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Comparison of different colorimetric assays and application of the optimized method for determining the liberated fluoride contents in various tea extracts

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Abstract: The appropriate intake of fluoride (F^-) is beneficial to human health; however, the over-consumption can result in various potentially harmful effects. This study compared different colorimetric reagents, i.e., aluminium-xylenol orange (Al-XO), zirconium-xylenol orange (Zr-XO), and zirconium-alizarin red S (Zr-ARS), for fluoride measurements by the UV-Vis, in terms of reaction mechanisms, method sensitivity, and interferences from aluminium and ferric ions. The colorimetric procedures were optimized, and the analytical methods were evaluated. The goodness of linearity (R^2 > 0.998) was obtained for all three assays within the concentration range of 1.0-20.0 mg/L fluoride in deionized water, in which the method sensitivity followed the descending order of Zr-XO > Al-XO > Zr-ARS. The Zr-XO was applied for determining the fluoride in different tea extracts in water (90 °C and 60-minute-brewing) and black tea demonstrated the highest fluoride content (3.0-3.6 mg/L). The effects of brewing time and temperature on the release of fluoride in the tea extracts were also investigated, indicating these are critical factors for the fluoride extraction. This study highlighted the application potentials of the UV-Vis measurement as a simple, convenient, and cheap analytical approach and discussed different colorimetric reagents used for fluoride determination in tea extracts in the context that the UV-Vis spectrophotometers are commonly equipped in most laboratories.

Key words: fluoride, tea extracts, Al-XO, Zr-XO, Zr-ARS

1. Introduction

Fluoride is an essential micronutrient, and the appropriate daily intake of this mineral can perform many benefits, e.g., preventing tooth decay as well as benefits for bones and tissues. However, over-consumption of fluoride can perform potential harms to human health.[1,2] Several foods rich in fluoride include tea, seafood, beans, fruits (oranges, mandarins, etc.), eggs, and milk, in which tea is known to perform the highest fluoride content.[3] In tea plants, fluoride forms complexes with aluminium ions, helping it to be

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transferred from the roots to the leaves, where most of fluoride is stored.[4] This is why tea leaves, especially mature ones, may have high fluoride content, accounting for around 98 % of the fluoride in the entire tea plant.[5-7] Moreover, fluoride can be released into the extracts during the brewing period, making tea cups a potential source of the fluoride intake. To the best of our knowledge, there are no specific regulations regarding permissible fluoride content in tea. However, according to the World Health Organization (WHO), adults' maximum safe daily fluoride intake is 4.0 mg per day, which highlights the necessity of determining the fluoride contents in tea products and tea extracts.[8]

The degree of fluoride absorption and accumulation in tea leaves can vary depending on the soil types, environmental conditions, and tea types, e.g., green tea, oolong tea, and black tea.[9] For instance, black tea was reported to generally contain higher fluoride concentrations than those of green tea. However, the amount of fluoride consumers absorb from tea greatly depends on brewing conditions, e.g., time, temperature, and water sources used for brewing tea.[10,11] The study of Peng et al. determined fluoride contents in 558 tea products (average fluoride level) from six types, i.e., black tea (699.74 mg/kg), oolong tea (159.78 mg/kg), Pu-erh tea (101.67 mg/kg), green tea (63.04 mg/kg), and white tea (52.19 mg/kg), using the fluoride-selective electrode.[12] Another study by Koblar et al. also found that fluoride contents decreased in the order of black tea > green tea > Pu-erh tea > oolong tea.[13]

Several analytical methods have been developed based on the complex-forming ability of fluoride with other ions or molecules and the UV-Vis measurement. Those determination approaches are simple, cheap, convenient, and can be applied for different sample matrices, e.g., soils, water, foods, etc.[14] As mentioned, fluoride can form complexes with various metal ions, such as zirconium and aluminium, but these formations do not produce color for the UV-Vis measurement. Within this context, another compound, e.g., xylenol orange (XO) or alizarin red S (ARS), can be used to initially form a colored complex with the metal ion, called a colorimetric reagent, which is interrupted by the presence of fluoride.[15,16] The rising fluoride concentrations result in a decrease in the color intensity of the colorimetric reagent. The publication by Davis et al. demonstrated that a mixture of Zr-XO used at a 1:2 v/v ratio showed the most favorable reaction efficiency for fluoride quantification.[17] Additionally, the method based on a decrease in absorbance of the Zr-ARS at 520 nm was used to quantify fluoride in water.[18]

Although the UV-Vis measurements for fluoride have been well-published so far, most publications have selected one colorimetric reagent for their specific applications. This study compared and discussed the performance of three different common reagents, i.e., Al-XO, Zr-XO, and Zr-ARS, in terms of reaction mechanisms, method sensitivity, and the potential interferences from the presence of aluminium and ferric ions. The colorimetric procedures were also optimized prior to applying to the tea extracts. The analytical method performance was evaluated based on the guidelines from the Appendix F of AOAC (2016), including limit of detection/quantification (LOD/LOQ) estimation, calibration curves (linear regression equations), repeatability (intra-day precision), reproducibility (inter-day precision), and recovery tests. Different tea products belonging to various types were collected from the northern and southern parts of Vietnam for the method application. The tea extracts in water (90 °C and 60-minute-brewing) were obtained to approximately compare the variabilities of fluoride in different tea samples. Moreover, the effects of the brewing conditions on the release of fluoride in the tea extracts were investigated in terms of changing brewing temperature and time values to understand the fluoride contents right in the tea-cups consumers ingest to their bodies. The results contributed to the food section in terms of food quality assurance and control. Moreover, this study highlighted the application potentials of the UV-Vis measurement as a simple, convenient, and cheap analytical approach and compared different common colorimetric reagents used for fluoride determination in tea extracts in the context that the UV-Vis spectrophotometers are equipped in most laboratories.

2. Experimental

2.1. Chemicals and reagents

All the chemicals used in this study were of analytical grade and obtained from Merck (Germany). Sodium fluoride (NaF) was used as a standard for fluoride ions. Other reagents include xylenol orange tetrasodium salt (XO), alizarin red S (ARS), aluminium nitrate ($Al(NO_3)_3$), zirconyl chloride ($ZrOCl_2$), potassium nitrate (KNO_3), methanol (CH_3OH), nitric acid (HNO_3), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), and ascorbic acid ($C_6H_8O_6$).

Three stock colorimetric reagent solutions were prepared, i.e., Al-XO, Zr (IV)-XO (Zr-XO), and Zr (IV)-ARS (Zr-ARS). For the Al-XO reagent, $Al(NO_3)_3$ of 5 mM in 2 M HNO_3 was mixed with XO solution of 5 mM in DIW to obtain a 2.5 mM Al-XO mixture. For the Zr-XO reagent, a 0.3 mM $ZrOCl_2$ in 30 % w/v HCl was mixed with a 0.15 mM XO solution in DIW to get a 75 μM Zr-XO solution. For the Zr-ARS reagent, a 1.0 mM $ZrOCl_2$ in concentrated HCl and concentrated H_2SO_4 was added to a 0.5 mM ARS solution in DIW to get a 250 μM Zr-ARS solution.

2.2. Sample collection and extraction

We purchased 18 tea samples, which were reported to have originated from the south (green tea, oolong tea, and black tea, 03 samples/each type) and the north of Vietnam (green tea, Pu-erh tea, and black tea, 03 samples/each type).

To simplify the sample preparation procedure in this study, we did not use the digestion method to decompose the tea matrix. Moreover, the focus was not the total fluoride concentrations in the tea samples, but the fluoride contents released in the tea extracts obtained from different brewing conditions. However, to estimate the fluoride concentrations in different tea samples, the 60-minute extraction at 90 °C was used (briefly called "60-minute extraction"), assumed that this extraction condition could liberate the highest fluoride from the tea matrix under our experimental design. The extraction was described as: 2.5 g of tea was brewed in 100 mL of DIW at 90 °C for continuously 60 minutes. The fluoride result from 60-minute extraction was also used as a reference basis to evaluate the effects of brewing time and temperature on the release of fluoride in the tea extracts by calculating the extraction percentage (%).

$$\text{Extraction percentage} = \frac{\text{Concentration from each brewing condition}}{\text{Concentration from 60-minute extraction}} \times 100\%$$

The brewing time and temperature included 5, 10, 15, 20, 25, and 30 minutes (at 90 °C) and 10, 20, 30, 40, 50, 60, 70, 80, and 90 °C (for 30 minutes), respectively. The temperature was kept constant during the brewing period using a water bath. An adequate volume of the tea extract was loaded through the SPE-C18 cartridge (200 mg, 3 mL, Agilent), initially activated by 6.0 mL of methanol and 6 mL of DIW, to reduce the color prior to the colorimetric assays.

2.3. Three different colorimetric assays: Al-XO, Zr-XO, and Zr-ARS

For each colorimetric assay, different concentrations of reagent solutions were prepared by diluting the stock reagent solutions using 0.1 M KNO_3 (for Al-XO: 25, 50, 75, and 100 μM) or DIW (for Zr-XO: 12.5, 18.75, 37.5, and 50.0 μM; and Zr-ARS: 100, 150, 200, and 250 μM). For the UV-Vis measurement, 0.5 mL of fluoride standard was mixed with 4.0 mL of each colorimetric reagent (0.5 mL of DIW was used for blanks). The mixture was then calibrated to 5.0 mL by DIW, except for Al-XO method by 10 % (w/v) ascorbic acid. The reaction solutions were vigorously vortexed for one minute and let stand for 60 minutes at the ambient temperature before recording their absorbance values at 520 nm (Al-XO) and 528 (Zr-XO and Zr-ARS).[16-18]

The reaction time between the colorimetric reagent(s) and fluoride was also investigated, i.e., 10, 20, 30, 40, 50, 60, 70, 80, and 90 minutes. Moreover, the effects of Al^{3+} and Fe^{3+} on the colorimetric assays were evaluated. The Al concentrations were set at 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg/L, whereas the concentrations of Fe(III) were sequentially adjusted to 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/L. Three

different fluoride levels were used for these investigations, including 5.0 mg/L, 10.0 mg/L, and 15.0 mg/L.

2.4. Evaluation of analytical method

The optimized conditions were applied for evaluating the analytical performance regarding each colorimetric assay, in which the green tea samples with low fluoride levels was used as a representative matrix. The limit of detection and quantification (LOD and LOQ) were estimated by simultaneously conducting 10 replicates to apply in the following equations: LOD = 3.3 × SD/a and LOQ = 3LOD (SD is the standard deviation and a is the slope of the calibration). The quantification was based on the linear calibration in the concentration range of 1.0 to 100 mg/L. The repeatability was evaluated by calculating the relative standard deviation (RSD_r) for six replicates (n = 6) in one day. One-way ANOVA (significance level of 0.05) was used for assessing the reproducibility (RSD_R) obtained from two different researchers for each colorimetric assay. The recovery tests were conducted using the spiked samples, in which three different concentration levels of fluoride were spiked to the green tea samples, i.e., $0.5C_x$, C_x, and $1.5C_x$, and C_x is the estimated fluoride concentration achieved from 60-minute extraction (at 90 °C).

3. Results and Discussion

3.1. Effects of the colorimetric reagent concentrations on the sensitivity

The three colorimetric assays were based on a principle that a metal ion (Me) would form a complex with an organic component R, $Me[R]_n$, called "colorimetric reagent", which was less stable than the complex formed between the metal ion (Me) and fluoride. Therefore, the presence of fluoride in the solution would disrupt the $Me[R]_n$ complex, resulting in a decrease in the concentrations of $Me[R]_n$ (see the simplified reaction 1, 2, and 3). According to this principle, we could calculate fluoride concentrations.

$$Al\text{-}XO + 6F^- \rightarrow [AlF_6]^{3-} + XO \quad (1)$$

$$Zr\text{-}XO + 6F^- \rightarrow [ZrF_6]^{2-} + XO \quad (2)$$

Table 1. Optimization results of the colorimetric reagent concentrations for the Al-XO, Zr-XO, and Zr-ARS assays

Colorimetric assay	Reagent conc. (μM)	Slope (a)	Intercept (b)	R^2
Al-XO	25	0.009	0.078	0.825
	50	0.021	0.041	0.950
	75	0.029	0.023	0.987
	100	0.035	-0.002	0.999
Zr-XO	12.5	0.013	-0.004	0.997
	18.75	0.021	0.013	0.997
	37.5	0.025	0.049	0.997
	50	0.042	0.012	0.997
Zr-ARS	100	0.005	-0.007	0.995
	150	0.006	-0.002	0.998
	200	0.005	-0.010	0.984
	250	0.005	0.011	0.993

Linear equations, i.e., y = ax + b, whereas x and y represent for the fluoride concentration (1.0 mg/L to 20.0 mg/L) and analytical response (blank absorbance subtracted by the respective standard solution absorbance). Blank samples contain the colorimetric reagent without fluoride standard chemical.

$$Zr\text{-}ARS + 6F^- \rightarrow [ZrF_6]^{2-} + ARS \quad (3)$$

Regarding Al-XO, theoretically, the aluminium ion can react with XO to form two main complexes at a 1:1 or 1:2 (metal to ligand) ratio. However, the Al-XO complex was more stable than the $Al\text{-}(XO)_2$ complex.[16] Hence, we chose a 1:1 reaction ratio for the Al-XO reagent. The most appropriate reagent concentration for each method was selected based on the sensitivity, i.e., slope (a) of the linear equation. The results in *Table* 1 indicate that the concentrations of the colorimetric reagent performed certain effects on the method sensitivity, i.e., different slope values. Generally, the increase in the colorimetric reagent concentrations enhanced the method sensitivity (higher slope), except for Zr-ARS and the linearity (improved R^2). When the Al-XO concentrations changed from 25 μM to 100 μM, the slope increased around 3.9 times. Similarly, for Zr-XO, the slope was improved nearly 3.2 times according to its rising concentration from 12.5 μM to 50 μM. However, the sensitivity of Zr-ARS assay was less improved compared to the other assays regarding the higher Zr-ARS concentrations, and the highest

slope was recorded at 150 μM with favorable R^2. In summary, our findings show the optimized colorimetric reagent concentrations were of 100 μM, 50 μM, and 150 μM for Al-XO, Zr-XO, and Zr-ARS, respectively. Among these reagents, the Zr-ARS performed 6-7 times less sensitivity than the others (*Table* 1). The Al-XO and Zr-XO are favorable candidates for detecting low fluoride levels, in which the Zr-XO demonstrated 1.2 times higher sensitivity than Al-XO. This could be explained due to the formation constants, i.e., 1.0×10^{25} *vs.* 2.5×10^4 ($[ZrF_6]^{2-}$ *vs.* $[AlF_6]^{3-}$).[19,20] Due to the low contents of fluoride in tea samples, the Zr-XO would be used for the application to determine the fluoride released in different tea extracts in this study.

3.2. Effects of the reaction time on the sensitivity

The adequate time is necessary to obtain quantitative reactions between the colorimetric reagents and fluoride, i.e., the highest sensitivity and favorable repeatability. The results in *Fig.* 1 demonstrate that three colorimetric reagents shared a similar pattern for three different fluoride levels of 5.0 mg/L, 10.0 mg/L, and 15.0 mg/L, i.e., the absorbance was increased from 10 minutes to 50 minutes, then stabilized for the next 20 minutes. The recoveries for each condition were also calculated, indicating the most favorable recoveries for 50 minutes, 60 minutes, and 70 minutes. Therefore, in this study, 60-minute reaction time was applied before the UV-Vis measurement to evaluate the three colori- metric assays.

3.3. Effects of aluminium and ferric ions on the method selectivity

Fluoride ions can form complexes with a broad spectrum of metal ions, including alkaline earth (e.g., calcium and magnesium), group 3A (e.g., aluminium), transition metals (e.g., iron, copper, and zinc), and other heavy metals (e.g., lead and mercury).[21] Notably, the complexes fluoride formed with aluminium and ferric ions were particularly stable and potentially interfere with the analytical methods due to the competitive complex formation reactions.[22] Therefore, the influences of aluminium and ferric were necessarily investigated (*Table* 2).

The Al-XO and Zr-ARS assays shared a similar trend pattern regarding the rising Al^{3+} concentrations

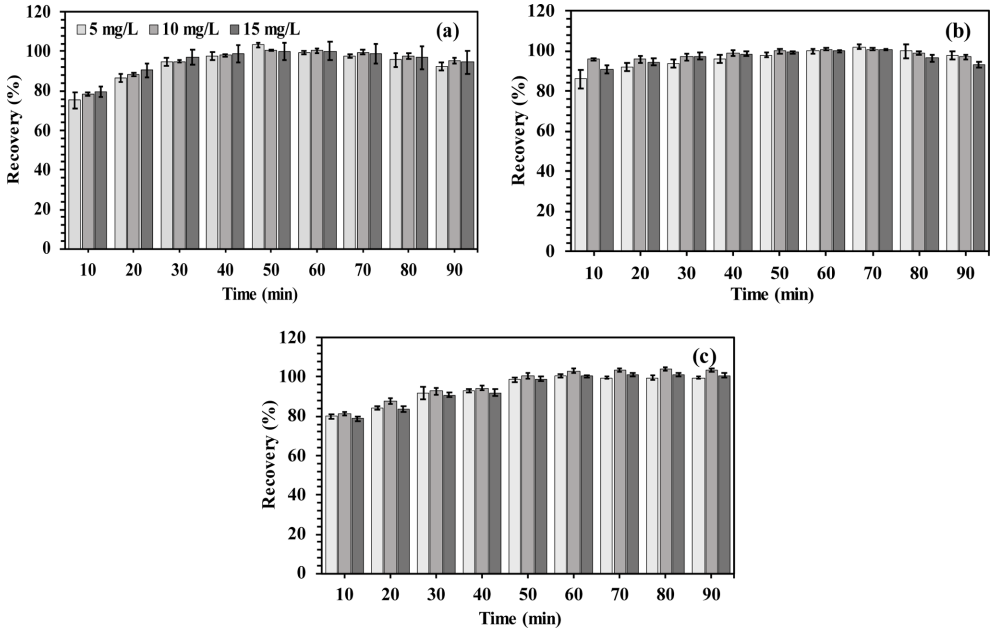

Fig. 1. Effects of the reaction time regarding three fluoride levels of 5.0 mg/L, 10.0 mg/L, and 15.0 mg/L. (a) Al-XO of 100 μM, (b) Zr-XO of 50 μM, and (c) Zr-ARS of 150 μM

Table 2. Effects of aluminium and ferric ions on the colorimetric assays

Colorimetric Assay	Al^{3+} (mg/L)	Recovery (%) For different fluoride concentrations			Fe^{3+} (mg/L)	Recovery (%) For different fluoride concentrations		
		5 mg/L	10 mg/L	15 mg/L		5 mg/L	10 mg/L	15 mg/L
Al-XO	1	92.7±0.5	86.1±1.2	85.3±1.1	0.25	94.7±1.0	90.3±1.2	89.3±0.3
	5	89.9±0.6	83.9±0.7	83.6±1.2	0.5	90.8±0.7	88.6±0.7	88.3±0.4
	10	86.2±0.8	79.9±1.1	82.1±2.2	1	88.2±2.4	82.7±1.9	85.5±1.2
	15	84.3±1.0	78.1±0.8	78.9±1.3	2	81.1±1.5	76.8±1.4	83.1±0.5
	20	80.2±1.4	75.2±0.3	76.6±1.2	3	76.2±1.0	74.9±0.2	76.0±0.2
	25	78.1±1.5	70.4±1.5	74.1±1.0	4	72.0±0.2	70.6±1.5	74.2±0.1
	30	72.8±1.7	67.9±1.1	68.8±0.3	5	71.5±1.0	67.6±1.5	68.1±1.0
Zr-XO	1	98.5±1.0	98.0±0.6	100±1.4	0.25	97.7±0.8	96.6±0.6	100±0.4
	5	99.4±0.7	97.8±1.0	99.8±1.1	0.5	98.7±1.3	95.9±0.2	99.7±1.3
	10	98.2±0.6	98.0±0.3	98.3±1.5	1	96.8±1.0	96.8±0.5	98.3±1.4
	15	98.5±1.1	95.9±0.8	97.8±1.3	2	97.5±1.1	95.3±1.2	97.4±0.6
	20	97.4±1.2	95.6±0.5	96.9±0.9	3	94.9±1.2	93.0±0.7	96.3±1.0
	25	97.3±0.4	95.3±0.4	96.4±0.5	4	89.9±2.5	90.0±1.9	92.3±0.8
	30	97.3±1.1	94.7±0.8	96.6±1.3	5	89.1±1.7	86.1±2.0	88.0±0.9
Zr-ARS	1	93.6±1.1	92.6±0.5	95.3±0.9	0.25	97.6±1.0	93.3±1.0	92.0±0.7
	5	90.2±3.3	93.3±1.5	97.6±0.5	0.5	94.6±1.2	93.9±0.8	92.2±0.4
	10	84.0±1.4	91.4±0.5	92.6±1.1	1	89.1±1.9	90.0±1.8	90.9±0.3
	15	81.3±0.5	91.1±0.7	87.2±0.6	2	85.4±1.0	85.7±1.9	87.9±1.6
	20	79.3±0.5	85.1±0.2	85.8±1.2	3	83.4±1.5	83.0±0.5	85.4±1.3
	25	72.5±0.3	83.3±2.0	82.7±1.4	4	75.2±2.9	78.4±0.9	81.9±1.1
	30	74.2±2.0	79.5±0.9	80.5±1.1	5	72.1±0.5	79.2±1.2	78.8±2.5

for 5.0 mg/L, 10.0 mg/L, and 15.0 mg/L fluoride, in which the increasing Al^{3+} resulted in poorer recoveries and the Al-XO was more affected. However, the Zr-XO assay was the least affected by the presence of Al^{3+} under the investigated concentration range of 1.0 mg/L to 30.0 mg/L, i.e., recoveries of higher than 95 % and only a slight decrease, i.e., 1-4 %, in recoveries were observed as the Al^{3+} concentration increased. The explanation could be based on the stability of the formed complexes in the reaction cocktails with the presence of Al^{3+} interference, including Zr-F, Al-F, Zr-XO, Al-XO, and Zr-ARS, in which the Zr-F is more stable or more favorably formed than Al-F complexes (the formation constants of $[ZrF_6]^{2-}$ and $[AlF_6]^{3-}$ are 1.0×10^{25} and 2.5×10^4, respectively). Therefore, the presence of Al^{3+} hardly disrupt the Zr-F, resulting in less influences.[20] With the Al^{3+} ranges in tea extracts, e.g., 0.06 mg/L to 16.82 mg/L,[23] 0.279 mg/L to 9.38 mg/L, 0.304 mg/L to 15.0 mg/L, and 0.246 mg/L to 20.5 mg/L for 1 g of tea brewed in 50 mL of water for 5 minutes, 60 minutes, and 24 hours, respectively,[24] and 0.15 mg/L to 2.23 mg/L for 2 g of tea brewed in 100 mL of water at 100 ºC for 30 minutes,[25] the Zr-XO assay can be effectively used as a cheap and simple method to quantify fluoride content in tea extracts without any significant effects by the presence of aluminium ion.

Similar tendency (to the case of Al^{3+}) was obtained for the ferric ion interference (0.25 mg/L to 5.0 mg/L) among the three colorimetric assays, in which the Zr-XO was the least affected (recoveries: 86.1 % to 101 %), followed by Zr-ARS (recoveries: 78.8 % and 97.6 %) and Al-XO (recoveries: 67.6 % to 94.7 %). The increasing ferric ion concentrations resulted in the decreased recoveries, emphasizing the effects of the ferric ion on the colorimetric assays. The explanation could be also based on the stability of the formed complexes in the reaction cocktails (as for Al^{3+}). The Zr-F complex exhibited higher stability than the complexes involving Fe^{3+} or Al^{3+} with fluoride. As a

Table 3. Analytical performance of the Al-XO, Zr-XO, and Zr-ARS assays

		Al-XO	Zr-XO	Zr-ARS
Equation		y = 0.0254x – 0.0021	y = 0.0444x – 0.0009	y = 0.0053 + 0.0065
R^2		0.9985	0.9983	0.9986
LOD (mg/L)		0.47	0.31	0.70
LOQ (mg/L)		1.42	1.00	2.13
RSD_r (%)		4.87	2.69	5.97
RSD_R (%)		4.92	2.69	5.97
Recovery (%)	$0.5C_x$	84.0±3.3	95.5±0.8	92.4±3.4
	C_x	85.2±3.3	95.7±1.1	93.5±4.2
	$1.5C_x$	85.8±2.0	95.0±0.8	97.7±4.1

result, the Zr(IV)-based methods were less affected by the ferric ion interference. Comparing the effects of Fe^{3+} and Al^{3+} at the same concentration of 5.0 mg/L, Fe^{3+} caused a more reduction in the recoveries (*vs.* Al^{3+}), i.e., the Al-XO showed a decrease of 33 % *vs.* 14 %, while the Zr-XO and Zr-ARS experienced reductions of 24 % *vs.* 2 % and 28 % *vs.* 10 %, respectively. The difference can be attributed to the varying formation constants of the complexes formed. The $[FeF_6]^{3-}$ complex exhibited a higher formation constant ($1.0 \times 10^{8.6}$) compared to $[AlF_6]^{3-}$ (2.5×10^4), but lower than $[ZrF^6]^{2-}$ (1.0×10^{25}).[26] Consequently, in the presence of Fe^{3+} in the solution, Fe^{3+} would form complexes with fluoride, and the presence of Al is insufficient to disrupt the $[FeF_6]^{3-}$ complex, thereby affecting the availability of free fluoride ions for reaction with the Al-XO reagent. On the other hand, the $[ZrF_6]^{2-}$ complex with higher stability can effectively compete and break the $[FeF_6]^{3-}$ complex. However, at excessively high Fe^{3+} concentrations, the competition of Zr-F formation by Fe^{3+} would be enhanced, resulting in the decreased reaction efficiency. A previous study has also indicated that the Al-XO assay was influenced by Fe^{3+},[16] while the Zr-XO assay was less affected by the ferric ion.[27] With the iron released in the tea extracts in boiled water mostly within or under the ferric ion investigation range in this study,[28-30] the Zr-XO was a favorable candidate due to the recoveries of higher than 95 % for ferrric ion concentrations of less than 1.0 mg/L (*Table* 2). Therefore, the Zr-XO method can be utilized for fluoride quantification in tea extracts in terms of minimized ferric ion effects.

3.4. Analytical method performance

In this study, the analytical methods based on three different colorimetric reagents were evaluated, including Al-XO, Zr-XO, and Zr-ARS assays (*Table* 3).

Three assays demonstrated the goodness of linearity ($R^2 > 0.998$) within the concentration range of 1.0-20.0 mg/L fluoride in DIW. The Zr-XO exhibited the highest sensitivity, followed by the Al-XO and Zr-ARS assays in terms of the obtained calibration slopes. This was also supported by the lower LOD and LOQ estimated from the Zr-XO compared to the other assays. The repeatability (RSD_r) and reproducibility (RSD_R) agreed with the Appendix F of AOAC (2016) for the three assays under the ppm concentration ranges, in terms of the analytical method performance. The recovery tests were conducted on the spiked green tea samples, proceeded to 60-minute extraction at 90 °C at three fluoride levels of $0.5C_x$, C_x, and $1.5C_x$ (C_x: estimated fluoride concentration in the extract). The Al-XO demonstrated the lower recoveries than the others (84.0-85.8 % *vs.* 92.4-97.7 %), which could be due to the potential interferences from the tea matrices, especially related to aluminium and ferric ions (discussed in the above part).

3.5. Application of spectrophotometric method for fluoride quantification in tea extracts

3.5.1. Comparison of fluoride contents in different tea types

Among different tea types, black teas demonstrated

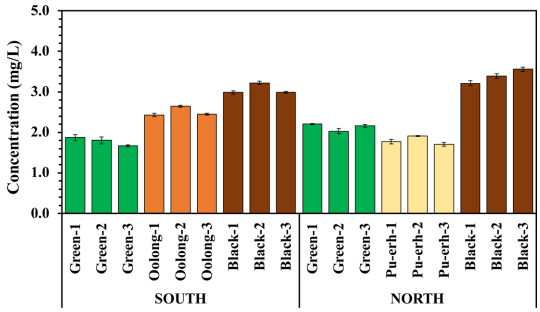

Fig. 2. Fluoride levels in different types of tea products, estimated from 60-minute extraction condition.

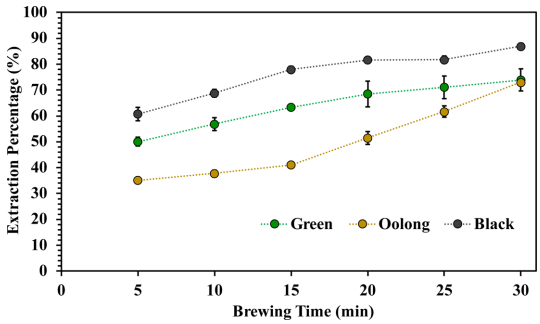

Fig. 3. Effects of brewing time on fluoride extraction percentage (tea samples from the Southern part, Vietnam).

the highest fluoride, while the lowest fluoride was observed for Pu-erh teas (*Fig*. 2). For the southern region, the fluoride contents performed a descending order of black tea > oolong tea > green tea. For the northern region, a decreasing order was observed: black tea > green tea > Pu-erh tea. The variabilities of fluoride among different tea types could be explained by the accumulation of fluoride according to the maturity of leaves, in which black teas were mostly produced from the most mature leaves, while green and Pu-erh teas were made from younger tea buds and leaves. A study of Szmagara et al. analyzed fluoride levels in 33 popular teas, from the Polish market also found that black tea had the highest fluoride in the extracts, with an average of 2.65 mg/L, followed by green tea, with an average of 1.19 mg/L.[31] Similarly, another study reported that black tea had the highest fluoride content among all tea types, ranging from 3 mg/L to 4 mg/L, followed by oolong tea with a range of 0.8 mg/L to 1.6 mg/L, and green tea with a range of 0.3 mg/L to 0.6 mg/L.[8] Furthermore, several other publications have reported similar trends.[32,33]

The accumulation of fluoride is also assisted by the presence of Al in soils, which further contributed to the differences in fluoride contents between the north and south (Vietnam) due to the soils (and Al in soils) are different. The Al-F complex was formed through the release of aluminosilicates from clay soils in an acidic environment, which was then absorbed by the tea plants.[34] This complex subsequently moved to the tea leaves and accumulated in higher quantities in older leaves.[35,36] Additionally, long-established tea plants tended to accumulate higher amounts of aluminium than tea plants with shorter cultivation periods.[37] The aluminium content in tea leaves was proportional to the accumulated fluoride content in the leaves.[36]

3.5.2. Effects of brewing conditions on the release of fluoride

Temperature and time are critical factors in the tea brewing process that can affect the extraction of various components from tea, including beneficial compounds such as polyphenols, flavonoids, amino acids, and potentially harmful substances like metals and fluoride. In this study, we evaluated the effects of brewing temperature and time on extraction of fluoride (using 60-minute extraction as a reference state). As the brewing time increased, the fluoride extraction percentage also increased gradually (*Fig*. 3), i.e., from 50.1 % to 73.9 %, from 35.1 % to 73.0 %, and from 60.7 % to 86.9 % for green, oolong, and black teas, respectively. However, under the same brewing conditions, black tea exhibited the highest fluoride extraction, while oolong tea showed the lowest. This can be attributed to the twisted structure of oolong tea leaves after the production, which requires more time for water to penetrate deep into the leaf structure and release fluoride. Therefore, during the 5- to 15-minute period, the released fluoride content only increased by approximately 6 %. However, during the 15- to 30-minute period, the fluoride content increased by around 32 %. A study by Koblar

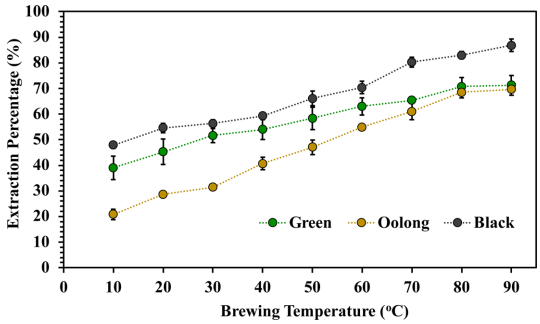

Fig. 4. Effects of temperature on fluoride extraction percentage (tea samples from the Southern part, Vietnam).

et al. reported that a single extraction process released approximately 55-90 % of the fluoride content in tea.[13] Another study by Maleki *et al.* found that increasing the brewing time from 3 minutes to 15 minutes resulted in an increased total fluoride content released into tea extracts for most tea types.[38] Similarly, another study reported that fluoride concentration increased with longer brewing time ranging from 5 to 30 minutes.[39]

The release of fluoride was also observed to be accelerated according to the rising brewing temperature (*Fig.* 4). The fluoride extraction percentage (60-minute extraction was also used as a reference) was higher in black tea (69.7 %-86.9 %) than green tea (39.0 %-71.2 %), and oolong tea also showed the lowest, ranging from 20.8 % to 69.7 %. Similar to the effects of brewing time, the twisted structure of oolong tea leaves required more time for water to penetrate the leaf structure and extract fluoride. A publication by Pattaravisitsate *et al*. revealed that boiling water (100 °C) led to higher infusible fluoride levels compared to warm water (80 °C) for black tea, green tea, and white tea but less effects for oolong tea (and herbal tea).[40] The study of Fung *et al*. also observed that the release of fluoride into tea extracts increased in accordance with higher temperature and longer infusion time.[41] Furthermore, several other published studies have reported similar results.[31,42]

4. Conclusions

This study compared different colorimetric reagents, i.e., Al-XO, Zr-XO, and Zr-ARS, for the fluoride determination using the UV-Vis measurement. The colorimetric reactions were discussed, including reagent concentrations and reaction time, in which 60-minute period was chosen. The effects of the potential interference ions were also investigated, indicating the Zr-XO was the most favorable candidate due to the higher recoveries compared to the other colorimetric assays. The methods for all three reagents were evaluated based on the Appendix F of AOAC (2016) for analytical method performance. The Zr-XO also performed the highest sensitivity and lower estimated LOD/LOQ, which was applied for the fluoride determination in different tea extracts. The variabilities of fluoride among different tea types could be explained by the accumulation of fluoride according to the maturity of leaves, and black tea performed the highest fluoride content since it is produced from the most mature leaves. The experiments of the brewing condition effect, i.e., time and temperature, indicated that the release of fluoride was accelerated according to the rising brewing time and temperature. This study highlighted the application potentials for the colorimetry as a simple, cheap, and convenient approach for many laboratories in the context that the UV-Vis is commonly equipped and does not require high expertise.

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