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To cite this article: A Agustanti *et al* 2018 *J. Phys.: Conf. Ser.* **1073** 022001

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The measurement of fluoride ion in anchovy (*Stolephorus insularis*) using ion selective electrode

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Abstract. Anchovy is a common fish found in all over Indonesia's sea. Anchovy, often stated, that it has high fluoride content as well as other ocean fishes, but to date there are limited study measuring the fluoride content in anchovy (*Stolephorus insularis*) established yet. The aim of this study is to measure fluoride content in *Stolephorus insularis* using Ion Selective Electrode (ISE). To measure fluoride ion with ISE, sample preparation was needed to obtain maximum fluoride readability. Sample preparation consisted of heating, shaking, digesting, and ashing. Samples were divided into two groups, one was heated using the oven at 800 for 5 minutes (oven group), and the other group was left fresh (non-oven group). The prepared samples were then measured with ISE. The results found that the fluoride concentration of non-oven and oven *Stolephorus insularis* ranged from 12.935 – 13.381 ppm and 15.416 – 24.914 ppm, respectively. It is concluded that ovened *Stolephorus insularis* has potential to be a natural source of fluoride.

1. Introduction

Fish generally contain lots of fluorides [1]. Anchovy is the type of fish most consumed by in Asia, because it is easy to get and affordable [2]. Anchovy contains calories, fats, proteins, carbohydrates, vitamins, minerals, including fluorides as trace elements. Anchovies that circulate on the market are commonly found in two types, namely *Stolephorus insularis* and *Stolephorus baganensis*. Both types of anchovies are sold in fresh form and dried form. However, this study analyzed *Stolephorus insularis*.

Fluoride is an essential mineral for the human body, it is also an important component in food [3]. In addition to the body's metabolism regulation, fluoride also serves to help deposit calcium in bones and teeth [4]. In the field of dentistry, fluoride plays a significant role in the prevention of caries because fluoride has a cariostatic effect that can suppress the solubility of enamel due to the acid produced by bacteria [5]. Fluoride is sourced from natural ingredients and is largely contained in marine products. It



shows that to obtain a source of fluoride for dental health is not difficult, especially in Indonesia which is a maritime country rich in seafood. Also, fluoridation through food is a cheap and easy way to do it. Therefore, anchovy that is the result of Indonesia's sea become one of alternative fluoride source which is cheap, easy to get, and spread all over Indonesia. So far, studies on the amount of fluoride in natural ingredients, especially in anchovies are scarce. This might be due to the highly reactive and unstable nature of fluoride. Therefore, in this study, the sample was divided into two different sample preparation methods, i.e., the heated sample in the oven and the sample that is not heated or left fresh (non-oven). This was done based on assumption that the heating process will increase the fluoride reactivity in anchovies. Also, the distribution of the sample is also based on the assumption that people consume more of the heated fish. It is hoped that at the end of this study, alternative food that is healthy, cheap, and rich in fluoride, can be introduced.

The fluoride isolation method needs to be done before measurements on the sample to overcome any disturbances that may be caused by other ions. The method of fluoride isolation conducted in this study was shaking, digesting, and ashing. The fluoride content in marine fish is generally known to be more than 1 ppm [6]. Based on these data, it was suspected that Anchovy contains fluoride in higher quantities. With these assumptions, fluoride measurements in this study used the Ion Selective Electrode (ISE) tool. ISE can measure fluoride from a range of 0.1 to more than 10mg/L, this tool tends to be more sensitive, easier to use, and more effectively compared to other means or tools [7].

2. Materials and Methods

This research was laboratory research conducted at Central Laboratory of Drug and Food Inspection, Agency for Drug and Food Control, Jakarta. In this study, the samples used were *Stolephorus insularis* which was preserved by salted, this sample was taken randomly from the market in Jakarta, Indonesia area. Based on the preparation method, samples were divided into two groups, i.e., non-oven and oven. In the non-oven group, *Stolephorus insularis* sample was directly smoothed using a blender (Moulinex type 53402, Moulinex, French) to form a grain-like grain of sand. While on oven group, samples of *Stolephorus insularis* are preheated in the oven (National type No.NT-T10N, National, Jakarta, Indonesia) at 800°C for 5 minutes until the color turns into yellowish gold, then mashed with a blender (Moulinex type 53402, Moulinex, French) until the grain of sand.

This sample then passed through the fluoride isolation stage which was divided into three different processes, namely: shaking, digesting, and ashing. In the shaking process, 2g of the sample was fed into a measuring flask and added with aqua demineralization up to 100ml, then shaken with a stirrer (Elma Transsonic type T460, Tovatech, Orange County, New Jersey, USA) for 15 minutes until homogeneous. In the digestion process, 1g of sample was inserted into plastic wrapper then 4ml irrigated mineralized and 2 ml of concentrated HCl 37%, and shaken until homogeneous. Beaker was placed on the water bath at a temperature of 800°C for 30 minutes, then removed and allowed to reach room temperature. The sample was then transferred into a measuring flask and added with aqua demineralization up to 100ml, then shaken until homogeneous. In the filling process, 5 grams of sample was fed into a porcelain crucible which was then placed onto the electric bath until it was smoke-free and the sample was black. The sample was then fed into a furnace (Naber type 2804, Naberthem, Lilienthal, Germany) and heated to a temperature of 5500°C to perfect ashing, which was white ash. The ash was allowed to reach room temperature, then added 15ml HCl 6N and dried over a water bath at 1100°C while shaking. Then 10ml 3N HCl was added while shaken, then placed on the water bath again to boiling. The cruses were lifted, and the sample was allowed to reach room temperature, then transferred into a measuring flask and added with microorganisms up to 100ml, then shaken until homogeneous.

Furthermore, Total Ionic Strength Adjuster Buffer (TISAB) II was made. 100 ml of aqua demineralization in measuring flask was added with 58gr NaCl and shaken until dissolved above the stirrer. Then 4 grams of cyclohexylenediamine-tetraacetic acid (CDTA) was added gradually while being shaken. The aqua demineralization was added until dissolved. In a fume hood, 57 ml of glacial acetic acid was added gradually until dissolved. pH of the solution was adjusted by adding 5M NaOH

(NaOH 20%) approximately 150ml gradually while shaking up to a pH ranging from 5.0-5.5. The pH control was measured using pH meters (Metrohm types 713 and 744, Metrohm, Herisau, Switzerland). When the desired temperature was obtained, the solution was allowed to reach room temperature and 1 liter of aqua demineralization added.

After samples and TISAB II solutions were ready, fluoride ion measurements were made using pH/ion meter devices (Metrohm types 713 and 744). The ISE is a method of measuring ions by using special electrodes. The ISE measurement principle is to measure millivolt (mV) changes due to the fluoride ion displacement activity before and after the addition of standard solutions. In this study used ISE with brand Metrohm type 6.0502.150. Samples and TISAB II were inserted into a plastic alarm with a ratio of 1: 1 (in this test used 20ml: 20ml), with volume represented by V_o and sample volume alone denoted by V_s . The electrodes were inserted into the beaker to set, and the beaker was shuffled during the measurement. The sample solution was measured and recorded the mV number after drift was lost and denoted as U_1 . After that, the standard fluoride (NaF) solution was added moderately (V_{std}) with a concentration of 0.001 mol/L (C_{std}) using Eppendorf, as measured, until the measurement difference (ΔmV) was at least ten mV, then measured until drift was lost. The difference was recorded then the factor value (increment factor = A) seen in the table, then performed the following calculation:

$$\text{Fluoride Levels (mol/L)} = \frac{A \times C_{std} \times V_{std} \times V_o}{V_o \times V_s}$$

The result of these calculations were then recorded and compared by using the calibration curve. The statistical analysis was done by performing independent t-test with 95% confidence level and p-value ≤ 0.05 .

3. Results

The measurement result of fluoride in in *Stolephorus insularis* with ISE method is shown in Table 1 below.

Table 1. Measurement of Fluoride Content in in *Stolephorus insularis*

Group	Sample Preparation	Fluoride Content $\mu\text{gF/g}$ in <i>Stolephorus insularis</i> (ppm)	
		Mean	SD
Non-oven	Shaking	13,380 ^{a, d, e*}	2,366
	Digesting	13,395 ^{b*, d, f*}	2,652
	Ashing	7,689 ^{c, e*, f*}	1,920
Oven	Shaking	15,416 ^{a, g*, h*}	2,654
	Digesting	24,914 ^{b*, g*, i*}	4,661
	Ashing	5,378 ^{c, h*, i*}	580

a-a, b-b, c-c, d-d, e-e, f-f, g-g, h-h, i-i tested by independent T-test statistic test

* show statistically significant differences

The result of statistic test shows that there are significant differences of fluoride content between non-oven and oven in *Stolephorus insularis* by digestion process, non-oven in *Stolephorus insularis* by shaking and ashing process, non-oven in *Stolephorus insularis* by digestion and ashing process, ovened in *Stolephorus insularis* with the process of shaking and digesting, ovened in *Stolephorus insularis* by shaking and ashing process, and ovened in *Stolephorus insularis* by digesting and ashing process.

4. Discussion

Fluoride is one of the halogen elements, considered as the most electronegative element, highly reactive and unstable, making it very difficult to handle and measure. Fluoride contained in nature is all present

in a bound form [7]. In this study, the measured fluoride should be in the form of free ions because the measurement method used was the ISE which can only measure fluoride in free form. Since the fluoride in anchovies is in a bound form, it is necessary that fluoride isolation methods be able to break the fluoride bond from its complex form. There are a variety of ways of isolating fluoride, including by adding acids or other substances, mechanically or physically, and by heating. The method of fluoride isolation conducted in this study was by shaking, digesting (with the addition of strong acid and warming), and ashing (by heating with very high temperature and acid addition). This study used the ISE method to measure fluoride levels in *Stolephorus insularis* because this method is a frequently used method, as well as colorimetric. Compared with the colorimetric method, ISE can measure fluoride in samples of 0.1 to >10 mgF/L (ppm), whereas the colorimetric method can only measure fluoride in samples of 0 to 1.4 mgF/L. The advantage of ISE methods from other methods is that this method specifically measures only fluoride ions, tends to be freer of intervention, easy to use, and does not take long in its use [8, 9]. In measuring the content of fluoride in *Stolephorus insularis*, this is not used glass beaker but which is used is a plastic beaker, because the nature of fluoride that can eat silicate so that can interfere with ISE reading. In using ISE, special reagents are needed to assist fluoride measurements. Reagent is TISAB. There are several kinds of TISAB namely TISAB 0, TISAB II, TISAB IV, TISAB C, and TISAB T.11 In this study selected TISAB II reagents because TISAB is freer from intervention because of the CDTA [10]. ISE membrane sensitivity needs to be considered, especially when cleaning the membrane between usages. The membrane should always be cleaned with a soft tissue slowly and gently. This was done to clean the membrane from the previous sample, so as not to interfere with ISE reading of fluoride in subsequent samples. Another thing to note is that if ISE is used continuously, it will be subject to thin film films that interfere with the accuracy of fluoride readings. Also, there may also be small defects or basins on the ISE membrane so that it can be filled by one sample solution and interfere with fluoride readings in the next sample.

In measuring the fluoride content with ISE, a pH meter with a wide millivolt (mV) scale was used. Free fluoride ion transfer activity is calculated by the fluoride electrode shown in pH meter in mV unit [11]. Measurement of sample solution ready for measurement with ISE reads fluoride ion activity in the sample solution as U1 in mV unit. Then, in the same sample drops a standard fluoride solution sufficiently, so the pH meter shows the value of U2. The difference between U1 and U2 is expected to have a value between 10-40 mV, to be read in the available table increment factor. Based on the results of the experiment, in addition to the standard solution of fluoride NaF 0.1M into the sample, with Eppendorf 10: 1 (smallest capacity), the difference of U1 with U2 is too large or exceeding 40 mV, so it cannot be seen increment factor (A) in the table. This results in the non-calculation of fluoride content by the formula for calculating fluoride levels (Metrohm). To obtain a good U1 with U2 difference, ranging from 10-20 mV, the standard solution used needs to be diluted 100 times with aqua demineralization, to obtain a standard solution of 0.001M NaF. From the results of this study, overall it is known that the fluoride contained in the samples of *Stolephorus insularis* that have been read out by ISE is greater than the fluoride found in non-oven anchovies. This is because the sample preparation of ovened Anchovy can be obtained better granulation of the sample and there is a reduction of moisture content by heating. With less water element (H₂O) in the sample of the *Stolephorus insularis*, it allows TISAB II to enter and react so that more free fluoride ions can be read. In contrast, in non-oven *Stolephorus insularis* samples, the H₂O content is more numerous, resulting in TISAB II meeting the H₂O molecule first, thereby lowering the performance of TISAB II to expose the fluoride. Also, in the presence of H₂O molecules, it results in a decrease in the potential of free fluoride to bind to water molecules, resulting in fewer fluoride ions readable by the ISE [12]. By ovened, the *Stolephorus insularis* sample gets heated, thus increasing fluoride reactivity to ISE and improving fluoride readings. Smooth grinding will expand the contact surface between samples with TISAB II so that fluoride readings by ISE will be better.

In the method of fluoride isolation by digesting, a higher reading of fluoride content was observed compared to the non-digestion fluoride isolation method. This is because, in the digestion method, the sample is added concentrated HCl (a more reactive strong acid group) which can substitute and break

the weak acid salt bonds. Increased fluoride readings on ISE by digestion method are also due to sample heating which can improve CDTA performance in breaking up the fluoride complex [13]. The decrease in fluoride levels in ashing fluoride isolation method can occur due to the heating process at high temperatures and for a long time, so that fluoride ions evaporate and sublime [14]. With the evaporation of fluoride ions, it will also decrease the fluoride ion concentration in the sample, so that the ISE reads decreases in number. Another possibility that can cause at least fluoride levels in the method of ashing due to the addition of 6N HCl and the addition of 3N HCl which is then reheated results in saturation of fluoride ions to bind back to their complex form and increase the number of fluoride ions evaporating.

5. Conclusion

Fluoride content of non-oven and oven in *Stolephorus insularis* are 13,380-13,935 ppm and 15,416-24,914 ppm. The oven sample preparation method is more effective than the non-oven; with the most effective method of fluoride isolation is by the digestion method.

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