

## Iodine Content in Breast Milk Samples in the Aegean Region in Turkey

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In this study, the iodine content in breast milk was measured by Sandell-Kolthoff's method. The milk was obtained from 40 healthy mothers, aged 20 to 37, from the pediatric division of Ege University hospital. All mothers were from Aegean region of Turkey. Comparisons were made with commercial milk samples. Destruction of organic matter by using alkaline ash method was carried out prior to determination by Sandell-Kolthoff reaction based on iodine's catalytic effect on the Ce(IV)-As(III) system. The iodine concentration ranges were within 19.1 and 94.2 µg/kg in breast milk samples. Results obtained in this study clearly showed that breast milk samples did not have iodine deficiency which can lead to goiter and iodine concentrations of market milk were at sufficient level in the Aegean region of Turkey.

**Key Words: Iodine determination, Breast milk, Commercial milk, Sandell-Kolthoff reaction.**

### INTRODUCTION

Iodine is an important trace element in human nutrition since it is an essential constituent of the hormones tetraiodothyronine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) which is produced in the thyroid gland. Iodine is found in relative abundance in marine plants and animals, in deposits of organic origin, in certain natural water, in sedimentary phosphate rock and in association with certain mineral deposits<sup>1</sup>. Most of the iodine ingested by humans comes from food of animal and plant origin. Sea fish, shell-fish and seaweed are natural source of iodine<sup>2</sup>. World health organization (WHO), united nations children's fund (UNICEF), international council for control of iodine deficiency disorders (ICCIDD) recommend a daily iodine requirement of 90 µg for preschool children (0 to 59 months); 120 µg for school children (6-12 years); 150 µg for adults (above 12 years) and 200 µg for pregnant and lactating women<sup>3</sup>.

Iodine deficiency can lead to goiter and cretinism, excessive intake to thyrotoxicosis<sup>4,5</sup>. A deficiency of iodine was identified in the early part of the 20th century as a contributing factor to formation of goiter in humans<sup>6</sup>.

The most common type of goiter occurs when the one's diet is deficient in iodine. This type of goiter, known as endemic goiter which is used when more than 10 % of population or 5 % of an adolescent group have goiter occurs mostly in areas where the food and water are naturally poor in iodine, notably inland areas and mountainous regions far from bodies of salt water. In endemic areas, the 24 h urinary excretion is less than 50 to 80  $\mu\text{g}$ <sup>7</sup>.

The effects of iodine deficiency on growth and development are now denoted by the term iodine deficiency disorder (IDD). Iodine deficiency at critical stages during pregnancy and early childhood results in impaired development of the brain and consequently mental function. IDD is among the easiest and cheapest of all disorders to prevent according to WHO. Cretinism, an IDD resulting from iodine deficiency during prenatal development, is detected by a reduced intellectual capacity and physiological impairment. Goiter, an IDD where the thyroid gland enlarges, is in response to the need to capture the limited amount of iodine circulating in blood more efficiently and is generally reversed with increased iodine intake. IDD effects are seen at all stages of development and particularly in the fetus, the neonate and the infant, *i.e.*, in periods of rapid growth. They result from the influence of a low maternal thyroxin level on the fetus and are associated with levels of intake of iodine less than 25 % of normal. Levels less than 50 % of normal are associated with goiter<sup>8-10</sup>.

IDD was seen in Europe prior to the 20th century, but with more varied diets and salt fortification it has been brought under control in most countries, but there is still in Europe, in countries such as Bulgaria, Germany, Greece, Italy, Poland, Romania, Spain and including Turkey<sup>11,12</sup>.

Many methods of analysis have been used in determination of the iodine content of foods. They are respectively high-performance liquid chromatography (HPLC), inductively coupled plasma-mass spectrometry (ICP-MS), intracavity laser spectrometry (ILS), energy-dispersive X-ray spectrometry (EDX-Ray) and the optimized potentiometric method and isotope dilution analysis (IDA)<sup>13-18</sup>. The most widely used method for determination of iodine is probably that based on its catalytic effect on the  $\text{Ce}^{4+}$ - $\text{As}^{3+}$  system, known as Sandell-Kolthoff reaction<sup>19,20</sup>. This method is frequently used in a large number of routine laboratories and this is an official method in official methods analysis of the association of official analytical chemists, AOAC<sup>21</sup>.

The objectives of the study were to obtain information on the actual iodine content of breast milk samples collected from the Aegean region of Turkey. In the present study, the amount of iodine in breast milk samples collected from west part of Turkey (Aegean region) was determined by Sandell-Kolthoff's method.

## EXPERIMENTAL

All breast-feeding mothers aged 20 to 37 (n = 80) in the pediatric division of Ege University hospital were asked to provide a breast milk sample. Consenting breast-feeding mothers (n = 40) were supplied with a detailed set of instructions and an electric breast pump (Mini Electric, Medela AG, Baar, Switzerland) on the second home visit. Women were asked to collect approximately 20 mL of breast milk into an acid-washed opaque polystyrene bottle. After collection, mothers were requested to place the breast milk sample in their home freezer until collected by a research assistant. Breast milk samples were then stored at -20 °C until analysis. Breast milk iodine was measured with the Sandell-Kolthoff reaction<sup>19,20</sup> with a UV-Visible recording spectrophotometer (Shimadzu UV-160A, Japan).

All reagents used in the study were analytical-reagent grade and they were obtained from Merck Chemical Co. The water used in the experiments was de-ionized.

**Iodine analysis in the breast milk samples:** The analytical method for total iodine was based on the alkaline digestion method of Sandell-Kolthoff. A volume of 1 mL was pipetted from the breast milk samples into 10 × 1.5 cm test tubes and mixed with 0.5 mL KOH and 0.5 mL zinc sulfate. The samples then were dried overnight in an oven at 150 °C. The ash was extracted with 6 mL of water by mechanical shaking for 0.5 h. After centrifugation, 2 mL aliquots were diluted with 3 mL hydrochloric acid (0.33 N). Arsenous oxide (1 mL) was added and the content of the tube vortexed, followed by warming in a water bath for 15 min at 40 °C. A volume of 1 mL of ceric ammonium sulfate was then added into all tubes with 15 s time intervals between additions. The tubes were transferred to a water bath at 40 °C for 15 min. Brucin (1 %) was added into 0.5 mL aliquots to each tube in the water bath. The tubes were next transferred to an oven heated to 105 °C for 15 min. The reduction in the colour of ceric ammonium sulfate with arsenous oxide in the presence of iodine as a catalyst was measured at 420 nm. A standard curve using potassium iodide solution containing 0.5-9.0 ng/mL iodine was prepared. The intensity of the developed colour was measured at 420 nm. The iodine concentration in breast milk samples was calculated from the calibration curve.

**Preparation of serum of mature human milk:** 2-3 Drops of 30 % TCA solution was added into 2 mL milk sample. After waiting at 40 °C for 5 min, the solution was centrifuged at 2500 g for 10 min. The supernatant fluid was transferred into a test tube. After washing with water, the residue was also re-centrifuged at 2500 g for 5 min. The present supernatant was transferred again into the test tube. The total volume of supernatant fluids was diluted to 4 mL with water. 2 mL Aliquot was used for determination of iodide in the serum.

**Calculation:** The calibration curve shown in Fig. 1 was used for determination of iodine concentration of breast milk and different commercial milk samples. The absorbance of the sample solutions is read against the concentrations of the standard solutions. The results are expressed in  $\mu\text{g}/\text{kg}$ .

## RESULTS AND DISCUSSION

For the investigated iodine concentration ranged of 0.5 to 9.0 ng/mL, a calibration curve was obtained from the absorbance versus the concentration (Fig. 1). The function of this curve is  $y = 0.0021x^2 - 0.0327x + 0.2645$ . Iodine concentrations in some different mature human milk samples collected from 40 healthy women were given in Tables 2 and 3. The range of iodine concentrations for 40 mature human milk samples was obtained between 26.4 and 93.2  $\mu\text{g}/\text{kg}$ . The mean value of all iodine concentrations of 40 samples was  $54.71 \pm 0.47 \mu\text{g}/\text{kg}$ . If the iodine concentration in mature human milk appears to be less than 3  $\mu\text{g}/\text{kg}$ , a disease called Endemic goiter which is caused by the lack of iodine will occur in the environment. According to the obtained results, mature human milk samples did not have iodine deficiency in this study.

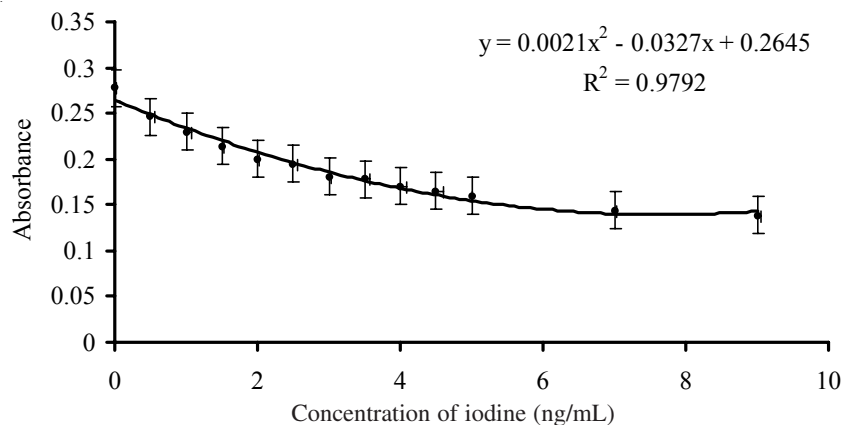


Fig. 1. Calibration curve

Table-1 shows the iodine concentrations for different market milk and two raw cow milk samples. The total amount of iodine values of market milk samples was measured between  $683.8 \pm 67$  and  $208.8 \pm 75 \mu\text{g}/\text{kg}$ . The mean value of iodine concentrations of market milk samples was  $459.56 \pm 77 \mu\text{g}/\text{kg}$ . Iodine concentrations of two raw cow milk samples collected from Izmir were measured as  $593.3 \pm 78$ - $419.1 \pm 63 \mu\text{g}/\text{kg}$ . The mean value of iodine concentrations of raw cow milk samples was  $506.2 \pm 71$

TABLE-1  
IODINE CONCENTRATION IN DIFFERENT COMMERCIAL AND  
RAW COW MILK SAMPLES

Sample	Iodine concentration ( $\mu\text{g}/\text{kg}$ ) (mean $\pm$ SD)
Raw cow milk-1	593.30 $\pm$ 78
Raw cow milk-2	419.10 $\pm$ 63
<b>Mean</b>	<b>506.20 <math>\pm</math> 71</b>
Pinar süt	394.20 $\pm$ 83
Gülüm süt	683.80 $\pm$ 67
Mis süt	412.30 $\pm$ 73
Sütas	598.70 $\pm$ 85
Dimes süt	208.80 $\pm$ 75
<b>Mean</b>	<b>459.56 <math>\pm</math> 77</b>

TABLE-2  
IODINE CONCENTRATION IN SOME DIFFERENT BREAST MILK  
SAMPLES COLLECTED FROM 24 HEALTHY WOMEN LIVING IN  
THE AEGEAN REGION OF TURKEY

Sample	Iodine concentration ( $\mu\text{g}/\text{kg}$ ) (mean $\pm$ SD)	Mother's age	Place of residence
1	55.80 $\pm$ 8.1	26	Menemen-Izmir
2	93.20 $\pm$ 6.4	25	Karsiyaka-Izmir
3	57.30 $\pm$ 5.3	26	Bornova-Izmir
4	64.40 $\pm$ 4.3	30	Çigli-Izmir
5	68.70 $\pm$ 9.3	32	Izmir
6	82.90 $\pm$ 3.5	22	Menderes-Izmir
7	77.50 $\pm$ 2.1	32	Afyon
8	46.80 $\pm$ 5.5	30	Usak
9	43.90 $\pm$ 4.3	20	Usak
10	60.70 $\pm$ 2.8	37	Izmir
11	39.50 $\pm$ 3.6	28	Bornova-Izmir
12	29.70 $\pm$ 6.7	24	Izmir
13	39.50 $\pm$ 1.2	29	Bornova-Izmir
14	48.10 $\pm$ 7.8	31	Çesme-Izmir
15	68.70 $\pm$ 3.2	34	Bornova-Izmir
16	76.80 $\pm$ 5.6	32	Bornova-Izmir
17	37.10 $\pm$ 4.5	22	Mugla
18	40.30 $\pm$ 1.1	32	Manisa
19	43.90 $\pm$ 3.4	20	Manisa
20	34.20 $\pm$ 2.2	32	Manisa
21	62.40 $\pm$ 5.6	26	Izmir
22	58.90 $\pm$ 3.4	33	Izmir
23	80.10 $\pm$ 7.7	34	Aliaga-Izmir
24	32.20 $\pm$ 7.6	21	Usak
<b>Mean</b>	<b>54.12 <math>\pm</math> 4.7</b>		

TABLE-3  
 TOTAL IODINE CONCENTRATION, IODINE CONCENTRATION IN  
 THE SERUM AND ORGANIC IODINE CONCENTRATIONS IN SOME  
 DIFFERENT BREAST MILK SAMPLES COLLECTED FROM 16  
 HEALTHY WOMEN LIVING IN THE AEGEAN REGION OF TURKEY

Sample	Total iodine concentration (µg/kg)	Iodine concentration in the serum (µg/kg)	Organic iodine concentration (µg/kg)
25	26.40 ± 8.7	24.4 ± 3.4	2.00 ± 0.8
26	31.00 ± 3.3	27.0 ± 5.6	4.00 ± 0.2
27	55.80 ± 2.3	49.3 ± 3.7	6.50 ± 0.1
28	64.40 ± 1.2	60.7 ± 6.6	3.70 ± 0.9
29	34.90 ± 2.2	32.9 ± 4.3	2.00 ± 0.6
30	55.80 ± 2.8	50.5 ± 2.2	8.50 ± 0.7
31	76.80 ± 6.7	64.4 ± 1.0	12.40 ± 0.4
32	36.00 ± 4.5	32.2 ± 2.3	3.80 ± 0.2
33	59.00 ± 5.4	50.5 ± 7.6	8.50 ± 0.2
34	57.40 ± 8.8	48.1 ± 3.5	9.30 ± 0.1
35	48.10 ± 2.1	44.9 ± 4.5	3.20 ± 0.3
36	80.20 ± 2.2	78.3 ± 3.4	1.90 ± 0.2
37	73.60 ± 5.6	71.3 ± 2.5	2.30 ± 0.6
38	90.30 ± 7.6	84.8 ± 1.6	5.50 ± 0.9
39	29.10 ± 6.8	22.7 ± 6.7	6.40 ± 0.6
40	65.80 ± 3.3	61.2 ± 6.1	4.60 ± 0.4
<b>Mean</b>	<b>55.29 ± 4.6</b>	<b>50.2 ± 4.1</b>	<b>5.09 ± 0.5</b>

µg/kg. WHO reported that the range of the iodine concentrations (µg/kg)<sup>9</sup> in milk should be within the range of 350-560 µg/kg. Although the Aegean region like most part of Turkey is known as iodine deficient area<sup>22-26</sup>, iodide concentration ranges in milk are found close to those of WHO in our region.

Endemic goiter is still a public health problem in Turkey. In 1935 and 1948, Atay and Onat showed endemic goiter in the Aegean region for the first time<sup>27,28</sup>. Although there was not sufficient data about the iodine status during the 1960s in Turkey, WHO reported that endemic goiter was not a significant health problem<sup>29</sup>. In 1987, Urgancioglu *et al.*<sup>30</sup> found a goiter rate of 30 % in the general population. Goiter prevalence of different cities in Turkey ranged from 92.0-93.2 % according to different groups for the last decade<sup>31-34</sup>. Hamulu *et al.*<sup>32</sup> found a goiter rate of 49 % in two cities of the western coast (Izmir and Aydin) for all age groups. During 1997-1999, Erdogan *et al.*<sup>34</sup> found a goiter rate between 6 and 58 % in 20 different cities of Turkey in children between 9 and 11 years of age.

In 1968, a salt iodization program was started in Turkey. However, its use is not yet common. Turkey produced 400 million kg salt per year and only 1 million kg of the crude product was refined and 17 % of these products had been iodized according to 1993 data<sup>35</sup>. In 1998, the salt iodization program became compulsory and iodinated salt consumption ratio increased to 57 %. However, there is still a large amount of noniodized and nonrefined salt consumption in Turkey. Noniodized and nonrefined local salt consumption is still common especially in high-risk areas. Iodized salt consumption ratio is 71 and 36 % in city centers and rural areas, respectively. Locally produced nonrefined salt consumption is still more common in rural areas. Recommended iodine concentration should be 50-70 mg/kg potassium iodide or 25-40 mg/kg potassium iodate<sup>33</sup>. Also, salts contain 100 mg/kg sodium thiosulphate as stabilizer. Iodide may easily be oxidized to iodine and it is known that iodine may sublime, so storage conditions of iodide in food such as iodized salt is important. Salt iodine content was measured in previous studies<sup>26,36</sup>. Salts consumed<sup>26</sup> in Turkey contain between 9 and 58 µg/g iodide and the mean iodide concentration is 27 µg/g. However, iodine content in salt may change with different storing and consuming conditions. Iodine deficiency control programs have not been successful up to recent years although there are sufficient technologies. The WHO has aimed to control iodine deficiency within the next 10 years. The areas that impose undeveloped economy and use their own products should be taken into consideration regarding the research program of iodine deficiency.

This study showed that range of iodine concentration in breast milk shows differences in the mothers who live in this region and more detailed studies should be handed all over the country. It is more likely that iodine concentrations of market milk seem sufficient in Izmir. As a result, a widespread consumption of milk may be a solution of iodine deficiency in this region. Therefore, the use of milk and milk supplements in the diet of children should be encouraged and iodine deficiency may be prevented by the use of iodized salt.

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#### REFERENCES

1. B.A. Lambery, *Eur. J. Clin. Nutr.*, **47**, 1 (1993).
2. J. Koops, H. Klomp and M.F.K. Mogot, *Neth. Milk Dairy J.*, **41**, 161 (1987).
3. World Health Organization Report, WHO, p. 49 (1996).
4. J.T. Dunn, *J. Clin. Endocr. Metab.*, **81**, 1332 (1996).
5. O. Ali, *Nutrition*, **11**, 517 (1995).

6. H.C. Holt, B.J. Demott and J.A. Bacon, *J. Food Protect.*, **52**, 115 (1989).
7. H.J. Biersack and F. Grunwald, *Seminars in Nuclear Medicine*, **25**, 92 (1995).
8. World Health Organization Report, WHO/NHD/01-1 (2001).
9. World Health Organization Report, p. 49 (1996).
10. T.A. Nichols, J.S. Morris, V.L. Spate, C.J. Tharp, C.K. Baskett, T.L. Horsman, M.M. Mason and T.P. Cheng, *J. Radioanal. Nucl. Chem.*, **236**, 65 (1998).
11. B.A. Lamberg, *Eur. J. Clin. Nutr.*, **47**, 1 (1993).
12. R.F. Hurrell, *Eur. J. Clin. Nutr.*, **51**, S9 (1997).
13. J. Rendl, S. Seybold and W. Borner, *Clin. Chem.*, **40**, 908 (1994).
14. P. Allain, Y. Mouras, C. Douge, L. Jaunault, T. Delaporte and C. Beaugrand, *Analyst*, **115**, 813 (1990).
15. V.S. Burakov, A.V. Isaevich, P.Ya. Misakov, P.A. Naumenkov and S.N. Raikov, *J. Anal. Atom Spectrom.*, **9**, 307 (1994).
16. M. Mwavra, D.G.S. Narayena and A.M. Kinyva, *J. Trace Elem. Elect. H.*, **8**, 115 (1994).
17. I. Blabina, M. Braizer, H. Bour, A. Dohl and G. Desment, *Ann. Biol. Clin.*, **52**, 261 (1994).
18. P. Ünak, S. Darcan, F. Yurt, Z. Biber and M. Çoker, *Biol. Trace Elem. Res.*, **71**, 463 (1999).
19. E.B. Sandell and I.M. Kolthoff, *J. Am. Chem. Soc.*, **56**, 56 (1934).
20. E.B. Sandell and I.M. Kolthoff, *Microchim. Acta*, **1**, 9 (1937).
21. V.A. Arlington, Official Methods Analysis of the Association of Official Analytical Chemists, AOAC, edn. 13, p. 115 (1967).
22. F. Delange, *Eur. J. Endocr.*, **143**, 189 (2000).
23. A.L. Orville and P.D. Whanger, RDA Workshop: New Approaches, Endpoints and Paradigms for RDAs of Mineral Elements, American Institute of Nutrition, p. 2427S (1996).
24. G. Aumont and J.C. Tressol, *Analyst*, **111**, 841 (1986).
25. H. Ozakay, P. Unak, Z. Biber and F. Yurt, *J. Radioanal. Nucl. Chem.*, **230**, 231 (1998).
26. Z. Biber, H. Ozakay, P. Ünak and F. Yurt, *J. Radioanal. Nucl. Chem.*, **240**, 395 (1999).
27. K.A. Atay, Ulusal Cerrahi Kurultayına Rapor, Kader Basimevi, Istanbul, Turkey (1935).
28. A.R. Onat, X. Milli Pediatri Kongresi Ankara, Thyroidea, Kader Basimevi, Istanbul, Turkey (1948).
29. F.C. Kelly and W.W. Senedden, World Health Organization, Geneva (1960).
30. I. Urgancioglu, H. Hatemi and I. Uslu, *Klinik Gelisim*, **7**, 36 (1987).
31. I. Bircan, *Akdeniz Üniversitesi Tıp Fak. Dergisi*, **54**, 79 (1989).
32. F. Hamulu, B. Karabulut, G. Özgen, G. Saydam, C. Yilmaz, M. Tüzün and T. Kabalak, *Turk. J. Endocr. Metabolis.*, **1**, 63 (1998).
33. H. Pekcan, G. Pekcan, M. Aykut and A. Ünal, *Kayseri Üniversitesi Gevher Nesibe Tıp Fak Mecmuasi*, **1**, 239 (1979).
34. M.F. Erdogan, G. Erdogan, H. Sav, S. Gülü and N. Kamel, *Biol. Trace Elem. Res.*, **79**, 121 (2001).
35. I. Bilabina, M. Braizer, H. Bour, A. Dohl and G. Desmet, *Ann. Biol. Clin.*, **52**, 261 (1994).
36. F.Z. Biber, P. Ünak and F. Yurt, *Isot. Environ. Health Stud.*, **38**, 87 (2002).