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INFUSIONS FROM EDIBLE FLOWERS AS A SOURCE OF FLUORIDE IN DIET

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ABSTRACT: One of the sources of fluoride in the human diet includes products of plant origin. The presence of this element has been observed, e.g., in drinks—juice, coffee, tea, herbal infusions, and alcoholic beverages. Fluoride content in a product can be influenced by the origin of the resource, the species, and the age and the morphological part of the plant. In the case of infusions, other factors that should be considered include the method, the number of infusions made from the same resource, the time of brewing, and the quality of water used to prepare the infusions. The aim of the study was to determine if infusions made from the flowers are an important source of fluoride in diet. Fluoride concentrations were measured by potentiometric method. The highest content of fluoride was observed in the infusions made from dandelion flowers at all of the temperatures used. Studies show that infusions made from edible plants are a source of fluoride. However, its content is not alarmingly high. The consumption of these infusions in an amount similar to the consumption of tea (1 - 2 glasses per day) does not entail the risk associated with excessive fluoride intake.

Keywords: Common poppy; Dandelion; Edible flowers; Fluoride content; Infusions; Mirabelle plum.

INTRODUCTION

The subject of fluoride (F) and its potentially toxic influence on the human organism is being raised more and more often in academic circles. In physiological concentrations, F affects the processes of hard tissue mineralization and the binding of magnesium, calcium, and phosphorus to hydroxyapatite. It can also substitute for the hydroxyl ions of enamel hydroxyapatites forming fluorhydroxyapatites and fluorapatites to create a structure which is harder, more crystalline, and more resistant to the influence of external factors.¹ Moreover, F supports the remineralization of enamel and limits the development of bacterial plaque.²

The abundance of F sources for the human organism is the reason why it is problematic to establish a daily dose of this element, and overexposure or chronic exposure to F can lead to side effects. Fluorosis is a chronic metabolic illness caused by the excessive intake of F and may involve dental fluorosis, skeletal fluorosis, and nonskeletal fluorosis.³⁻¹⁰ F influences bones by promoting anabolic processes within them.¹¹ Long-term exposure to the influence of F changes the amount and properties of numerous enzymes, especially metalloenzymes.¹² F can also find its way into soft tissues and interrupt the functions of numerous organs, including the nervous system, as it penetrates cell membranes relatively easily.¹³⁻¹⁵ The mechanism of F toxicity is multidirectional. Probably the most important role in this respect is played by the amplified production of free oxygen radicals.^{12,16,17} Sodium fluoride, which amplifies oxidative stress and increases the production of free oxygen radicals, is one of the factors that can support the development of atherosclerosis.¹²⁻¹⁸ Free radical processes also speed up the pathological changes in cells, which can lead to cell apoptosis or inflammations.^{16,17} Natural sources of F include the areas of active volcanoes, soil, and sea water. However, most of the F that exists in the air comes

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from urbanized areas (aluminum industry, glassworks and ironworks, superphosphate factories, etc.). The daily dose of F should not exceed 6 mg/24 hr. However, it is difficult to determine the intake as it refers to numerous sources. The daily exposure to F can be 0.46-5.4 mg/day, most of which (80–85%) originates from food, whereas the rest mainly comes from drinking water and toothpaste.¹⁹

One of the sources of F in the human diet includes products of plant origin. The presence of this element has been observed, e.g., in drinks—juice, nectar, coffee, tea, herbal infusions, and alcoholic beverages.²⁰⁻²³ The concentration may be a result of multiple factors. F content in a product can be influenced by the origin of the resource,²⁴ the species,²⁵ the subspecies,²⁶ the age of the plant, and the morphological part that was used in the preparation of the product.²⁷ In the case of infusions, other factors that should be considered include the method,²³ the number of infusions made from the same resource,²⁸ the time of brewing,²⁰ and the quality of water used to prepare the infusions. Furthermore, the type of packaging of the product,²⁹ the method of resource processing,³⁰ and flavor additives³¹ can also have an influence on F content.

In Europe, about 130 species of herbal plants are grown, mainly in the Mediterranean. In Poland, about 70 species of herbs are grown, of which a large part has been cultivated by breeding domestic varieties (over 20). Poland is one of the leading European countries in the cultivation of herbs and spices which is connected with a long-standing tradition and their use in traditional herbal medicines.³²⁻³³ Herbal plantations in Poland cover an area of about 30,000 ha and the Polish herbal industry uses about 130 species of plants from the natural collection and about 60 species from crops, which gives about 10,000 tons of raw material from the natural state and about 50 thousand tons of field crops.³⁴ Polish companies producing herbal and fruit teas are selling more and more abroad and in non-European markets.³⁵ The market for herbal and fruit teas, produced ecologically, grows year by year.³⁵ Edible plants have become more popular as plant resources recently. They are used as additions to meals mainly due to their aesthetic value, but they are also a source of bioactive substances. The potentially healthy properties of phytotherapy have been discussed more often recently, and the resource is often added to diets, e.g., in the form of infusions. In recent years, the sale of black teas in Poland has been falling (43% of market share), in favor of fruit (14%) and herbal teas (11%). It follows that Poles consume them more than green tea (9%) or Earl Gray (10%).³⁶⁻³⁷

Due to the widespread presence of F in plant resources and the growing popularity of edible plants, it makes sense to analyze whether the consumption of these plants contributes to a significant increase of the supply of this element in the diet, and what factors may influence F concentration. The aim of the study was to determine the influence of the preparation temperature on the F content of infusions made from the flowers of the dandelion, the mirabelle plum, and the common poppy.

MATERIAL AND METHODS

Plant material: The plant material of dandelion (*Taraxacum officinale*), the mirabelle plum (*Prunus domestica* subsp. *syriaca*), and the common poppy (*Papaver rhoeas* L.) was taken from areas located in the West Pomeranian Voivodeship in north-western Poland, from spring to autumn 2017. Directly after picking, the plant material was cleaned, then frozen at temperature -20° C and lyophilised in the

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apparatus Alpha 1-2 LD plus (the pressure of 0.735 mm Hg, temperature -20° C). The dried material was homogenised (homogeniser for food analysis FOSS 2094) and was used to prepare infusions.

The preparation of the infusion: 0.5 g of a plain material sample was transferred to a conical flask to which 100 mL of distilled water at given temperature (70°C, 80°C, and 90°C) was added. The flask with the infusion was closed and rotated with a speed of 180 rpm (Brunswick model EXCELLA E24) for 10 min. After brewing, the plant parts were separated from the infusion through filtration. All the infusions were prepared in triplicate.

Determining fluoride content: The infusions were poured into a plastic tube, labelled, and frozen at -20° C until the determination of F levels. F concentrations in individual samples were measured by the potentiometric method with a fluoride ion-selective electrode (Orion 9409 BN, Thermo Scientific, USA). 1.0 mL of the sample was transferred to a plastic tube and 1.0 mL of TISAB II was then added to this solution. After mixing, the potential difference of each sample was measured for 10 min; 5 min before the addition of the appropriate standard, and 5 min after the addition. According to the work of Łukomska et al.,³⁸ the F content in the samples was calculated based on the difference of potentials measured in each sample and the concentration of the added standard. The electrode was calibrated using standard solutions. The correctness of the analytical procedure was controlled by comparing the concentration of F in the NaF solutions with the known concentrations: 0.1, 1.0, 10.0 mg/L (Orion Company, USA).

Statistical analysis: In all the experiments, three samples were analysed and all the assays were carried out at least in triplicate. The statistical analysis was performed using Stat Soft Statistica 13.0 and Microsoft Excel 2010. The results are expressed as mean values and standard deviation (SD). For F content one-way analysis of variance (ANOVA) and the Tukey post-hoc test were used. Differences were considered significant at $p \le 0.05$.

RESULTS

F content in the infusions made from poppy flowers (Figure 1) was from 0.034 mg/ L (at 90°C) to 0.0637 mg/L (at 80°C). The differences between the results achieved at 70°C and 80°C and between the results acquired at 80°C and 90°C were statistically significant.

In the case of the infusions made from the mirabelle plum flowers (Figure 2), the highest content of F (0.0565 mg/L) was observed in the infusion prepared at 70°C, whereas the lowest (0.0338 mg/L) was at 80°C. Only the results achieved at 70°C and 80°C differed in a statistically significant manner (p = 0.034).

F content in infusions made from the plants of the dandelion (Figure 3) was from 0.0605 mg/L to 0.2529 mg/L (for 90°C and 70°C, respectively). F content in the infusion prepared at 70°C was statistically significantly different from other cases (p = 0.003 and p = 0.0007).

The highest content of F was observed in the infusions made from dandelion flowers at all of the temperatures (Figure 4). The lowest concentration of this element was observed in the infusions made from the flowers of the mirabelle plum at 80°C (0.0338 mg/L) and the common poppy at 90°C (0.0339 mg/L).

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Figure 1. Fluoride content in common poppy infusion. *Significant difference at p≤0.05.



Figure 2. Fluoride content in mirabelle plum infusion. *Significant difference at p≤0.05.





Figure 3. Fluoride content in dandelion infusion. *Significant difference at p≤0.05.





Most of the differences between the values achieved for specific species were statistically significant (Table).

Flowers	Infusion temperature (°C)		
	70	80	90
Common poppy vs mirabelle plum	ns	*	*
Common poppy vs dandelion	*	*	*
Mirabelle plum vs dandelion	*	*	ns

 Table. Fluoride content in infusions of common poppy, mirabelle plum and dandelion (significance of differences)

^{*}Significant difference at $p \le 0.05$; ns = not significant.

The relation between F content in the infusions and temperature they were prepared at has been analyzed. The only statistically significant negative correlation (r = -0.750) was observed in the case of dandelion flowers, which means that fluoride content decreased with the increase of the temperature used to prepare the infusion. In reference to the infusions made from the common poppy and the mirabelle plum, the relations were not significant (r = -0.305 and r = -0.109, respectively), which means that the temperature at which the infusions were prepared did not have an influence on fluoride content.

DISCUSSION

Our study shows that infusions made from edible flowers are a source of F. However, it has to be considered whether the concentration of this element is low or if the introduction of infusions to diets can pose a risk of excessive F intake. Drinks like tea are consumed by most of the population with an intake of 1 to 3 glasses a day. On the basis of our results, it can be determined that one glass of a poppy flower infusion would contain from 0.009 mg to 0.016 mg of F (depending on the temperature of preparation), one glass of a mirabelle plum flower infusion would contain from 0.014 mg of F, and one glass of dandelion flower infusion would contain between 0.015 to 0.063 mg. The recommended dietary allowance (RDA) in reference to F is 3 mg/day,³⁹ so one glass of such an infusion would provide 0.5–2.1% of the RDA.

Telesiński et al.²⁶ studied the flowers of the chamomile. The results they achieved indicated F levels of 2.26–5.39 mg/kg dw, depending on the subtype. In the present study, the content of F was analyzed in infusions prepared with a plant concentration of 0.5% (0.5 g of a plain material sample in 100 mL of distilled water). Assuming that the entirety of F passes on from the resource to the infusion, if the results were to be calculated with comparable units, we would achieve the values from 6.8 mg/kg dw to 50.6 mg/kg dw, indicating a significantly higher F content in the studied flowers in comparison to the content present in chamomile. F content in infusions made from chamomile flowers was also studied by Emekli-Alturfan et al.⁴⁰ whose results showed a F content at the level of 0.03 mg/L, whereas Chan and Koh found a result

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of 0.05 mg/L.²⁰ Chan and Koh prepared the infusions using 1 g of dw per 100 mL of water.²⁰ Therefore, the concentration they achieved was twice as high. The study by Emekli-Alturfan et al. does not provide the weight of the resource.⁴⁰ The authors used one bag of dry matter per 100 mL of water, on the basis of which it can be assumed that they achieved the infusions with 1.5-2% concentration. Based on this information, it can be concluded that the flowers we studied were richer in terms of F content in comparison to the chamomile mentioned above.

Emekli-Alturfan et al.,⁴⁰ Buzalaf et al.,⁴¹ and Malinowska et al.⁴² studied 1.5–2% infusions of various types of black tea. In these cases, F content was $1.21-3.56 \mu g/mL$, 0.57-3.53 mg/L, and 0.32-6.87 mg/L. These values, after a calculation that takes into account the concentration of infusions, are higher than the content of this element in common poppy and mirabelle plum infusions and fruit infusions indicate F content at the level between 0.02 and 0.04 mg/L.⁴⁰ The study by Malinowska et al. pointed to the following concentrations of F: green tea 0.59–2.52 mg/L, green tea with additions 0.08–1.7 mg/L, oolong or pu-erh 0.39–2.85 mg/L, white tea 0.37–0.69 mg/L, and herbal tea 0.02–0.14 mg/L.⁴² In that study, herbal tea types were the only ones characterized by F concentrations that were lower than those present in the infusions made from edible flowers that we studied. The content of this element in the remaining tea types was similar to the content observed in our work.

Numerous studies used infusions with the concentration of 1% w/v. The results show the following levels of F in various tea types: black: $0.32-0.43 \text{ mg/mL}^{43}$, green: $0.008 \text{ mg/L}^{25}-3 \text{ mg/kg}$,²⁵ Ceylon tea: 0.32-1.69 mg/L,⁴⁴ and herbal infusions: 0.05-0.393 mg/L.^{20,25}

In our study, the results achieved for the mirabelle plum and the common poppy were similar to the F content present in herbal infusions, but lower than in most of the tea types. In the case of the dandelion, F levels were similar to black tea,⁴³ mint infusions,²⁵ and Ceylon tea.⁴⁴ Gupta and Sandesh achieved F content in tea (brewed using tea bags) at the level between 1.67 and 2.67 mg/L; using tea leaves: 1.0–3.0 mg/L; and using granulated tea: 1.45–3.81 mg/L.³⁰ Maleki et al. observed levels from 0.75 to 3.29 mg/L for the tea-bag flavored version of green tea and between 0.13 and 0.56 mg/L for tea made from leaves.⁴³ Considering the concentrations of these infusions, it can be concluded that those made from edible plants in most cases contain less F than tea infusions.

Łukomska et al. showed that F content in 20% infusions (i.e., 40 times higher concentration than the one used in the present study) of yerba mate was between 0.08 and 0.16 mg/L.³⁸ After converting this value in order to compare it with our infusions, it can be concluded that the flowers of the common poppy, the mirabelle plum, and the dandelion are a much bigger source of F than yerba mate. Ready-made drinks such as juice, iced tea, nectars, and fizzy or alcoholic drinks are also a source of F. The content of this element in iced tea was between 0.03 and 3.35 ppm⁴⁵ and from 0.09 to 0.81 µg/mL.⁴¹ Fojo et al. observed F content at the level of 0.40 mg/L in soft drinks, 0.37 mg/L in juice, 0.33 mg/L in nectars, and 0.29 mg/L in concentrates,²¹ whereas Heilman et al. observed the average F content in fizzy drinks at 0.34 ppm.⁴⁷ F content was also labeled in alcoholic drinks. Paz et al. showed that the content of F was from 0.048 to 0.703 mg/L in wines made from ecological vines

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and from 0.033 to 0.346 mg/L in those made using conventional means.⁴⁷ Taking into account the color of wine, it has been shown that F content in red wines was between 0.13 mg/L and 2.87 mg/L, in white wines between 0.19 mg/L and 2.23 mg/L, and in pink wines at 0.17 mg/L.^{48,49} Studies show that beer is also a source of F. According to various authors, F content was from 0.067 to 1.12 mg/L.^{29,50,51} According to Goschorska et al. the lowest concentration of this element was found in vodkas (about 0.05 mg/100 g), whereas the highest was present in wine and beer (about 0.15 mg/100 g). The contents were highly influenced by the amount of water used in a given alcoholic drink.²²

The amount of F content in a plant resource depends on numerous factors. On the basis of our study it can be concluded that one such factor is the species of a given plant. Peng et al. also observed that the type of resource is important.⁵² From all tea types that they studied, the leaves of oolong had the highest \overline{F} content, whereas puehr and black tea had lower concentrations. The lowest concentration of this element was detected in green and white tea. Lu et al. observed that the age of the resource is also important—old tea leaves accumulate much more F than young ones.⁵³ On the basis of this information, some scientists claim that the quality of tea can be a reflection of its F content.³² The studies by Fung et al. confirm this relation and show that in other plant parts (root and stalk) this element is also present, but in significantly lower concentrations, indicating that F content also depends on the morphological part of the resource.²⁷ The process of F extraction from its resource into an infusion depends on numerous factors. Studies by Pehrsson et al. show that leaves contribute about 70-80% of F in an infusion, whereas the remaining 20-30% of this element comes from water.²⁴ It is without doubt that one of the basic factors that influences the extraction process is the concentration of the infusion. The number of infusions made from the same resource is also an important factor. Łukomska et al. and Fung et al. observed that F content in infusions decreased with every subsequent brewing.^{28,38} Emekli-Alturfan et al. observed a small positive influence of the time of brewing on F content in herbal infusions and fruit infusions, which was also confirmed by the studies by Chan and Koh and Fung et al.^{20,28,40} Malinowska et al. observed that the time of brewing was much longer in the case of black tea than other tea types.⁴² Chan and Koh concluded that the brand of the tea, its type, and the presence of caffeine also have an influence on F content-higher concentration was observed in decaffeinated tea.²⁰ Gupta and Sandesh and Wolska et al. also showed that the type of resource and the method of its preparation are also important factors that influence F content.^{23,30} Our studies show that F content in the infusions made from edible plants also depends on temperature, as there are statistically significant differences between temperatures, but only in the case of dandelion flowers it can be determined that the relation is inversely proportional.

CONCLUSIONS

F content in the flowers of the common poppy, the mirabelle plum, and the dandelion has been labeled, contributing to the knowledge about the concentration of this element in plant resources and about the influence of temperature on F transfer to infusions. The differences in F content in reference to specific flower species have been observed, and the highest concentration was discovered in the infusions made from flowers of the dandelion. In the case of this species, there was a statistically

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significant and directly proportional relation between F content and the temperature used to prepare the infusion. Dandelion plants are typical plants of open meadow or grassland ecosystems and visible forests with varying degrees of moisture. When used for therapeutic or consumer purposes, dandelion plants are usually collected from the positions on which they naturally occur, therefore the areas should be free from any sources of pollution. For our study, dandelion flowers were collected from flood plains near the city. Although no industry is located in this area, and the area is located far from roads, periodic runoff of surface water from urban areas may cause an increased accumulation of some elements in the soil, including F. In addition, these plants have the ability to selectively accumulate certain elements, and numerous environmental factors, including soil properties, can affect their mineral composition.

Our studies show that infusions made from edible plants are a source of F. However, its content is not alarmingly high. The consumption of these infusions in an amount similar to the consumption of tea (1-2 glasses per day) does not entail the risk associated with excessive F intake.

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