flask, dissolving and diluting to volume with H2O. From the basic standard solution of fluoride work solutions were created at 0.0015, 0.005, 0.12, 0.25 respectively. To each 10 mL 1 N HCl, 25 mL sodium acetate solution, 25 mL sodium citrate solution was added, diluted to volume with H2O and mixed.

Samples

Samples used in this study originating from nine different parts of the world including. Aleksinac, Kikinda, Opatija, Rumenka, Three samples from different sites of Soko Banja and tow samples obtained from Musrata and Western Mountain Libya.

Sample preparation

The preparation of the sample was rather simple. As basis for process defining AOAC method for fluoride determination in cattle feed was used. The composite sample (30g) was used to obtain 3 representative samples of 8g, analyzed separately.

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Each sample was placed into a 200 ml flask containing 60 ml of hydrochloric acid was added. The mixture was stirred for 20 minutes on a magnetic stirrer. The mixture was then filtrated and transferred into a 300 ml flask. 100 ml of sodium acetate solution was added to the filtrate and the filtrate was diluted with double distilled water (to which concentration?). Before the analysis, 50 ml of TISAB was added to each of the prepared solutions.

Results and discussions

Determination of equilibrium time

According to experimental design steps, the errors in potentiometric determination are greatest if the reading of potential concentration was taken in the first moments of contact between the solution and the electrode, and smallest if the reading is done after equilibrium time. The equilibrium time depends on many factors and is different for all systems. Before examining experimental factors it was necessary to determine equilibrium time for the applied system and different fluoride concentrations. Standard fluoride solutions contained 0.0015, 0.005, 0.05, 0.12, 0.25 ppm, respectively.

Optimal time for potential measurement was selected according to its reproducibility. For every fluoride content, a measurement of potential was done under different equilibrations time, with times between 30 and 300s. For most standard solutions, best reproducibility was reached at 120s. Potential was measured at 120 s, and its relative standard deviation was calculated accordingly for three measurements. According to calculated reproducibility, an equilibration time of 120s was adopted taking into consideration satisfactory duration of analysis, as well as good reproducibility relative standard deviation (RSD = 1,3%).

Linearity

For a quantitative analysis of fluoride it was necessary to determine the range of concentrations in which linear dependence of electrode potential could be correlated good with assumed linear function. The testing was done in the range between 0.005 to 0.25 ppm. Fiure 3

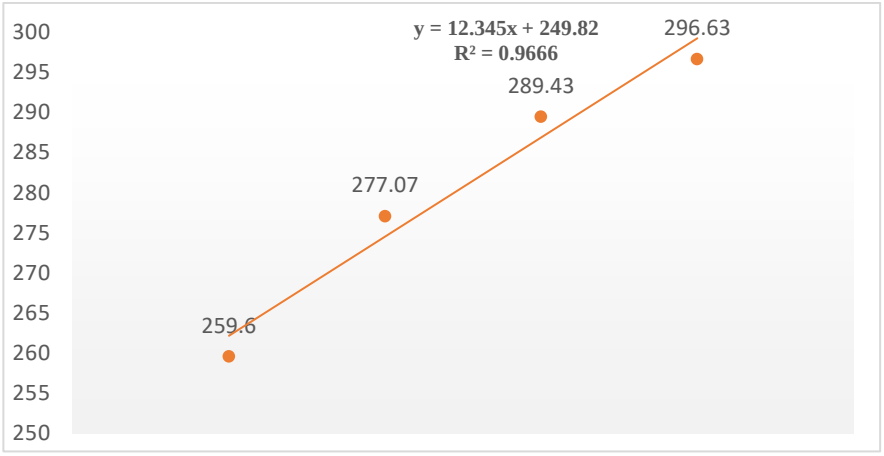

Figure 3: Linearity of the analytical fluoride signal.

Reproducibility

Analytical reproducibility (repeatability) is defined according to 3 successive analyses of fluoride solution with the concentration of 0.005 0.05, 0.12 and 0.25 ppm (figure 3). For a relatively low fluoride content at 0.005 ppm, an excellent reproducibility was achieved, with the relative standard deviation (RSD) below 0.005%.

Determination of reproductively with different fluoride contents has shown that the system is extremely reproductive, since, independently from the fluoride content, the RSD value was very low, (below 0.0035%) in all cases. With the growth of fluoride concentration, a rise in reproductively was noticed.

Determination of sensitivity

Limit of detection (LOD)

Limit of detection (LOD) represents the lowest analyte concentration that can be reliably
detected. According to IUPAC nomenclature, limit of detection is the lowest quantity of a substance that can be distinguished from that in the absence of that substance (blank) within confidence limit (generally 1 %) (Nic M., et al 2006) .

The criteria of three standard deviations of the analytical signal were used in this paper. Based on the equation from picture 10 and the value of three standard deviations of the analytical signal of the blank (SD = 2.90 mV), the limit of detection was determined to be 10-3 ppm of fluoride.

Limit of quantification (LOQ)

Limit of quantification is the limit at which we can tell the difference, reasonably, between two different values. LOQ tends to be very different from lab to lab, therefore, the Practical Quantitation Limit (PQL) was used in this study. The limit of quantification was determined to be 0.0019 ppb.

4. Determination of fluoride in rosemary samples

All rosemary samples were analyzed in three replicates by optimized procedure. The results of the sample analysis concerning concentration of fluoride in rosemary are
shown in figure 4. Presented values represent the mean calculated value +/- RSD. Flourid concentration was calculated for three success measurements and ranged from 0.07-1.8 ppm with SD of 0.001 to 0.009. Data analysis showed a significant differences between means at (P=.0001) using SPSS statistic version 25 software and One-way ANOVA test

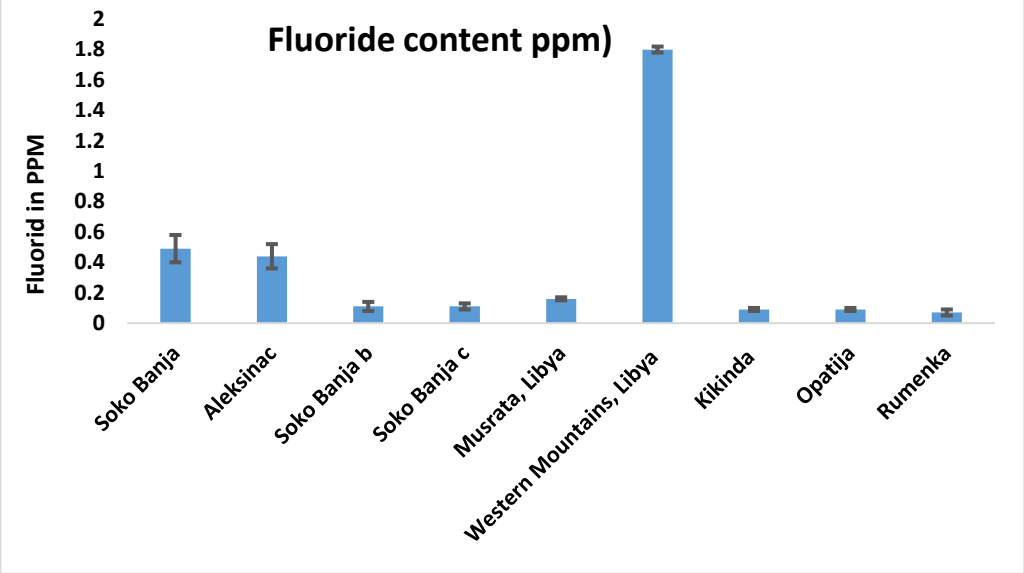

Figure 4: Fluoride concentration in tested samples

Discussion.

Consumption of spicy and medical plants are widely distributed. However, in addition to organic material, plants can also contain heavy metals from the surrounding polluted environment (Truong., 2020), and accumulate different element with concentration higher than its content in the environment and up to 20 000 times (Ref). Fluoride content in different sources was widely investigated using different detection methods. However, detection of fluoride using potentiometric method not yet been globally established. This method was adopted in order to find faster, trustable and low coasting method to detect different elements including fluoride in water and consumable materials. Šucman & Bednář, . (2012) have evaluated this method with Certified Reference Material (SRM Fluoride in Vegetation NIST 2695 - Low Level, USA) and the results were significantly similar.

Prolonged exposure to high concentration may result in fluorosis especially in childre. Godel,. (2002) reported an increased fluorosis from13.5% to 41.4% in children exposed to water contains less than 0.3 ppm and greater than 1.2 ppm respectively. Generally, in recent study, the detected concentrations in tested plants are under this levels with exception of Soko Banja, Aleksinac and Libyan western mountain samples which contained 0.49, 0.44 and1.8 ppm respectively. However, returning back to the recommend daily intake, these levels are still less than the advised doses. Šucman & Bednář, . (2012) investigated the concentration of fluoride in eighteen spice samples in Czech Republic and found that mean content of fluoride ranged from 3.95

mg/kg (dried garlic) to 26.08 mg/kg (mustard seeds). Fluoride levels were also investigated in 11 spice samples widely consumed by Ethiopian people were investigated and the fluoride levels the highest in thyme leaves and black cumin seeds (8.57±0.11 8.14±0.15 mg/kg) and the lowest was found in coriander seeds (2.14±0.04 mg/kg) (Nigus and Chandravanshi, 2016). This levels are much higher than that recorded by our study, taking into account that the daily consumption of Rosemary as aspic or meal supplement contain a fairly small amount of fluoride compared to the currently recommended daily fluoride intake of 0.05 mg/kg body weight/day (Nigus and Chandravanshi, 2016).

Conclusions

The recent work defines the potentiometric method for fluoride determination with the application of fluoride-selective electrode which is a simple procedure of sample preparation compared with different techniques. To avoid the interference from other ions, sample extraction was implied with hydrochloric acid and addition of TISAB at filtration step. The limit of detection was 10-9 mg/100g of fluoride (0.0019 ppb). The highest determined fluoride content was found in samples from Libyan Western Mountains and Soko Banja (Serbia), implying that it is natural and comes from the soil, rather than from anthropogenic sources. The potentiometric method is simple, selective, and relatively low cost when it comes to instruments and can be proposed for fluoride determination in other plant and spice samples.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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