https://doi.org/10.17221/85/2018-CJFS

Effects of different pH, temperature and foils on aluminum leaching from baked fish by ICP-MS

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Citation: Inan-Eroglu E., Gulec A., Ayaz A. (2019): Effects of different pH, temperature and foils on aluminum leaching from baked fish by ICP-MS. Czech J. Food Sci., 37: 165–172.

Abstract: The effect of different type of fish, marination methods, temperature, and cooking time as well as foil type on Al leaching into baked fish, was determinated. Two fish types, Al foils, and marinating ingredients were procured from a hypermarket. Fish was cooked in the baking tray of oven, using two foil types, at 150°C for 40 min and at 200°C for 20 min. Al content was determined. It was found that increase in acidity caused significant increase in Al leaching. When the relationship between temperature-time and Al leaching based on meat type was analysed, it was found that when the temperature was increased the extent of Al leaching was higher in salmon. Even in the highest estimated weekly exposure to Al (1.228 ± 0.1631 mg/kg per week), Al PTWI suggested by JECFA was not exceeded. Although using different Al foil did not leach significant amounts of Al into the fish and exposure values of Al did not exceed the PTWI, exposure of Al from fish samples may be dangerous to vulnerable groups such as children, elderly and people with kidney disease.

Keywords: aluminum; baking; dietary exposure; haddock; salmon

Aluminum (Al) is the third most abundant element after oxygen and silicone and the most common metal in the earth's crust. Although Al is ubiquitous in the environment, it is nonessential element for biological systems (Exley 2003).

Al toxicity is well established in chronic renal failure patients (Sharma *et al.* 2005). Al exposure had been associated with neurological disorders including dialysis encephalopathy syndrome (Bansal & Bansal 2014), Alzheimer's disease (Kawahara & Kato-Negishi 2011), Parkinson disease (Laabdar *et al.* 2016) and multiple sclerosis (Exley *et al.* 2006); skeletal diseases (Chappard *et al.* 2016); hematopoietic diseases (Jeffery *et al.* 1996); adverse respiratory and immunologic health effects (Willhite *et al.* 2014; Zhu *et al.* 2014). Although the relationship between Al and its health effects are controversial, more and more questions are raised about health effects due to public concerns of Al. Al enters into human body through drinking water, food, kitchen utensils, and pharmaceuticals (Yildiz *et al.* 2017). Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2011) has established the provisional tolerable weekly intake (PTWI) as 2 mg Al/kg of body weight (*BW*) per week. The estimation of dietary intake of Al is between 3 and 30 mg/day (Vasudevaraju *et al.* 2008). Al is widely used in kitchen utensils, because of its properties including low specific weight, excellent thermal and electrical conductivity, flexibility, resistance to oxidation, and superior barrier quality (Müller *et al.* 1998; Ranau *et al.* 2001; Joshi *et al.* 2003). Thus, widespread usage of Al in kitchen utensils makes them a potential source of dietary Al (Turhan 2006). The extend of Al leaching from kitchen utensils is highly dependent on the acidity of the food and/or cooking medium, cooking time and temperature, food composition, and the presence of complexing species (Ranau *et al.* 2001; Veríssimo *et al.* 2006). The Al leaching processes from kitchen utensils can be described by the following chemical reaction: $Al_2O_3 + 6\,H^+ \;\; 2Al^{3+} + 3H_2O$, where Al_2O_3 is a protective film.

This chemical reaction occurs on the surface of the Al utensils (Bi 1996).

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The use of Al foil with meat and fish in baking process is a common practice in order to protect food against direct heat effect and prevent water uptake (Ranau *et al.* 2001; Turhan 2006). It is important to determine the Al leaching into foods from Al foils since there is a relationship between Al and the specific diseases mentioned above. Although leaching from regular Al foils has been established, there are no studies about leaching from foils, whose one side is baking paper, and the other, Al. In this context the aim of this study was to determine the effect of different types of fish, marination methods, temperature, and cooking time as well as foil type on Al leaching into baked fish.

MATERIAL AND METHODS

Sample preparation and cooking process. Salmon and haddock, foils of the same brand, ingredients for marination (Riviera olive oil, milk, salt, and white pepper) were purchased from a hypermarket in Ankara, Turkey. Fishes were placed immediately into an icebox (Igloo Ice Peak) and transported in an air-conditioned car to research laboratory at Nutrition and Dietetics Department, Hacettepe University (Turkey). All fish samples were stored at –20°C until used. The frozen fishes were thawed at 4°C overnight in refrigerator, prior to cooking on the morning of the study. Thawing water was not used.

For each type of fish, 2.5 kg of meats were purchased and all types of meat were divided into 250 g portions. One portion was analysed as raw fresh sample without any marination. Other portions were marinated with two different marination methods. Marination method *X*: Riviera olive oil (25 g), salt (3.75 g), and white pepper (1.25 g). Marination method *Y*: Riviera olive oil (25 g), salt (3.75 g), white pepper (1.25 g), and milk (25 ml). The portions were stored at 4°C in a refrigerator for 10–12 h and baked in a house-type embedded oven (Arcelik, Turkey) in a baking mode after being wrapped in two different types of foils (30 × 35 cm) at 150°C for 40 min and at 200°C for 20 min, then removed and cooled. The Al content of marination solution was not analysed.

Following types of foils were used for baking meat: Foil (1): Al foil; Foil (2): Baking paper (interior surface) + Al foil (exterior surface).

Thus, the Al content of 18 samples in total, including cooked and raw samples, was detected (a schematic representation of this can be found in Figure 1). The samples were homogenized using a kitchen blender and were transferred into disposable plastic tubes for analysis. All meat samples were frozen at –20°C until measurement of Al content.

Proximate composition and pH analysis. The protein, fat, and ash contents of raw samples were determined according to the methods described by AOAC (2012). Homogenized samples of each species were individually analysed in triplicate; each value is provided as the mean ± standard deviation.

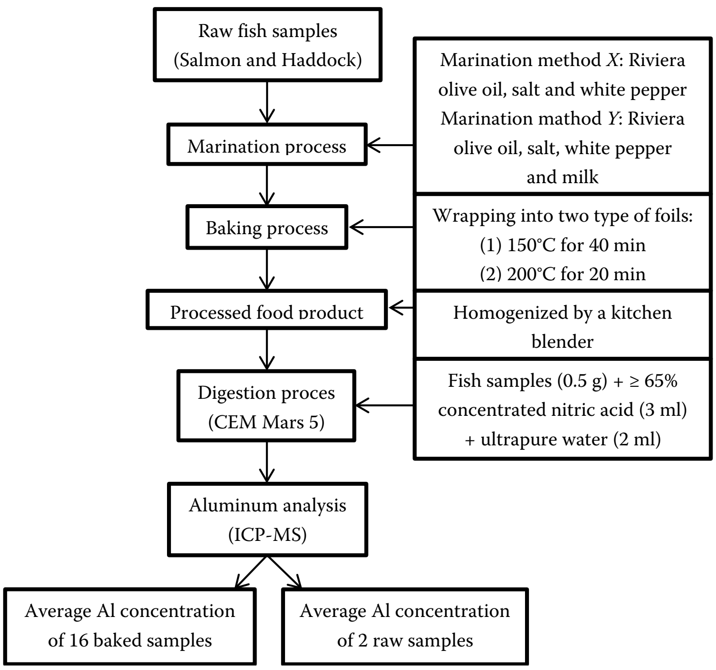

Figure 1. Schematic representation of the experimental design

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The protein content of raw meats was determined by using the Kjeldahl method, and the lipid content was determined by using Soxhlet method. Moisture content was detected by using Sartorious® MA150 (Sartorius; Germany). A value of pH was measured with a pH meter (2020 edge®; Hanna Instruments, UK) which was calibrated by using standard buffers of pH 4, 7, and 10 at room temperature. All parameters were determined in triplicate.

Inductively coupled plasma mass spectrometer (ICP-MS) analysis. The quadrupole ICP-MS Thermo Electron X series II (Thermo Electron Corporation, USA) was used in this study.

All chemicals and solutions used during Al analysis were of analytic grade. Nitric acid (HNO_3, ≥ 65%, for ultrapure/trace element analyses) was purchased from Merck (Darmstadt, Germany). A water purification system (analytic grade water from Barnstead; Thermolyne Nanopure Diamond Analytical Ultrapure Water System, USA) was used to obtain ultra-deionized water (resistivity, ≥ 18 MΩ). Al single element standard (1000 µg/ml in 2% HNO_3) was obtained from High Purity Standards (USA). Ultrapure grade (99.998%) argon (Ar) gas (230 bar in 15°C, 12.06 m^3) was acquired from Ankara Gas (Ankara, Turkey). Oyster Tissue (1566b) was used as a standard reference material (SRM) for meat (NIST, USA).

Before ICP-MS analysis, the samples were digested by using a closed microwave digestion system CEM Mars 5 (CEM Corporation, USA). Before analysis, 0.5 g of the meat samples was weighed in ICP-MS using 50-ml Teflon XP-1500 Plus bombs. For trace analyses, 5 ml of ≥ 65% concentrated nitric acid and 2 ml of ultrapure water were added. The organic component was digested by using the microwave heating program in the CEM Mars microwave oven according to the NMKL method No. 186 (2007). After the samples were cooled to room temperature, they were diluted to 25 ml with ultrapure water, and then put in the ICP-MS for determination of Al content.

For analysis, the signal optimization and preliminary performance controls of the device were calibrated by using 10 ppb tune solutions. Device calibrators were prepared with 50 ppb tune solutions. Operation conditions for ICP-MS were as follows: The radio frequency (*RF*) power was 1430 W. Argon gas flow rates for the cool, auxiliary, and nebulizer flow were 13 l/min, 0.80 l/min and 0.80 l/min, respectively. All samples were processed in triplicate, each sample was read five times, and the mean values were calculated. The calibration blank (1% HNO_3) was analysed and assessed as an unknown sample about 20-times. The limit of detection (LOD) was detected by tripling the standard deviation and the limit of quantification (LOQ) was detected by decoupling the standard deviation. The LOD and LOQ values for Al were found to be 0.74 and 2.47 ng/ml, respectively. The Al content of the standard reference material (SRM) for meat (1566b Oyster Tissue; NIST, USA) was 197.2 ± 6 ppm (mg/kg); the measured value was 197.4 ± 2 ppm (mg/kg).

Estimation of risk exposure to Al from fish samples. In order to compare Al intake of individuals with the PTWI value that determined by JECFA (2011) as 2 mg/kg per week, Al exposure of individuals was calculated by using Al levels in wet matter of fish samples. The body weight data (kg) for women and men was taken from the Turkish Nutrition and Health Survey to evaluate the exposure of Al from fish samples. According to the Turkish Nutrition and Health Survey (2010) mean weight for female adults (19 age and above) and male adults (19 age and above) were taken as 70.9 and 77.2 kg, respectively. Al exposure was assessed by considering that individuals consumed 250 g of fish samples. Estimated weekly intake (EWI) was calculated as suggested by the World Health Organization (2009). EWI (mg/kg *BW*/week) of individuals was calculated as following Equation 1:

$$\text{EWI} = [\text{Al}_c \times 250\ \text{g} \times 7\ (d/w)]/BW \qquad (1)$$

where: *BW* – body weight (kg); Al_c – a mean of aluminium concentration (mg/kg)

Surface study. Scanning electron microscope (SEM) (Quanta 400F; FEI Philips) attached with energy dispersive X-ray (EDX) was used for the surface analysis of foil samples. Magnification was 500×. Foils were baked at 150°C for 40 min and at 200°C for 20 min without wrapping fish to see the effect of the temperature to the foils. Foils were also analysed as blank without exposure any temperature treatment. The result of the analysis helps in indicating any leaching of Al because of the temperature.

Statistical analysis. As a descriptive statistic for the measured variables, the mean value and standard deviation were used. The protein, fat, moisture, and ash contents of the fish are shown as percentages (%). Based on the cooking temperature and marination methods, the percentage of Al leaching into the fish in case of different types of Al foils was compared by using the Mann Whitney-U test. For the different meat

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samples baked by using Foil (1) and (2), the effect of cooking temperature and time and marination methods on Al leaching as well as the relationship between the change in the Al content of cooked samples, fat content of raw samples, and pH of cooked samples were assessed by using Spearman's correlation test. The lowest significance level was determined to be 0.05. All statistical analysis was performed using IBM SPSS Statistics 22.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

Proximate composition and pH values. The protein, fat, ash, moisture, pH and Al content in the dry matter (*DM*) of raw fish samples are presented in Table 1. The protein and fat contents of raw salmon samples were 17.85 ± 0.87 and 18.06 ± 0.56%, respectively. The same parameter for haddock was 13.00 ± 1.48 and 0.27 ± 0.003%, respectively. Similar results for salmon and haddock have been reported by the Food Standards Agency (Finglas & Roe 2015).

The ash content of raw salmon and haddock were 1.23 ± 0.08 and 1.24 ± 0.06%, respectively. The moisture content of the raw salmon and haddock sample was found to be 42.21 ± 2.23 and 66.93 ± 2.64%, respectively. In a study conducted with Atlantic bonito, the ash and moisture content of the fish were reported as 1.76 ± 0.15 and 67.71 ± 0.40%, respectively (Koral *et al.* 2010).

An important factor for determining the quality of meat – pH value, is related to the water-binding capacity of the muscle protein of the meat (Miller 2014). In parallel with the literature (Ólafsdóttir *et al.* 2006), in this study pH value of raw salmon and haddock samples were 6.34 ± 0.01 and 6.76 ± 0.01, respectively.

The Al content of raw salmon and haddock samples was 67.00 ± 4.45 and 86.79 ± 6.14 mg/kg, respectively. The Al contents of the meats vary depending on the multifactorial causes. It was reported that Al contents of meat can be different due to Al content of the soil, water and air, the application of animal feed and animal raising (Semwal *et al.* 2006; Goran *et al.* 2016). In a study to determine the Al content of foods in Greece, the average content of Al in fish was found 0.62 mg/kg (Bratakos *et al.* 2012). In a study that conducted in Hong Kong, the mean Al content of fish meat and seafood was determined 4.9 mg/kg (Chen *et al.* 2014). In a study in Belgium that determined the Al exposure levels through the use of Al kitchen utensils, the amount of Al in fish was found 0.62 mg/kg (Fekete *et al.* 2013).

Al content of baked meat samples. Percentage change in Al content of fish samples baked with different marination methods at 150°C for 40 min are given in Figure 2. In the haddock meat cooked at 150°C for 40 min with the application of marination method *X* and *Y* using Foil (2), Al leaching was significantly higher compared to salmon meat. There was not any significant difference in the case of Foil (1) at 150°C for 40 minutes. Percentage change

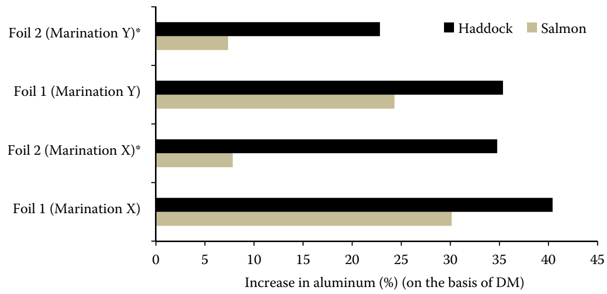

Figure 2. The changes in Al content (%) of haddock and salmon caused by different marination methods at 150°C

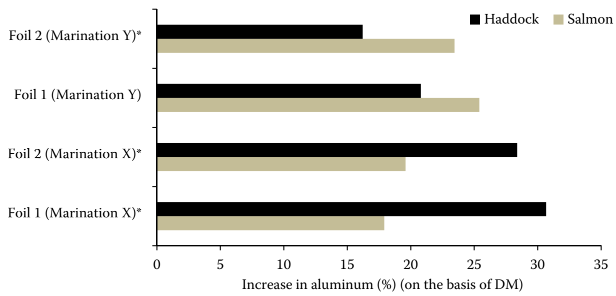

Figure 3. The changes in Al content (%) of haddock and salmon caused by different marination methods at 200°C

Table 1. Protein, fat, ash, and moisture content (%), pH, and Al content in the dry matter (DM) of raw fish samples

Meat type	Protein	Fat	Ash	Moisture	pH	Al
	(%)					(mg/kg *DM*)
Salmon	17.85 ± 0.87	18.06 ± 0.56	1.23 ± 0.08	41.21 ± 2.23	6.34 ± 0.01	67.00 ± 4.45
Haddock	13.00 ± 1.48	0.27 ± 0.003	1.24 ± 0.06	66.93 ± 2.64	6.76 ± 0.01	86.79 ± 6.14

DM – dry matter; values are presented as a mean ± sd

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in Al content of fish samples baked with different marination methods at 200°C for 20 min are given in Figure 3. In the haddock meat cooked at 200°C for 20 min with the application of marination method *X* using Foil (1) and Foil (2), Al leaching was significantly higher compared to salmon meat. Opposite of these results it was found that in the salmon meat cooked at 200°C for 20 min with the application of marination method *Y* using Foil (2), Al leaching was significantly higher compared to haddock meat. It is thought that the reason why the Al leaching in the haddock meat is higher compared to the salmon meat is due to the fact that the salmon slices are thicker than the haddock fillets. Thus, the exposure area to the Al foils of the haddock filet is wider than the salmon slices. Al Juhaiman (2015) showed that Al leaching increased with Al exposure area into meat baked with Al foil. Opposite of the results of this study, Ranau *et al.* (2001) has found that Al leaching was higher in the fatty and medium fatty fishes compared to lean fishes, baked at 200°C for 20 min, wrapped in the Al foil.

It is well established that higher acidity causes an increase Al leaching in cooking procedures with Al kitchen utensils (Joshi *et al.* 2003; Veríssimo *et al.* 2006; Mohammad *et al.* 2011). When the relationship between pH and percentage change in Al content based on meat type was analysed, it was found that increase in acidity caused significant increase in Al leaching (haddock cooked with Foil (2), (r = –372, P < 0.05). In parallel to this study, Bassioni *et al.* (2012) analysed Al leaching into ground meat cooked in Al foil in different acidic solutions (tomato juice, citric acid, or cider vinegar) and found that Al leaching was increased in lower pH.

When the relationship between temperature-time and Al leaching based on meat type was analysed, it was found that when the temperature was increased while cooking salmon in Foil (2) (r = 0.728, P < 0.05) and when the time of cooking was increased in case of in Foil (1) (r = 0.363, P < 0.05), the extent of Al leaching was higher. In the literature, there are studies showed that Al leaching into foods increased with increasing temperature and time. Ranau *et al.* (2001) determined Al leaching into foods based on different cooking methods. In the study, 4 different types of fish (codfish, coalfish, seabass and mackerel), wrapped with Al foil, were baked as plain or with vinegar and salt or were grilled as plain or with onion rings and 1–2 g of mixed spices by using coal. As a result of baking and grilling process, it was

Table 2. Estimated weekly exposure to Al (mg/kg per week) for adults

Fish type	Marination method	Female				Male			
		150°C for 40 min		200°C for 20 min		150°C for 40 min		200°C for 20 min	
		1	2	1	2	1	2	1	2
Salmon	*X*	1.228 ± 0.0125	1.055 ± 0.0480	1.146 ± 0.1158	1.095 ± 0.1121	1.128 ± 0.0115	0.969 ± 0.0441	1.053 ± 0.1063	1.005 ± 0.1030
	Y	1.228 ± 0.1631	1.079 ± 0.0865	1.118 ± 0.0603	1.105 ± 0.1168	1.128 ± 0.1498	0.991 ± 0.0795	1.027 ± 0.0554	1.015 ± 0.1073
Haddock	*X*	0.999 ± 0.0832	0.917 ± 0.1408	0.961 ± 0.0261	0.852 ± 0.0238	0.917 ± 0.0765	0.842 ± 0.1293	0.882 ± 0.0240	0.782 ± 0.0219
	Y	0.907 ± 0.1354	0.894 ± 0.1520	0.871 ± 0.0523	0.828 ± 0.0232	0.833 ± 0.1244	0.821 ± 0.1396	0.799 ± 0.0480	0.761 ± 0.0213

Y – Riviera olive oil, salt, and white pepper; *X* – Riviera olive oil, salt, white pepper, and milk; 1 – foil 1; 2 – foil 2

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detected that there were more Al migrations in the grilled fishes due to the fact that the grilled fishes were cooked under higher temperatures. Turhan (2006) analysed the effect of temperature and time on Al leaching in different types of meat (beef, water buffalo, mutton, chicken breast and drumstick, and turkey breast and drumstick) which were baked at 150°C for 60 min, at 200°C for 40 min, and at 250°C for 20 min by wrapping Al foil and found that maximum Al leaching into meats was at 250°C for 20 min baking. On the other side, Ekanem *et al.* (2009) analysed the effect of time in the same cooking temperature. In the study, beef was baked at 100°C for 60, 90, 120, 150, and 180 min, wrapped in Al foil and it was observed that Al leaching increased with the increase of cooking time.

Estimation of exposure to Al from fish samples. Estimated weekly exposure to Al (mg/kg per week) for adults is given in Table 2. In every scenario the estimated weekly exposure to Al did not exceed the Al PTWI. The highest Al exposure was found in salmon cooked wrapping Foil (1) with the application of marination *X* and *Y* at 150°C for 40 min in female adults as 1.228 ± 0.0125 mg/kg per week and 1.228 ± 0.1631 mg per kg per week, respectively. The lowest Al exposure was found in haddock cooked wrapping Foil (2) with the application of marination *Y* at 200°C for 20 min in male adults as 0.761 ± 0.0213 mg/kg per week.

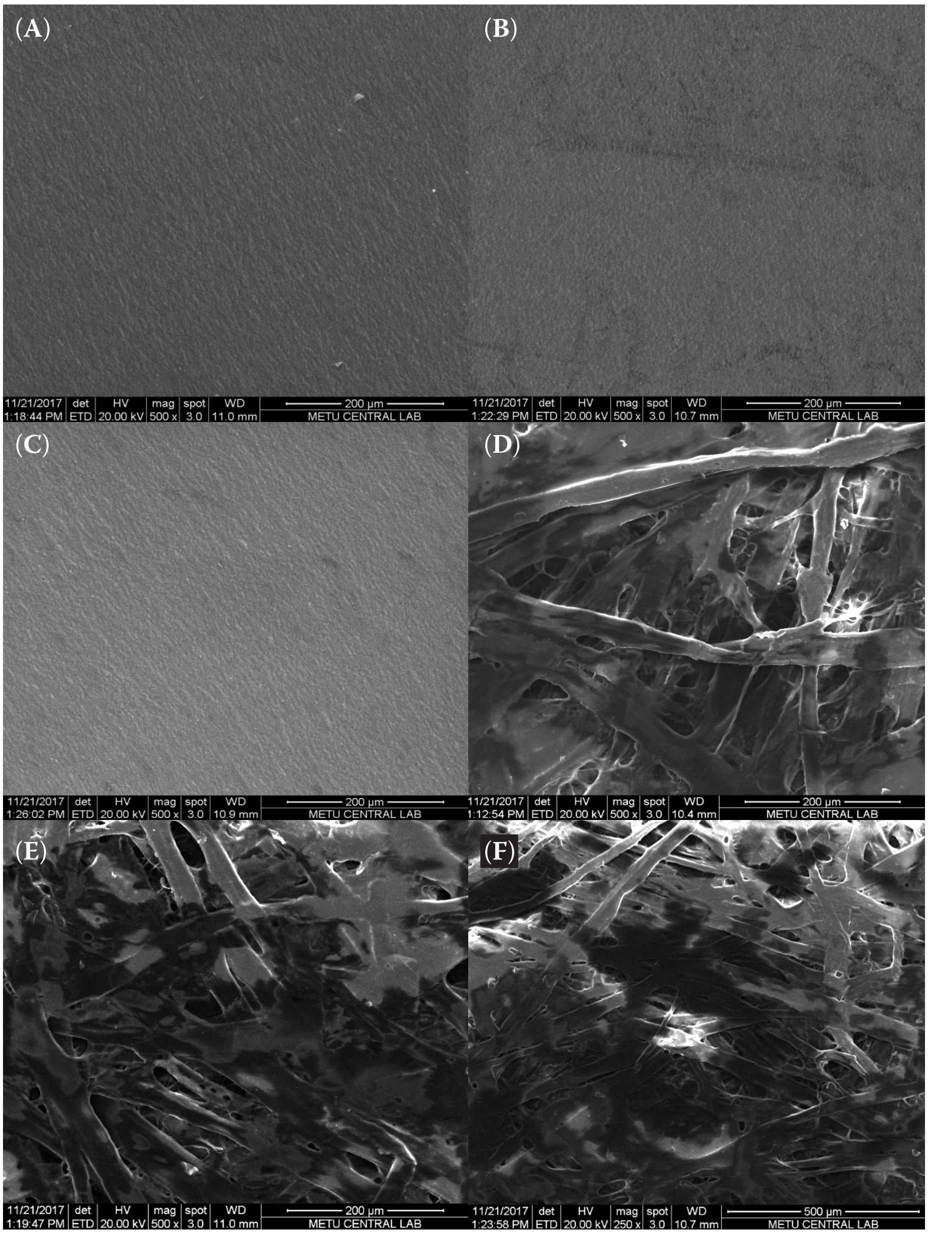

Figure 4. SEM image of foil samples. (**A**) Foil (1) without treatment; (**B**) Foil (1) baked at 150°C for 40 min; (**C**) Foil (1) baked at 200°C for 20 min; (**D**) Foil (2) without treatment; (**E**) Foil (2) baked at 150°C for 40 min; (**F**) Foil (2) baked at 200°C for 20 min

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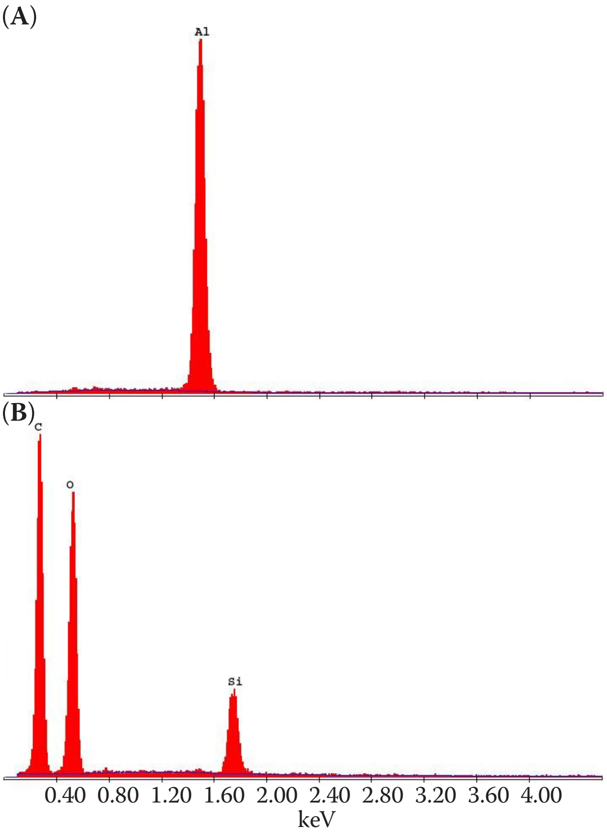

Figure 5. EDX spectrum of foils

Exposure to Al was found less in Foil (2) than Foil (1) both in female adults and male adults.

Similar to this study, other studies in the literature reported that the exposure Al from fish did not exceed the PTWI value (Fekete *et al.* 2013; Chen *et al.* 2014). In a study, it was reported that the Al exposure from fish was 3% of the weekly exposure of Al (Chen *et al.* 2014). Fekete *et al.* (2013) showed that the Al exposure made a contribution to PTWI by only 0.11%.

Surface study. The SEM micrographs results in Figure 4A indicated that the surface morphology of Foil (1) was smooth. Although there were little differences in the surface of Foil (1) after the temperature treatment at 150°C for 40 min (Figure 4B) and 200°C for 20 min (Figure 4C), these differences are not thought to increase the Al leaching into foods. The results in Figure 4D, E and F showed that there were not differences between temperature treatments in the surface of Foil (2). These results demonstrated that the structures of foil samples did not change by different temperature treatment and thus changes in temperature did not cause any Al leaching into foods as much as marination did.

EDX spectrum results showed that Foil (1) contained only Al (Figure 5A) and Foil (2) contained Carbon (C), Oxygen (O) and Silicon (Si) (Figure 5B). Al was not determined in the interior surface of Foil (2). This situation explains the reason that Al leaching into food from Foil (1) is higher than the Al leaching into food from Foil (2). Barrier properties of the interior surface of Foil (2) prevents the direct contact of food with the exterior surface of Al foil, thus Al leaching into foods from foil reduces.

CONCLUSIONS

From the result of the study, the Al leaching from different types of Al foils into salmon and haddock has varied in different temperature-time and marination applications. Although based on the present results, using different Al foil did not leach significant amounts of Al into the fish and exposure values of Al did not exceed the Al PTWI suggested in the 2011 JECFA report, exposure of Al from fish samples may be dangerous to vulnerable groups such as children, elderly and people with kidney disease. And also, the long term effects of Al exposure from kitchen utensils are not known exactly. The community should be informed about the use of Al kitchen utensils and the vulnerable groups should be monitored continuously.

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Received: 2018–03–29
Accepted after corrections: 2019–04–20