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Research Article

ESSENTIAL ELEMENTS AND HEAVY METAL LEVELS IN SHEEP MILK AND ITS DAIRY PRODUCTS

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Abstract: *Milk and various dairy products are among the basic foods used in nutrition. However, milk and dairy products can contain many environmental pollutants such as pesticides, detergents, drug residues, heavy metals that may pose technological risks and are dangerous for human health. The aim of this study is to reveal the change of the amounts of essential elements and heavy metals in sheep's milk, yoghurt, buttermilk, and butter which are produced from the same milk. For this purpose, yoghurt, buttermilk, and butter were made from sheep's milk. Then, in milk and dairy products, Sodium (Na), Magnesium (Mg), Potassium (K), Manganese (Mn), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se)), Cadmium (Cd), Lead (Pb) amounts were examined by ICP-MS. The amount of As, Cd, Pb in all samples were determined under the limit of detection (LOD). The amounts of Na, Mg, K, Mn, Cu, Zn, Se in milk were determined as 785, 92, 1537, 30,8, 73,5, 2683,5, 381,5 ppb, respectively. The amounts of Na, Mg, K, Mn, Cu, Zn, Se in yogurt were determined as 554,5, 121,5, 1516,5, 29,3, 71,5, 3692, 405 ppb, respectively. The amounts of Na, Mg, K, Mn, Cu, Zn, and Se in buttermilk were determined as 40175, 56,5, 553,5, 111,5, 1230, 2506,5, 447 ppb, respectively. The amounts of Na, Mg, K, Mn, Cu, Zn, Se in butter were determined as 98,2, 31,7, 223,1, 10,1, 24,6, 203,5, 282,5 ppb, respectively. According to these data, changes in the amount of essential elements were observed when milk was transformed into its products. It was determined that there were no heavy metals in sheep milk and products grown in this region.*

Keywords: *Sheep milk, Dairy product, Element, Heavy metal, ICP-MS*

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1.Introduction

Milk is produced in the milk glands of female mammals to feed their offspring. Various foods derived from milk are called "dairy products" such as cheese, cream, yoghurt, buttermilk, butter [1]. Milk and dairy products are among the basic foods used in human nutrition. Milk and dairy products are among the foods that are highly preferred by consumers because they have high biological values rich in nutrients, are thought to have little health risk, and are especially easily accessible. However, milk and dairy products may contain many environmental pollutants such as pesticides, detergents, drug residues, heavy metals that may pose technological risks and are dangerous for human health [2].

The heavy metal term is a general nomenclature given to heavy metals with an atomic number greater than 20 or a volume occupying one cubic centimeter more than five grams [3]. Heavy metals tend to accumulate in the tissues of the mammalian body, reaching toxic values over time and may cause serious health problems [4]. Heavy metals enter the human body through digestion, respiration, and skin. Acute, subacute and chronic intoxication symptoms (such as microcytic anemia, liver necrosis, memory retardation, speech, and voice disorders) occur depending on overdose, frequency, and duration of intake [5].

The level of elements found in milk and dairy products is very important due to their essential or toxic effects. For example, although Cd, As, Pb are toxic, Cu, Se Zn, Cr, Na, Mg, Mn are essential and are toxic only at high doses. Among the elements that have negative effects on human health, Pb, As and Cd are the most dangerous [6, 7]. Considering that milk and dairy products are one of the basic foods, the possibility of having high amounts of lead, cadmium, copper, and zinc residues in milk poses a serious risk. Children are more sensitive to heavy metals than adults. Because heavy metals accumulate in the tissues and very small amounts of milk can cause serious effects on the health of children. For example, slowing down in mental development, decrease in concentration can negatively affect kidney and heart health [8, 9].

Heavy metal contamination of milk and dairy products; The feed consumed by the animals from which milk is obtained can pass directly to the milk as a result of contamination with the animal through the water they drink and the air they breathe [10]. In addition, contamination can occur from machinery and equipment that come into contact with dairy products during the production and storage of milk and dairy products. Contamination may occur during technological processes or from metal containers and operational water used to preserve milk and milk products. Even if there is no heavy metal contamination in the milk to be used in technology, heavy metal can be detected after milk is processed (cheese, yogurt, buttermilk, butter). This is the result of metals in the composition of containers used in the production of acidic dairy products, dissolving into the product. The main elements in water and metallic contamination used in the business are copper, zinc, iron, tin, lead, arsenic, and cadmium [11, 12].

Akin et al. (2003) reported that the high level of aluminum they detected in raw milk may be caused by tools and equipment made of aluminum metal in addition to the feed consumed by animals [13]. Yuzbasi (2001) found that the amount of lead in the milk to be processed into cheddar cheese decreased significantly in cheddar cheeses after production, there was no change in the amount of copper, but the amount of cadmium increased [10]. Temurci and Güner. (2006) reported that when they examined heavy metal levels in milk and cheese obtained from these milks, they found that aluminum, chromium, copper, and iron amounts in cheese samples were higher than milk samples [14]. In this study, we aimed to demonstrate the change of essential element and heavy metal amounts in sheep's milk, yoghurt, buttermilk, and butter that we produce from this milk with the ICP-MS device.

2. Material Method

In this study, samples of milk belonging to ivesi breed sheep taken from the farm of Harran University, and samples of yoghurt, buttermilk, and butter produced from this milk formed the material of the study (Figure 1-3) [15].

Raw milk
↓
Standardization
↓
Homojenizasyon
↓
Heat Treatment
↓
Cooling
↓
Inoculation
↓
Incubation
↓
Cooling

Figure 1. Yoghurt production stages

Yoghurt
↓
Addition of water
(50:50 v/v)
↓
Addition of salt
(c.a. 0.5%)
↓
Mixing
↓
Buttermilk

Figure 2. Buttermilk production stages

Yoghurt
↓
Addition of water
(70:30 v/v)
↓
Churning (10 ^{0}C)
↓
Washing

Figure 3. Butter production stages

After dairy products are prepared in the laboratory, 1 gram of milk, cheese, yoghurt, and butter samples were weighed after the homogenization process and taken into the sample containers of the microwave device. 4 mL of 65% (v / v) nitric acid (HNO3) and 2 mL of 30% (v / v) hydrogen peroxide (H2O2) were added with a pipette and placed in the microwave device. The samples were burned in the microwave device with the predetermined program (Table 1). After burning the samples cooled, they were taken into sterile tubes and diluted with ultrapure water.

Table 1. Burning process steps in microwave device

Step	**Temperature (°C)**	**Time (min)**
1	90	8
2	170	10
3	210	25

Elemental and heavy metal analyzes of the samples were performed with the Agilent brand, 7500ce series ICP-MS (Tokyo, Japan) device in the Mersin University Advanced Technology Education, Research and Application Center laboratory.

3. Results and Discussion

In this study, the element and heavy metal amount of each sample analyzed by ICP-MS is shown in Table 2. and Figure 4.

Table 2. The element and heavy metal content of sheep milk and its dairy products

Element	**Milk**	**Yoghurt**	**Buttermilk**	**Butter**
Na (ppb)	785	554.5	40175	98.2
Mg (ppb)	92	121.5	56.5	31.7
K (ppb)	1537	1516.5	553.5	223.1
Mn (ppb)	30.8	29.3	111.5	10.1
Cu (ppb)	73.5	71.5	1230	24.6
Zn (ppb)	2683.5	3692	2506.5	203.5
As (ppb)	<LOD	<LOD	<LOD	<LOD
Se (ppb)	381.5	405	447	282.5
Cd (ppb)	<LOD	<LOD	<LOD	<LOD
Pb (ppb)	<LOD	<LOD	<LOD	<LOD

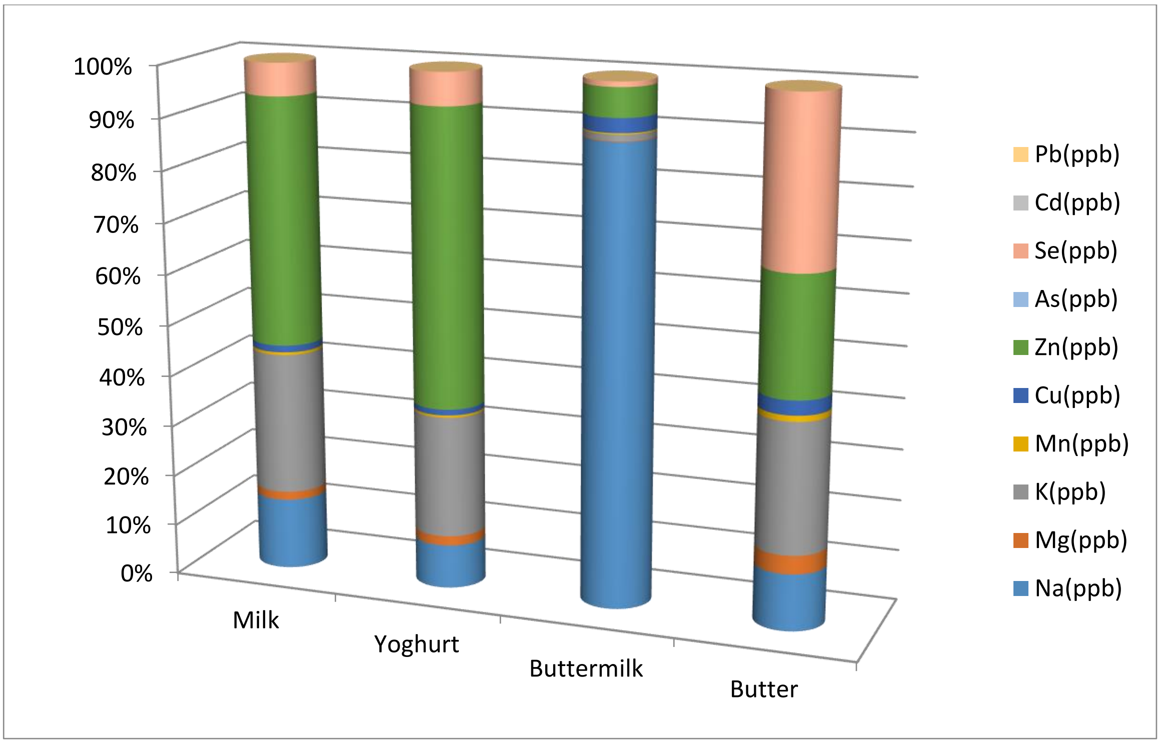

Figure 4. The element and heavy metal content of sheep milk and its dairy products

Micronutrients such as copper, magnesium, potassium, and zinc are essential for many biological functions. The deficiencies of such elements in the body contribute significantly to the emergence of many diseases. However, if these elements are found in foods at higher levels, they can have negative effects on human health [16]. The trace element content and heavy metal contents of milk and dairy products may vary depending on the lactation stage, the nutritional status of the animal, environmental and genetic factors, and possible contamination during production [17]. In this study we conducted, firstly, the mineral and heavy metal amounts in milk and then in dairy products produced from that milk were examined. There was a change in the amount of minerals seen (yoghurt, buttermilk, butter) (Table 2, Figüre 4.).

Na has many beneficial effects in the body, such as osmotic pressure, electrolyte balance, acid-base balance, and electrochemical impulse transmission across nerve and muscle membranes [18]. There is also some Na in milk and dairy products. However, the amount of Na in milk and dairy products varies according to the lactation period, nutritional status and the process applied to the milk. In a study conducted by Güler (2007), heavy metal analyzes were made on yoghurts produced from goat milk and goat milk collected from Hatay. Na levels were determined as 433 ppm for raw milk, 520 ppm for strained yogurt, and 5147 ppm for salted strained yogurt, respectively [19]. In our study, the amount of Na was 785 ppb in milk, 554,5 ppb in yogurt, 40 175 ppb in buttermilk, and 98,2 ppb in butter. Na amount was determined in the highest buttermilk. The reason for the high amount of Na in buttermilk is due to the addition of NaCl during construction.

Mg has many functions involved in more than 300 reactions in the body. Mg is found in most foods. It is a good source of Mg in milk and dairy products. There is about 100 mg of Mg in one liter [20]. The amount of Mg varies according to the lactation period, the animal's diet and the process applied to the milk. In a study conducted by Güler (2007), heavy metal analyzes were made on yoghurts produced from goat milk and goat milk collected from Hatay. Mg levels were determined as 510 ppm for raw milk, 587 ppm for strained yogurt, and 838 ppm for salted strained yogurt, respectively [19]. In our study, the amount of Mg was 92 ppb in milk, 121,5 ppb in yoghurt made from milk, 56,5 ppb in buttermilk, and 31,7 ppb in butter. The amount of Mg was found the most in yogurt and the least in butter.

K has many beneficial effects osmotic pressure, electrolyte balance, acid-base balance, nerve impulses, contraction of the heart and other muscles, protein synthesis, conversion of glucose into glycogen in the body [18]. Therefore, K should be taken from outside with food. The amount of K changes according to the lactation period, the animal's diet and the process applied to the milk. In a study conducted by Güler (2007), heavy metal analyzes were made on yoghurts produced from goat milk and goat milk collected from Hatay. K levels were determined as 409 ppm for raw milk, 511 ppm for strained yogurt, and 554 ppm for salted strained yogurt, respectively [19]. In our study, the amount of K was 1537 ppb in milk, 1516,5 ppb in yoghurt made from milk, 553,5 ppb in buttermilk, and 223,1 ppb in butter. K amount was determined mostly in yoghurt. The low amount of K element in butter and buttermilk suggests that it may be due to the production process.

Mn participates in cofactor, reproduction, and bone structure in many enzymatic reactions in the body and is necessary because it has many functions such as regulating brain functions. Therefore, Mn should be taken from outside with food [18]. It contains some Mn element in milk and dairy products. The amount of Mn varies according to the lactation period, the animal's feeding, and the process applied to the milk. Enb et al. (2009), in their study with buffalo and cow milk, determined the amount of Mn in buffalo milk as 0.076 mg/kg and the amount of Mn in cow milk as 0.056 mg/kg. In the same study, the highest amount of Mn in dairy products such as cream and butter was determined as 0.316 mg/kg in butter and 0.234 mg/kg in cream [21]. Kaya et al. (2008) reported metal concentrations in yoghurt made from cow's milk in a controlled environment as 0.01-0.179 mg/kg for Mn [22]. In our study, the amount of Mn was 30,8 ppb in milk, 29,3 ppb in yoghurt made from milk, 11,5 ppb in buttermilk, and 10.1 ppb in butter. While Mn amounts were higher in milk and yoghurt, the amount of Mn in buttermilk and butter decreased.

Cu is required as a trace element necessary for adequate growth, cardiovascular system, lungs, neuronendocrine function, and iron metabolism [23] Excess Cu taken into the body due to contamination or other reasons may cause poisoning effect and cause hypercoupremia. Generally, the Cu level contained in milk is at minimum levels but With subsequent contamination, Cu in milk and dairy products can be seen at maximum level [24]. Kaya et al. (2008) reported metal concentrations in yogurt made from cow's milk in a controlled environment as 0.011-0.498 mg / kg for Cu [22]. Temurci and Güner. (2006) reported that when they examined heavy metal levels in milk and cheese obtained from these milks, the amount of Cu in cheese samples was higher than in milk samples [14]. Yuzbasi (2001) reported that the amount of Cu in milk to be processed into cheddar cheese does not change in cheddar cheese after production [10]. Gördes Baş (2020) reported that the Cu amount in yoghurt and buttermilk offered for consumption was 0.03 mg/kg in yoghurt and 0.01 mg/kg in buttermilk [25]. In a study conducted by Kan and Küçükkurt (2018), Cu amount was reported as 0.09 mg/kg in cream and 0.02 mg / L in cream milk [26]. In this study we conducted, Cu amount was 73,5 ppb in milk, 71,5 ppb in yoghurt made from milk, 1230 ppb in buttermilk, and 24,6 ppb in butter. The high amount of Cu in buttermilk is thought to be due to the use of metal containers in the production of buttermilk and adding water from the outside. The probable reason for the high Cu content in buttermilk is due to the added salt or water.

Zn is involved in many physiological processes such as nucleic acid and protein synthesis, cellular replication, insulin secretion, sexual maturation, and strengthening of the immune system [27]. Enb et al. (2009) found the Zn value in milk as 4,350 mg / kg, in yogurt 4,059 mg / kg, in cream 19,570 mg / kg and in butter 29,363 mg / kg in their heavy metal analysis by collecting milk from buffalo and cow milk and obtaining dairy products [21]. Gördes Baş (2020) reported that the amount of Zn in yoghurt and buttermilk offered for consumption is 3.05 mg / kg in yoghurt and 1.66 mg / kg in buttermilk [25]. In a study conducted by Güler (2007), heavy metal analyzes were made on yoghurts produced from goat milk and goat milk collected from Hatay. Zn levels were determined as 4.68 ppm for raw milk, 6.85 ppm for strained yogurt, and 9.00 ppm for salted strained yogurt, respectively [19]. In a study by Kan and Küçükkurt (2018), the amount of Zn was reported as 8.27 mg/kg in cream and 2.37mg / L in skim milk [26]. In this study, the amount of Zn was determined as 2683,5 ppb in milk, 3692 ppb in yogurt made from milk, 2506,5 ppb in buttermilk, and 203.5 ppb in butter. The Zn amount was found in the highest yogurt and the lowest in butter. As the Zn element attaches to the casein micelles in yoghurt production, it was detected at a higher rate in yoghurt.

Se plays an important role in immunity, antioxidant system, DNA synthesis, and DNA repair. The recommended daily intake of Se is 55 µg. It is an important source of Se in milk and dairy products [20]. Setting et al. (2007) investigated some heavy metals in various dairy products and found the highest Se value in Tulum cheese with 0.434 mg/kg and then in butter with 0.315 mg/kg [28]. In a study conducted by Güler (2007), heavy metal analyzes were made on yoghurts produced from goat milk and goat milk collected from Hatay. Se levels were determined as 7.59 ppm for raw milk, 10.77 ppm for strained yogurt, and 12.20 ppm for salted strained yogurt, respectively [19]. In a study by Kan and Küçükkurt (2018), the amount of Se was reported as 0.94 mg/kg in skim milk and 0.17 mg / L in skim milk [26]. In our study, the amount of Se was 381,5 ppb in milk, 405 ppb in yogurt made from milk, 447 ppb in buttermilk, and 282,5 ppb in butter.

The amounts of Se in all samples were determined at close levels.

Since heavy metals cause acute and chronic health problems, national and international food organizations have introduced regulations to prevent contamination. However, in the communiqué of the Turkish Food Codex on determining the maximum levels of certain contaminants in foodstuffs [29], the highest acceptable values for milk and dairy products were determined as 0.020 mg/kg for Pb, but no limit was specified for other metals. In this study, the amounts of Pb, As and Cd in milk, yoghurt, buttermilk, and butter were analyzed. These heavy metals were not detected in any of the samples in the analysis results.

The presence of Pb in milk and dairy products is from environmental sources (atmosphere, vehicle exhausts, urban waste, etc.). Since Pb is toxic and has negative effects on human health, the Codex Alimentarius Commission [30] determined the Pb amount at the level of 0.02 mg/kg for milk and dairy products. Also, The Turkish Food Codex states that the highest acceptable Pb for milk and dairy products is 0.020 mg/kg [29]. In a study conducted by Güler (2007), heavy metal analyzes were made on yoghurts produced from goat milk and goat milk collected from Hatay. Pb levels were determined as 0.06 ppm for raw milk, 0.11 ppm for strained yoghurt, and 1,3 ppm for salted strained yogurt, respectively [19]. Kaya et al. (2008) reported metal concentrations in yogurt made from cow's milk in a controlled environment as 0.019-0.126 mg/kg for Pb [22]. In a study conducted by Hernandez and Park

(2014), they reported the Pb amount of 3 different yoghurts made from goat milk obtained from a market as 4,003-4,280ppm [31]. Coni et al. (1999) in a study conducted in Italy; Pb amount is 0.006 ± 0.003 ppm in sheep milk, 0.016 ± 0.006 ppm in curd obtained from sheep milk, 0.003 ± 0.001 ppm in whey obtained from sheep's milk, 0.019 ± 0.006 ppm in Pecorino cheese obtained from sheep milk, 0.002 in Ricatta cheese obtained from sheep milk. It has been reported as ± 0.001 [32]. Yuzbasi (2001) reported that the amount of Pb in the milk to be processed into cheddar cheese decreased significantly in post-production cheddar cheese [10]. In a study by Kan and Küçükkurt (2018), it was reported that the amount of Pb shifts and the average values are the same in under-cream milk, while the standard deviation and maximum value are higher in under-cream milk [26]. Gördes Baş (2020) reported that he did not detect Pb in yoghurt and buttermilk offered for consumption [25]. In our study, the amount of Pb was found below the detection limits (<LOD) in milk and yoghurt made from milk, buttermilk, and butter.

Cd can contaminate milk and dairy products from the environment (soil, fertilizer, atmosphere). It is considered the most important food contaminant. Cd is important because it shows high toxicity and has negative effects on human health (8). Turkish Food Codex does not set any limit for Cd. In a study conducted by Güler (2007), heavy metal analyzes were made on yoghurts produced from goat milk and goat milk collected from Hatay. Cd levels were determined as 0.63 ppm for raw milk, 1.01 ppm for strained yoghurt, and 1.00 ppm for salted strained yogurt, respectively [19]. In a study conducted by Hernandez and Park (2014), they reported the Cd amount of 3 different yoghurts made from goat milk obtained from a supermarket as 0.614-0.700 ppm [31]. Coni et al. (1999) in a study conducted in Italy; The amount of Cd in sheep's milk is 0.058 ± 0.019 ppm. It has been reported as 0.048 ± 0.016 ppm in curd obtained from sheep milk, 0.076 ± 0.035 ppm in whey obtained from sheep milk, 0.025 ± 0.009 ppm in Pecorino cheese obtained from sheep milk, and 0.043 ± 0.013 ppm in Ricatta cheese obtained from sheep milk [32]. Yuzbasi (2001) reported that the amount of Cd in the milk to be processed into cheddar cheese increased in cheddar cheese after production [10]. In a study by Kan and Küçükkurt (2018), the amount of Cd in cream and cream milk was found below the detectable value [26]. Gördes Baş (2020) reported that he did not detect Pb in yoghurt and buttermilk offered for consumption [25]. In this study we conducted, the amount of Cd was found below the detection limits (<LOD) in milk and yoghurt, buttermilk and butter made from milk.

As it is common in nature and increasing exposure to environmental arsenic today, high arsenic content in some products has increased. As is also contaminated with milk and dairy products from the environment (1). In a study by Kan and Küçükkurt (2018), the amount of As was reported as 0.14 mg/kg in skim milk and 0.04mg / L in skim milk [26]. In a study conducted by Güler (2007), heavy metal analyzes were made on goat milk collected from Hatay and yoghurts produced from goat milk [19]. Arsenic has not been detected in any product. Gördes Baş (2020) reported that he did not detect As in yoghurt and buttermilk offered for consumption [25]. In our study, the amount of As was found below the detection limits (<LOD) in yoghurt, buttermilk, and butter made from milk and milk.

4. Conclusion

According to these data, changes in the amount of essential elements were observed when milk was transformed into its products. It was determined that there were no heavy metals in sheep milk and products grown in this area. This study shows that sheep's milk and products can contribute significantly to the supply of elements in the human diet. It also provides important information on essential elements and heavy metal concentration about the safety and quality standards of sheep's milk, yoghurt, buttermilk, and butter.

The compliance to the Research and Publication Ethics: This study was carried out in accordance with the rules of research and publication ethics.

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