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ORGINAL RESEARCH

Quantitative Determination of The Heavy Metals (Lead, Zinc And Manganese) in White Cheese Produced in Ergene Basin by Using Atomic Absorption Spectrophotometry

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SUMMARY

It is well known that heavy metals are harmful for human health appearing only after exceeding the specific concentrations of each metal. This study was carried out to determine the concentrations of heavy metals in cheese that was produced in diaries of Ergene Basin.

The dairies near the Ergene River were investigated and suitable ones were selected. Since the sampling process directly affects the sensitivity of the results, particular attention was given to this process. A total of twelve samples of cheese were collected from different diaries and each samples were digested three times by dry ashing and wet ashing methods and totally 108 cheese samples were obtained for metal analyses. The lead, manganese content of the cheese samples were measured by graphite furnace atomic absorption spectrophotometer and zinc content of the cheese samples were measured by flame atomic absorption spectrophotometer.

Contents of heavy metals in the examined samples were compared with the excepted limit values in the literature and the results were evaluated. Experimental data shows that the concentrations of heavy metals are in the range of accepted limit values.

Keywords: Cheese, Ergene basin, Atomic absorption spectrophotometry, Heavy metals

INTRODUCTION

Ergene Basin is located in a region containing many industrial facilities and highways. In this study, by taking into consideration this characteristic of the region, effects of environmental pollution caused by heavy metal contents on one of the dairy products, white cheese have been investigated.

As it is known, Ergene Basin is a region where many industrial facilities have been established, waste water from these facilities reaches to the river, settlements as well as highways and traffic becoming more intense every day. From this aspect, where heavy metals such as lead accumulate intensely in the nature, impacted foods can be considered as a natural consequence of this situation (1,2).

Nutrition of animals is very important in production of milk, the raw material of cheese. In the event that animals are fed in a natural environment, they are affected from pollutants in the environment substantially; on the other hand, the ratio of harmful chemicals can be much higher than the limit values in production of processed feeds. Consequently, animals accumulate such metals in their milk and detrimental heavy

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metals can be contaminated with the milk through animal nutrition (3,4). In summary humans are exposed to heavy metals by consuming contaminated milk and milk product. This work was undertaken to determine their heavy metals levels in cheese samples collected from Ergene Basin manufacturers.

In atomic absorption spectrophotometry of the prepared cheese samples solutions, lead, zinc and manganese quantities were determined and obtained results were compared with the data in literature and then evaluated accordingly (5,6,7).

MATERIALS AND METHODS

The dairies near the Ergene River were searched and suitable ones were stabilized. It was very important to get the examples because the results could be effected directly (8). One sample from each of the twelve dairy plants was taken and then zinc solubilized by dry ash method in order to obtain three solutions, and in total thirty six cheese samples solutions were obtained. By wet ash method, it was obtained seventy two more cheese sample solutions by solubilization of three pieces for lead and manganese from the same samples. Thus, in total, hundred and eight cheese sample solutions were prepared.

All reagents were of analytical reagent grade unless otherwise stated. Bi-distilled water was used for all dilutions. HNO_3, $HClO_4$ and HCl were of suprapure quality (Merck, Darmstadt, Germany).

A Shimadzu AA-6800 F model atomic absorption spectrometer with deuterium background corrector was used in this work. Pb and Mn in samples were determined by HGA graphite furnace, using argon as the inert gas. Zn was carried out in an air/acetylene flame.

Dry Digestion

Cheese sample (weight 1,000 g) that was designated of humidity percentage, was weighed in acid washed porcelain capsule. The sample was burned and cooled for two hours in oven at 250°C that was raised to 500° C by the rate of 50°C every 30 min. 25,00 mL HCl was added on sample. It was heated for 15 minute in water bath by enclosing with a watch glass. 5,00 mL HNO_3 was added and the silica dissappeared by heating to 250°C on the hot plate. In order to dissolve the residue 5,00 mL concentrated HCl was added. It was filled with 50,00 mL distillate water and was heated again for one hour in water bath to be dissolved completely. 250,0 mL volumetric flask was filtered from blue band filter paper (No. 44) and up to mark with bi-distilled water. Blanck was also prepared the same way (5,7,9).

Wet Digestion

1,000 g of ground dried plant sample was taken and it was placed in a small beaker. 10,00 mL of concentrated HNO_3 was added and allowed to stand overnight. It was heated carefully on a hot plate until the production of red NO_2 furnes had ceased. The beaker was cooled and added a small amount (2,00-4,00 mL) of 70% $HClO_4$. It was heated again and allowed to evaporate to a small volume. Finally the sample was transferred to a 50,00 mL flask and was diluted to volume with bi-distilled water (10,11,12).

Determination of Moisture Content and Dry Matter

A cheese sample was sliced into~5mm thick sheets and weighed on a tare weighed glass container. The samples were kept in drying oven at 100°C for 2 hours until a constant weighed was attained. The moisture content and dry matter were determined from weight loss (%w/w dry matter).

EXPERIMENTAL

Preparation of Standard Solution for Lead

Titrisol Merck Art. 1.09969 Pb 1000 $mg.L^{-1}$ was used as standard solution.

Drawing the Calibration Curve

Standard solutions were prepared by including specified volumes from lead stock standard solution. The absorption values of these solutions were read at 283.3 nm by atomic absorption spectrophotometry. By the help of the values obtained, lead calibration curve was drawn (Fig.1).

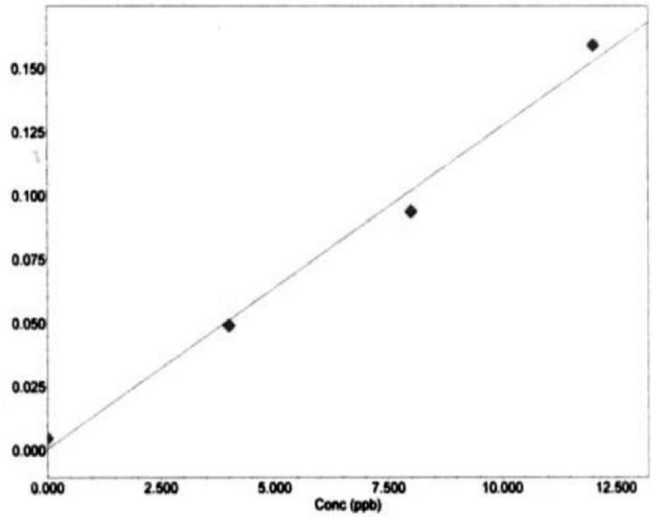

Figure 1. Lead calibration curve

Lead Quantification by Atomic Absorption Spectrophotometry (AAS)

The sample solution, obtained by wet ash method from cheese, lead concentrations in the solution were determined by Atomic Absorbtion Spectrophotometer which adjusted at 283.3 nm. From the results obtained, lead quantity in cheese samples solution was found in terms of µg/kg (Table 1).

Table 1. Results of lead quantification obtained from white cheese samples (µg/kg)

Cheese Samples	Experimental Results			$\overline{X}$	SS	% 90 GS*
	1st Experiment	2nd Experiment	3rd Experiment			
1	0.35460	0.36320	0.36720	0.36167	0.00707	0.361670 ∓ 0.01192
2	0.42726	0.41620	0.42260	0.42202	0.00707	0.42202 ∓ 0.01192
3	0.32920	0.32242	0.33426	0.32863	0.00707	0.32863∓0.01192
4	0.77870	0.76922	0.76518	0.77103	0.00816	0.77103 ∓ 0.01376
5	0.31350	0.33760	0.32450	0.32520	0.01291	0.32520 ∓ 0.02176
6	0.72493	0.70962	0.70517	0.71324	0.01155	0.71324 ∓ 0.01947
7	0.22236	0.21242	0.21228	0.21569	0.00408	0.21569 ∓ 0.00688
8	0.20613	0.20642	0.20627	0.20627	0.00577	0.20627 ∓ 0.00973
9	0.21409	0.21462	0.21521	0.21531	0.00408	0.21531 ∓ 0.00688
10	0.23218	0.22359	0.23104	0.22894	0.00577	0.22894 ∓ 0.00973
11	0.24607	0.24472	0.24642	0.24574	0.00408	0.24574 ∓ 0.00688
12	0.22983	0.22836	0.22975	0.22931	0.00408	0.22931 ∓ 0.00688

*GS: Safe Limits

Preparation of Standard Solution for Zinc

Titrisol Merck Art. 1.09953 Zn 1000 $mg.L^{-1}$ was used as standard solution.

Drawing the Calibration Curve

Standard solutions were prepared by including specified volumes from zinc stock standard solution. The absorption values of these solutions were read at 213.9 nm by atomic absorption spectrophotometry. By the help of the values obtained, zinc calibration curve was drawn (Fig.2).

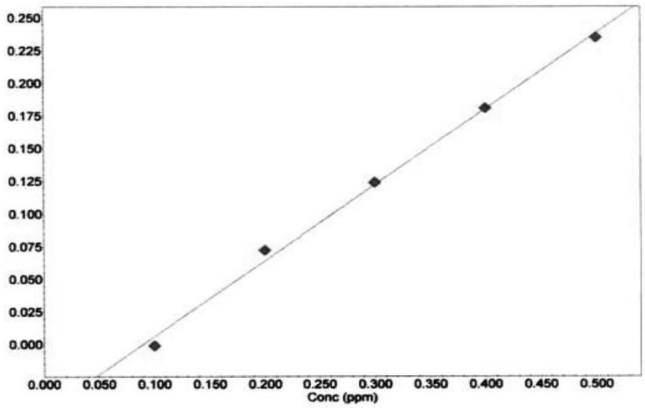

Figure 2. Zinc calibration curve

Zinc Quantification by Atomic Absorption Spectrophotometry (AAS)

The sample solution, obtained by dry ash method from cheese, zinc concentrations in the solution were determined by Atomic Absorbtion Spectrophotometer which adjusted at 213.9 nm. From the results obtained, zinc quantity in cheese samples solution was found in terms of mg/kg (Table 2).

Table 2. Results of zinc quantification obtained from white cheese samples (mg/kg)

Cheese Samples	Experimental Results			$\overline{X}$	SS	% 90 GS*
	1st Experiment	2nd Experiment	3rd Experiment			
1	0.40226	0.39780	0.42970	0.40992	0.01732	0.40992 ∓ 0.02920
2	0.44520	0.45800	0.46210	0.44510	0.00913	0.44510 ∓ 0.01539
3	0.43650	0.40360	0.42200	0.42070	0.01648	0.42070 ∓ 0.02778
4	0.26560	0.27840	0.25210	0.26537	0.01304	0.26537 ∓ 0.02198
5	0.32480	0.33350	0.31420	0.32417	0.00966	0.32417 ∓ 0.01628
6	0.42090	0.44410	0.43630	0.43377	0.01169	0.43377 ∓ 0.01971
7	0.48670	0.49750	0.50045	0.49488	0.00695	0.49488 ∓ 0.01172
8	0.39587	0.38610	0.37611	0.38603	0.00931	0.38603 ∓ 0.01570
9	0.40399	0.41112	0.41849	0.41123	0.00730	0.41123 ∓ 0.01231
10	0.26179	0.25643	0.26386	0.26069	0.00365	0.26069 ∓ 0.00615
11	0.32875	0.34538	0.32461	0.33291	0.01065	0.33291 ∓ 0.01795
12	0.39184	0.38859	0.39704	0.39249	0.00483	0.39249 ∓ 0.00814

*GS: Safe Limits

Preparation of Standard Solution for Manganese

Titrisol Merck Art. 1.09988 Mn 1000 $mg.L^{-1}$ was used as standard solution.

Drawing the Calibration Curve

Standard solutions were prepared by including specified volumes from manganese stock standard solution. The absorption values of these solutions were read at 279.5 nm by atomic absorption spectrophotometry. By the help of the values obtained, manganese calibration curve was drawn (Fig.3).

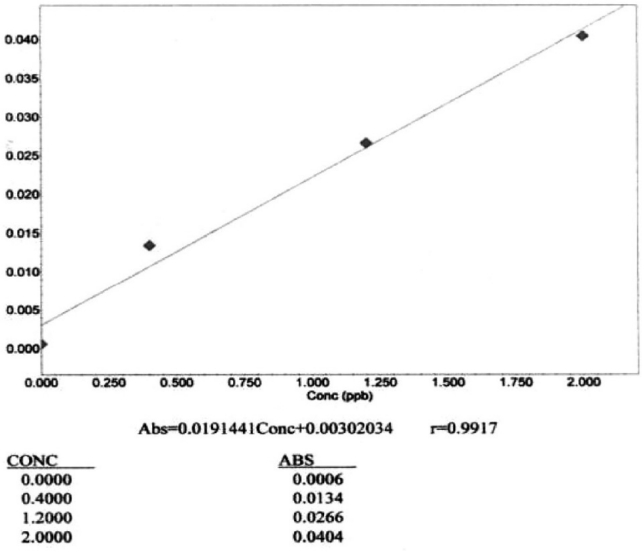

Figure 3. Manganese calibration curve

Manganese Quantification by Atomic Absorption Spectrophotometry (AAS)

The sample solution, obtained by wet ash method from cheese, manganese concentrations in the solution were determined by Atomic Absorbtion Spectrophotometer which adjusted at 279.5 nm. From the results obtained, manganese quantity in cheese samples solution was found in terms of µg/kg (Table 3).

RESULT AND DISCUSSION

Heavy metals have bio-toxic effects in human biochemistry. These metals are released into the environment especially industrial activities, which will cause harm to living organism animals also accumulate such metals in their tissues and milk so humans are exposed to heavy metals along their cycles of food chain. In this study, cheese products produced in dairy plants established in Ergene Basin were investigated and heavy metal contents in cheese were analyzed. For this purpose, dairy plants around Ergene River were searched and appropriate ones were determined. As precision in sampling would directly affect sensitivity of the results, a particular attention was shown to sampling process.

Table 3. Results of manganese quantification obtained from white cheese samples (µg/kg)

Cheese Samples	Experimental Results			$\overline{X}$	SS	% 90 GS*
	1st Experiment	2nd Experiment	3rd Experiment			
1	1.2622	1.2473	1.2514	1.2536	0.01080	1.2536 ∓ 0.01821
2	1.5280	1.5049	1.5175	1.5168	0.01275	1.5168 ∓ 0.02150
3	1.1212	1.1356	1.1295	1.1288	0.00577	1.1288 ∓ 0.00973
4	1.4856	1.4773	1.4910	1.4846	0.00913	1.4846 ∓ 0.01539
5	1.5922	1.5826	1.6014	1.5921	0.01000	1.5921 ∓ 0.01686
6	1.8319	1.8392	1.8295	1.8335	0.01291	1.8335 ∓ 0.02176
7	1.2819	1.2822	1.2820	1.2820	0.00817	1.2820 ∓ 0.01377
8	1.6192	1.6438	1.6437	1.6355	0.01155	1.6355 ∓ 0.01947
9	1.7167	1.7165	1.7166	1.7166	0.01000	1.7166 ∓ 0.01686
10	1.7279	1.7543	1.7401	1.7408	0.01633	1.7408 ∓ 0.02753
11	1.4858	1.4448	1.4867	1.4724	0.02550	1.4724 ∓ 0.04299
12	1.8324	1.8322	1.8325	1.8324	0.01291	1.8324 ∓ 0.02176

*GS: Safe Limits

In tables, heavy metal concentrations are indicated as mg or µg metal quantity / kg dry substance.

In atomic absorption spectrophotometry, flame and graphite furnace system configurations were used. In flame furnace, zinc quantities were measured in ppm level. In graphite furnace, lead and manganese quantities were measured in ppb level.

According to the evaluation of the results obtained, it is seen that contents of lead, zinc and manganese, which are all heavy metals, in white cheese samples taken from twelve different points in Ergene Basin are within the safe limits specified in the literature that do not treat human health.

According to international standards, maximum amount of lead is stated as 2mg/kg (12,13). The lead amount in cheese sample that is taken from Ergene River is found very low when compared to literature review (Table 1). According to international standards, maximum amount of zinc is stated as 50mg/kg (12,13). In this study, zinc amount in cheese sample that is taken from Ergene River is found within limits (Table 2). And according to international standards, maximum amount of manganese is given as 0.5 mg/kg (12,13). The manganese amount in cheese sample of Ergene River is situated within limits (Table 3).

In this respect, heavy metal pollution in milk used in cheese production has not occurred at all or occurred at the minimum level. Therefore, determined quantities of lead, zinc and manganese heavy metals in produced cheese are within the limits that do not treat human health. Correctness of this fact has been proved by the experimental results obtained.

Ergene Havzasında Üretilen Beyaz Peynirlerde Atomik Absorpsiyon Spektrofotometri Yöntemiyle Nicel Ağır Metal (Kurşun, Çinko, Manganez) Tayini

ÖZET

Ağır metallerin insan sağlığı açısından zararlı etkileri olduğu bilinmektedir. Bu zararlı etkiler her metal için belirli konsantrasyonlardan sonra ortaya çıkmaktadır. Bu çalışma, Ergene Havzasındaki süt işletmelerinde üretilen peynirlerde incelemeler yapılarak içerdikleri ağır metal miktarlarının araştırılması amacıyla yapılmıştır.

Ergene Nehri civarındaki süt işletmeleri araştırılmış ve uygun olanları seçilmiştir. Örneklerin alınmasındaki hassasiyet, elde edilecek sonuçların duyarlılığını doğrudan etkileyeceğinden bu konuya özellikle dikkat edilmiştir. Toplam on iki adet peynir örneği farklı süt işletmelerinden toplanmış ve her bir örnek kuru ve yaş külleme yöntemi ile üçer kez çözünürleştirilmiş, ağır metal analizleri için 108 peynir örneği elde edilmiştir. Örneklerin kurşun, mangan içerikleri grafit fırın kullanılarak atomik absorbsiyon spektrofotometresinde, çinko içerikleri ise alevli atomik absorbsiyon spektrofotometresinde ölçülmüştür. Hazırlanan örneklerdeki ağır metal içerikleri literatürde verilen limit değerlerle karşılaştırılmış ve sonuçlar değerlendirilmiştir. Deneysel veriler örneklerdeki ağır metal konsantrasyonlarının sınırlar içinde olduğunu göstermiştir.

Anahtar Kelimeler: Peynir, Ergene havzası, Atomik absorbsiyon spektrofotometrisi, Ağır metaller

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