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## **Spectroscopy Letters**

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597299>

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**To cite this Article** Yaman, Mehmet , Durak, Muhammed and Bakirdere, Sezgin(2005) 'Comparison of Dry, Wet, and Microwave Ashing Methods for the Determination of Al, Zn, and Fe in Yogurt Samples by Atomic Absorption Spectrometry', *Spectroscopy Letters*, 38: 4, 405 — 417

**To link to this Article:** DOI: 10.1081/SL-200062997

**URL:** <http://dx.doi.org/10.1081/SL-200062997>

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## Comparison of Dry, Wet, and Microwave Ashing Methods for the Determination of Al, Zn, and Fe in Yogurt Samples by Atomic Absorption Spectrometry

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**Abstract:** Digestion methods such as dry and wet ashing and microwave oven were examined at various conditions to determine a rapid, reliable, and simple digestion procedure for yogurt. Digestion by microwave oven was found to be an excellent method in comparison with dry and wet ashing methods when only Al and Zn in yogurt samples were determined. Iron in this matrix is not completely digested by the microwave oven method at the examined conditions. From the obtained results, yogurt can also be a good source of essential nutrients such as minerals in human diet, especially zinc. Aluminum concentrations in yogurt samples fermented in Al containers were found to be significantly higher than in plastic containers. Al concentrations of yogurt taken from the bottom of the container were found to be higher than from the center and top of Al containers. The determinations of metals were carried out via an atomic absorption spectrophotometer using calibration curve and standard additions methods. Aluminum concentrations in yogurt samples produced in big production centers were found to be significantly higher than the other yogurt samples produced in-house when plastic containers were used.

**Keywords:** Aluminum, atomic absorption, dry and wet ashing, iron, microwave, yogurt, zinc

Received 5 January 2004, Accepted 7 December 2004

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## INTRODUCTION

The essential trace elements have four major functions as stabilizers, as elements of structure, as essential elements for hormonal function, and as cofactors in enzymes.<sup>[1]</sup> For example, zinc is a component of more than 300 proteins and more than 100 DNA-binding proteins with zinc fingers.<sup>[2]</sup> Zn is the prosthetic group of some metalloenzymes containing superoxide dismutase (SOD), which is an important antioxidant enzyme for cellular protection from reactive oxygen species (ROS).<sup>[3]</sup> Iron is found in hemoglobin and is also a component of myoglobin. On the other hand, excessive exposure to the essential trace metals described above can cause some diseases. The dietary Recommended Daily Allowances (RDA) for adults of 60 kg per person by authorized agencies are in the ranges of 8 to 18 mg for Fe and 8 to 11 mg for Zn as dependent on sex, age, and some situations such as pregnancy.<sup>[4]</sup>

Aluminum has been implicated as an etiological factor in some pathologies such as encephalopathy, bone disease, and anemia related to dialysis treatment.<sup>[3]</sup> In addition, it has been hypothesized to be a cofactor in the etiopathogenesis of some neurodegenerative diseases, including Alzheimer disease.<sup>[5]</sup> Furthermore, an increased concentration of Al in infant formulas and in solutions for home parenteral nutrition has been associated with neurological consequences and metabolic bone disease, characterized by low-bone formation rate, respectively.<sup>[6]</sup> The provisional tolerable weekly intakes (PTWI) were established as  $7.0 \text{ mg Al kg}^{-1}$  of body weight for adults by the Joint Food and Agricultural Organization (FAO)–World Health Organization (WHO) Expert Committee on Food Additives.<sup>[7]</sup>

Except occupational sources, the main sources of trace metals described above are foods and beverages. For all these reasons, it is recommended that the trace metal contents should be declared in all food preparations. In addition, measurement of trace metals must be undertaken with great care. Published reports on trace metal concentrations in food and beverage samples such as yogurt and milk generally include the results obtained by using atomic absorption spectrometry (AAS) and inductively coupled plasma–atomic emission spectrometry (ICP-AES).<sup>[8–15]</sup>

Yogurt is gaining in popularity due to its acceptability for consumers as well as its nutritional properties and potentially beneficial effects in human health. Yogurt can also be a good source of essential nutrients such as minerals in the human diet. On the other hand, undesirable metals may enter yogurt, and the levels of the nutritional metals may increase in the fermentation procedure depending on the conditions such as container material (aluminum, plastic, or steel). The time passed after production due to the probability of being sour and the sampling positions from the container (i.e., bottom, central, and top) may also increase undesirable metals in yogurt, as well as transportation to consumer. There are not studies to evaluate the extent and nature of mineral redistribution arising from yogurt processing.<sup>[9]</sup>

In the ashing procedures, sample digestion is often the most time-consuming step and involves some potential problems such as incomplete dissolution, precipitation of insoluble analyte, contamination, and losses of some volatile elements. Sometimes, 15 and 24 hr for dry ashing<sup>[8,9]</sup> and one night for both wet and dry ashing<sup>[10,11]</sup> were applied. In order to prevent the losses of elements, closed digestion bombs are used. However, this procedure requires a prolonged time for complete dissolution. An alternative wet digestion technique, use of a microwave oven for rapid sample digestion, seemed an attractive procedure. The time of several hours needed for the conventional wet digestion procedures was reduced to minutes by using microwave oven. Analytical applications of the microwave digestion method include both commercial domestic microwave oven<sup>[16,17]</sup> for biological samples and commercial microwave oven equipped with temperature and pressure regulators.<sup>[18]</sup>

In this study, digestion methods such as dry, wet, and microwave oven were examined to determine the best method. Al, Zn, and Fe concentrations in Turkish yogurt samples fermented in different containers made from aluminum, plastic, and steel materials were determined by flame atomic absorption spectrometry (FAAS).

## EXPERIMENTAL

An ATI UNICAM 929 model atomic absorption spectrophotometer equipped with ATI UNICAM hollow cathode lamps was used for the metal determinations. The optimum conditions for AAS are given in Table 1. A domestic microwave oven (Kenwood) was used for the digestion of yogurt.

Unless stated otherwise, all chemicals used were of analytical-reagent grade. Throughout all analytical work, double-distilled water was used. All glass apparatus (Pyrex) was kept permanently full of 1 mol L<sup>-1</sup> nitric acid when not in use. In the digestion procedure, platinum dish, Pyrex glass, concentrated nitric acid (65%, Merck) and hydrogen peroxide (35%, Merck) were used. Stock solutions of metals (1000 mg L<sup>-1</sup>) were prepared by dissolving their nitrate salts in suitable volume of 1.0 mol L<sup>-1</sup> HNO<sub>3</sub>.

**Table 1.** Operating parameters for FAAS

Parameter	Zn	Fe	Al
Wavelength, nm	213.9	248.3	309.3
HCL current, mA	9.5	15	7.5
Acetylene flow rate, L/min	0.5	0.5	4.2
N <sub>2</sub> O flow rate, L/min	—	—	4.7
Air flow rate, L/min	4.0	4.0	—
Slit, nm	0.5	0.2	0.5

## Preparation of Samples

Yogurt samples fermented in the containers made from aluminum, plastic, and steel materials were taken from different local shops in Elazig, Turkey. Yogurt samples are generally made with cow-origin milk and scarcely with sheep-origin milk in this region in summer. In this study, two samples of yogurt fermented in plastic material were produced in two big production centers, while the others were produced in individual houses.

## Dry Ashing

Yogurt samples (3.0–5.0 g) were transferred to a platinum dish and dried in an oven at 100°C until dried. The dried samples were ashed at desired temperatures such as 450°C, 475°C, and 500°C for various ashing periods changed from 1 to 4 hr. Minimum volumes of the mixture of nitric acid–hydrogen peroxide (1/1) were added to the ashed samples and dried with occasional stirring on a hot plate with low heat. When dried, 1.5 mL of 1.0 mol L<sup>-1</sup> HNO<sub>3</sub> was added and centrifuged. The clear solutions were analyzed by FAAS. Ten-times dilution was made for zinc determination. The blank digests were carried out in the same way.

## Wet Ashing

Yogurt samples (3.0–5.0 g) were transferred to a Pyrex glass, 3.0–5.0 mL of the mixture of concentrated HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (1/1) was added, and the mixtures were dried on the heater with stirring. The same procedure was reapplied by using the same mixture described above. Various solvent/sample ratios from 1:1 to 3:1 were examined. When dried, 1.5 mL of 1.0 mol L<sup>-1</sup> HNO<sub>3</sub> was added and centrifuged. The clear solution was analyzed by FAAS. Ten-times dilution was made for zinc determination. The blank digests were carried out in the same way.

## Digestion by Using Microwave Oven

For this purpose, various digestion reagents such as HNO<sub>3</sub>, HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (2/1), and HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (1/1) were examined, separately. Yogurt (1.0 g) was transferred to a Teflon bomb, and then the mixture of the digestion reagent (2.0 mL) was added. The Teflon bomb was placed into the microwave oven, and radiation was applied using the power of 270, 360, and 450 W for 3.0 min, separately. After cooling, 1.0 mL of the same mixture was also added, and radiation at the same power was repeated for 3.0 min. After cooling for 4 min, 1.0 mL of 0.1 mol L<sup>-1</sup> HNO<sub>3</sub> was added, and the mixture was transferred into a Pyrex tube. After centrifugation, the clear solution was measured by FAAS. The blank digests were carried out in the same way.

RESULTS AND DISCUSSION

The calibration graphs obtained for the studied three metals were rectilinear in the concentration ranges described in Figures 4–6. For the determination of the best digestion method, dry and wet ashing and the microwave oven methods were examined at the various conditions. In the wet ashing procedure, different proportions of solvent/sample were tested. It is seen that twofold of the solvent/sample was sufficient for complete digestion (Table 2). The observed concentrations of Al, Zn, and Fe in the same yogurt samples were compared to the concentrations in dry ashing and the microwave oven methods in Table 2.

In the wet ashing method, the metal levels in reagent blanks at the measurement step were found (as  $\text{ng mL}^{-1}$ ) 70 for Fe, 50 for Zn, and 300 for Al with standard deviations of 20, 14, and 50, respectively. The limit of detection in wet ashing method (as  $\text{ng mL}^{-1}$ ), defined as three times the standard deviations of the blanks, were therefore 60, 42, and  $150 \text{ ng mL}^{-1}$ ,

**Table 2.** Comparison of dry and wet ashing and microwave oven digestion methods for metal determinations in yogurt samples ( $n = 3$ )

Digestion method (the proportion of solvent/sample)	Al ( $\text{mg kg}^{-1}$ )	Zn ( $\text{mg kg}^{-1}$ )	Fe ( $\text{mg kg}^{-1}$ )
Dry ashing, 3 hr at $500^{\circ}\text{C}$	$55 \pm 4$	$3.5 \pm 0.3$	$2.5 \pm 0.1$
Wet ash- $\text{HNO}_3/\text{H}_2\text{O}_2$ (1/1) (1-fold)	$51 \pm 4$	$2.9 \pm 0.2$	$2.4 \pm 0.2$
Wet ash- $\text{HNO}_3/\text{H}_2\text{O}_2$ (1/1) (1.5-fold)	$55 \pm 4$	$3.3 \pm 0.3$	$2.5 \pm 0.1$
Wet ash- $\text{HNO}_3/\text{H}_2\text{O}_2$ (1/1) (2-fold)	$56 \pm 5$	$3.3 \pm 0.3$	$2.5 \pm 0.1$
Wet ash- $\text{HNO}_3/\text{H}_2\text{O}_2$ (1/1) (2.5-fold)	$55 \pm 5$	$3.0 \pm 0.2$	$2.5 \pm 0.1$
Wet ash- $\text{HNO}_3/\text{H}_2\text{O}_2$ (1/1) (3-fold)	$53 \pm 4$	$2.8 \pm 0.2$	$2.5 \pm 0.1$
Skim milk powder-BCR 151	Using dry ashing		49.1
Certified value =	Using wet ashing		48.9
50.1 $\text{mg Fe/kg} \pm 1.3$			
Microwave oven			
Digestion reagent			
Conc. $\text{HNO}_3$	$46 \pm 3^a$	$2.0 \pm 0.2^a$	$1.5 \pm 0.1^a$
	$47 \pm 3^b$	$2.1 \pm 0.2^b$	$1.5 \pm 0.1^b$
	$46 \pm 2^c$	$3.0 \pm 0.2^c$	$1.5 \pm 0.1^c$
Conc. $\text{HNO}_3/\text{H}_2\text{O}_2$ (2/1)	$50 \pm 3^a$	$2.4 \pm 0.2^a$	$0.9 \pm 0.1^a$
	$49 \pm 3^b$	$3.0 \pm 0.2^b$	$1.2 \pm 0.1^b$
	$54 \pm 3^c$	$2.9 \pm 0.1^c$	$1.2 \pm 0.1^c$
Conc. $\text{HNO}_3/\text{H}_2\text{O}_2$ (1/1)	$51 \pm 3^a$	$2.8 \pm 0.2^a$	$1.1 \pm 0.1^a$
	$55 \pm 2^b$	$2.8 \pm 0.2^b$	$1.3 \pm 0.1^b$
	$55 \pm 3^c$	$3.2 \pm 0.2^c$	$1.6 \pm 0.1^c$

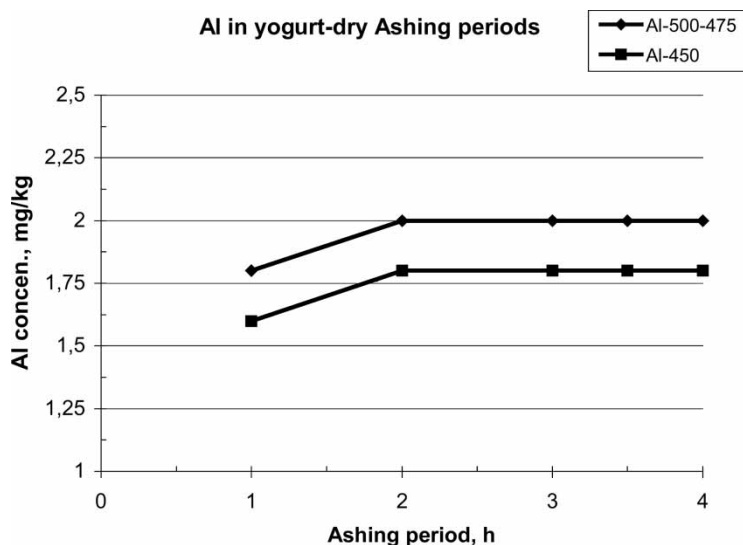
<sup>a</sup>At 270 W power.

<sup>b</sup>At 360 W power.

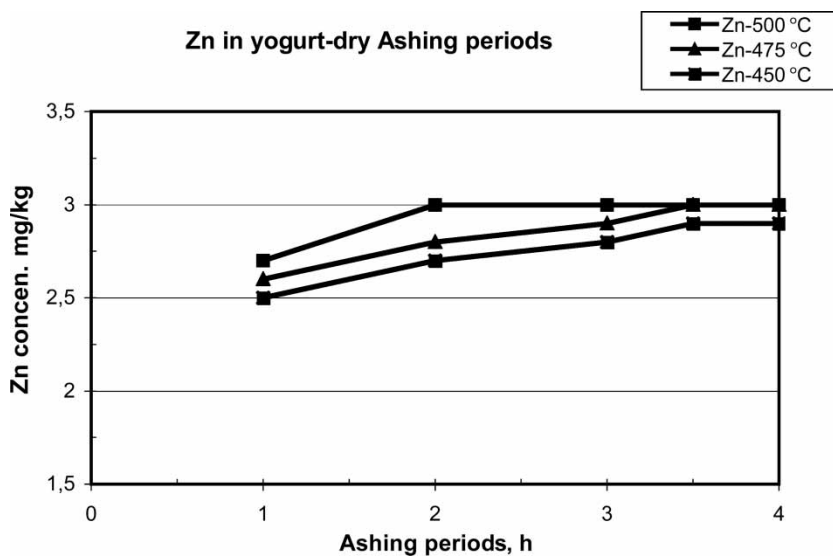
<sup>c</sup>At 450 W power.

respectively. The corresponding blank levels for dry ashing method were found ( $\text{ng mL}^{-1}$ ) as 40, 30, and 200 with standard deviations of 10, 6, and 40, respectively. Thus, the limit of detection in dry ashing method (as  $\text{ng mL}^{-1}$ ), defined as described above, were estimated 30, 18, and  $120 \text{ ng mL}^{-1}$ , respectively. The blank levels of microwave oven method were found ( $\text{ng mL}^{-1}$ ) as 30 for Fe, 20 for Zn, and 100 for Al with standard deviations of 7, 4, and 25, respectively. Similar to the above definition, the limit of detection in microwave oven method (as  $\text{ng mL}^{-1}$ ) was therefore 21, 12, and 75 ( $\text{ng mL}^{-1}$ ), respectively. The effects of contamination for Al and Fe were eliminated by subtracting values obtained for blanks of each method. The subtraction of the blank values obtained for Zn is not necessary because of its dilution requirement.

To determine the volatilization loss and the optimum conditions during dry ashing procedure, three different temperatures including  $450^\circ\text{C}$ ,  $475^\circ\text{C}$ , and  $500^\circ\text{C}$  were studied by using various oxidation periods in ranges of 1.0–4.0 hr. It is seen that Al concentrations reached to the maximum values in 2–4 hr ranges for  $475^\circ\text{C}$  and  $500^\circ\text{C}$  (Fig. 1). It is interesting that the obtained Al levels in yogurt using these two temperatures were cross to each other. For zinc, the optimum oxidation periods were found in the range of 2–3 hr at the  $500^\circ\text{C}$  (Fig. 2). Iron concentrations were found as maximum in the range of 3–4 hr at the  $500^\circ\text{C}$  (Fig. 3), while an actual plateau was not achieved at  $475^\circ\text{C}$  due to uncompleted ashing periods. Al, Fe, and Zn concentrations of the same yogurt samples by using dry ashing were found close to the obtained levels by using wet ashing method. In the

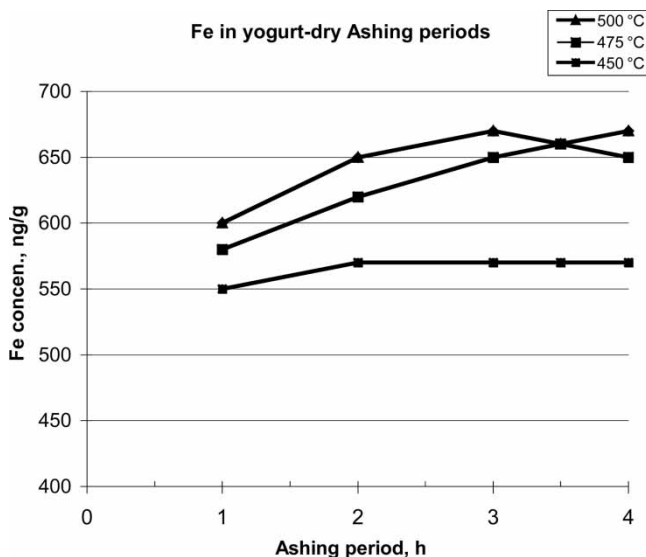


**Figure 1.** Effect of dry ashing periods on Al concentrations in yogurt sample.



**Figure 2.** Effect of dry ashing periods on Zn concentrations in yogurt sample.

microwave oven digestion, the maximum Al and Zn concentrations were obtained by using the mixture of  $\text{HNO}_3/\text{H}_2\text{O}_2$  (1/1) at 450 W, and these levels were close to the obtained levels for dry ashing concentrations. The obtained iron concentrations are identical by using both  $\text{HNO}_3$  and



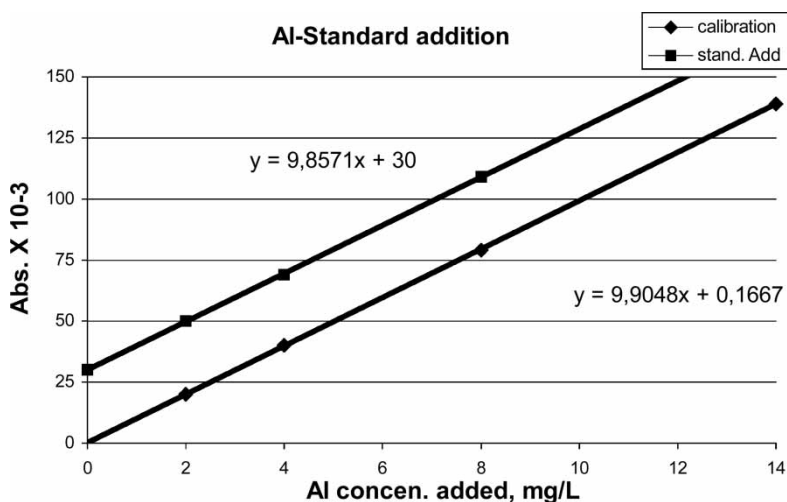
**Figure 3.** Effect of dry ashing periods on Fe concentrations in yogurt sample.



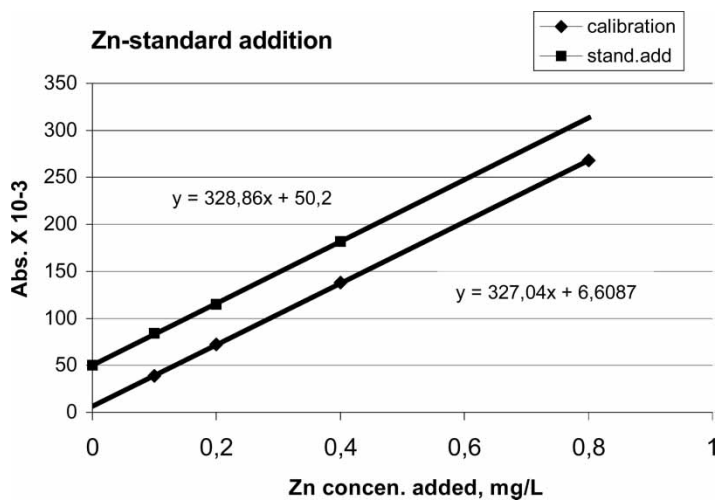
the mixture of  $\text{HNO}_3/\text{H}_2\text{O}_2$  (1/1) at 450 W, but these concentrations were significantly lower than the dry ashing values.

The accuracy of the methods was studied by examining the Certified Reference Material (skim milk powder-BCR 151). The results for iron are given in the Table 2. It can be seen that the recoveries were found to be 98% for Fe using both dry ashing at 500°C and wet ashing methods. In addition, the recoveries of metals from the yogurt samples fortified with the studied elements were found. The spiked metals to the sample are in the range of 0.3–1.0  $\text{mg kg}^{-1}$  related to the metal concentrations. It was found that at least 90% of metals added to yogurt sample were recovered. In addition, to overcome enhancement or suppression due to the presence of major components of the yogurt matrix, calibration solutions were performed within the sample matrix itself. Standard additions of Al, Zn, and Fe to the yogurt samples were made. The slopes of the calibration graphs were compared with the slopes obtained by the standard additions method (Figs. 4–6). These results indicate the absence of chemical interferences because the slopes of calibration graphs are the same with that obtained with standard additions for all metals. Thus, it is not necessary to apply the standard additions method to yogurt samples.

Due to the above results, dry ashing at 500°C for 3.0 hr was preferred for the digestions of all other samples. It is seen from Table 3 that Al concentrations in yogurt samples fermented in containers made from Al were importantly higher ( $p = 0.017$ ) than in the plastic containers. Similarly, Al concentrations in yogurt samples fermented in containers made from aluminum were significantly higher ( $p = 0.016$ ) than in the steel containers.

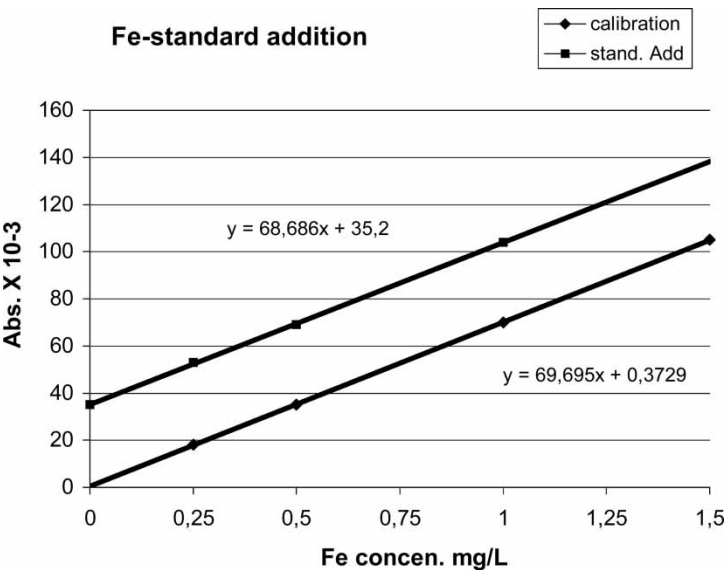


**Figure 4.** Calibration graphs obtained with the standard additions method and with standards.



**Figure 5.** Calibration graphs obtained with the standard additions method and with standards.

Yogurt samples were taken from the top, center, and bottom of Al containers to examine whether high Al levels are sourced from the Al containers or not. Al levels of yogurt taken from the bottom of aluminum containers were found to be significantly higher ( $p < 0.01$ ) than the Al levels of yogurt taken from the



**Figure 6.** Calibration graphs obtained with the standard additions method and with standards.

**Table 3.** Comparison of metal concentrations in yogurt samples fermented in containers made from different materials according to sampling from bottom, center, and top (n = 3)

Container	Bottom, center, or top	Al (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )
Al	Bottom (soured)	231 ± 13	6.7 ± 0.4	6.15 ± 0.32
Al	Bottom	57 ± 4	3.8 ± 0.2	0.39 ± 0.03
Al	Bottom	55 ± 4	3.5 ± 0.3	2.51 ± 0.14
Al	Bottom	19 ± 1	4.1 ± 0.2	0.39 ± 0.03
Al	Bottom	24 ± 1	3.6 ± 0.3	0.71 ± 0.05
Al	Bottom	26 ± 2	3.2 ± 0.2	0.66 ± 0.07
Al	Bottom	15 ± 1	3.4 ± 0.3	0.55 ± 0.06
Al	Center	2.2 ± 0.2	4.1 ± 0.2	0.88 ± 0.06
Al	Center	1.4 ± 0.1	4.2 ± 0.2	0.32 ± 0.03
Al	Center	2.4 ± 0.3	4.3 ± 0.2	0.67 ± 0.06
Al	Center	2.9 ± 0.2	3.2 ± 0.2	0.36 ± 0.04
Al	Center	4.1 ± 0.3	3.5 ± 0.2	0.23 ± 0.03
Al	Top	2.2 ± 0.2	3.9 ± 0.2	0.29 ± 0.02
Al	Top	2.1 ± 0.2	4.9 ± 0.3	0.72 ± 0.06
Al	Top	2.0 ± 0.2	4.6 ± 0.3	0.45 ± 0.04
Al	Top	1.8 ± 0.2	4.7 ± 0.3	0.42 ± 0.04
Plastic	Center	0.25 ± 0.06	3.1 ± 0.2	0.43 ± 0.04
Plastic	Center	0.38 ± 0.08	3.4 ± 0.2	0.45 ± 0.04
Plastic	Center	0.48 ± 0.06	3.0 ± 0.2	0.44 ± 0.03
Plastic	Center	0.31 ± 0.06	3.8 ± 0.2	0.62 ± 0.06
Plastic	Center	0.32 ± 0.05	3.1 ± 0.2	0.25 ± 0.03
Plastic	Top	0.18 ± 0.03	3.9 ± 0.2	0.28 ± 0.02
Plastic	Top	0.17 ± 0.04	4.4 ± 0.2	0.51 ± 0.04
Plastic <sup>a</sup>	Top	4.2 ± 0.1	4.7 ± 0.2	0.39 ± 0.03
Plastic <sup>a</sup>	Top	4.2 ± 0.2	5.2 ± 0.2	0.40 ± 0.03
Steel	Top	1.20 ± 0.01	4.1 ± 0.2	0.40 ± 0.03
Steel	Top	0.85 ± 0.01	3.8 ± 0.2	0.34 ± 0.03
Steel	Top	0.92 ± 0.01	3.7 ± 0.2	0.38 ± 0.03

<sup>a</sup>These samples were taken from big production centers.

center and top of Al containers. These results can be sourced from releasing of Al from both the bottom and part of edge of the Al container. High Al levels (4.2 mg kg<sup>-1</sup>) in two plastic containers can be attributed to the containers used during the applied process such as heating of the milk before fermentation processing in big production centers. It is not necessary to take the sampling from the bottom of plastic container because the plastic containers cannot be the source of high Al concentration in yogurt. In addition, yogurt samples were not taken from the bottom and center positions of the steel containers because Al levels in yogurt fermented in these containers were not high.

In the literature, data related to the trace metal levels in yogurt matrices were in the range of 3.5–7.3 for Zn and 0.4–0.5 for Fe ( $\text{mg kg}^{-1}$ ).<sup>[8,9]</sup> On the other hand, it is described that Zn in yogurt is more available in comparison with raw milk due to the formation of lactic acid in fermentation procedure.<sup>[19]</sup> Except Al, the studied metal concentrations do not importantly change as dependent on the container materials. We think that the observed unimportant differences ( $p = 0.77$ ) between the zinc concentrations of Al containers and plastic containers except for soured sample were sourced from the yogurt origin. Similarly, important differences were not observed ( $p = 0.20$ ) between the iron concentrations of Al containers and plastic containers, except for two samples together with soured sample. It is described that zinc concentration of milk samples varies with respect to the animal species such as buffalo, cow, goat, and sheep,<sup>[20]</sup> but any differences were not described for Al in the literature. In addition, Zn and Fe concentrations in the soured yogurt sample taken from the bottom of Al container were significantly higher than in all other yogurt samples as similar to the Al concentrations. This can also be attributed to the releasing of these two metals from Al container by effect of acidity. Iron and Zn concentrations as high as 2300 and 800  $\text{mg kg}^{-1}$  in Al containers provide this opinion.

Al levels of yogurt samples fermented in Al containers were found in ranges of 15–57  $\text{mg kg}^{-1}$  for bottom except soured yoghurt (231  $\text{mg kg}^{-1}$ ) and lower for center and top sampling positions. According to the limitation of 1.0  $\text{mg kg}^{-1}$  Al daily suggested by FAO-WHO,<sup>[7]</sup> in one day the eating of more than 380 g of yogurt soured in the bottom of an Al container exceeds this limitation. It is concluded that the contribution from yogurt to total intake of the studied metals is toxicologically insignificant in regard to RDA values.<sup>[4]</sup> Furthermore, yogurt is a major source of the nutritional metals such as Zn and Fe dependent on their bioavailable forms, especially for zinc.<sup>[19]</sup> The obtained Zn and Fe concentrations were in the ranges of the literature values described above.

## CONCLUSIONS

From the results, it is seen that Al and Zn determinations in yogurt samples can successfully be carried out by using FAAS and microwave digestion method. For this digestion method, 6 min were necessary and the blank values were too low. But, iron in the yogurt samples was not completely digested by using microwave oven at the applied conditions, and it should be digested by using dry and wet ashing methods.

In addition, yogurt should not be kept in an aluminum container for a long time because Al is released from the Al container at a dangerous level for health. It was observed that zinc and iron do not systematically change depending on the studied conditions, except the souring.

## ACKNOWLEDGMENTS

This work was financially supported by The Scientific and Technical Research Council of Turkey.

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