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Specification of Mineral Composition, Heavy Metal Content, Microbiological Characteristics of a Traditional Anatolian Cheese-Aho Cheese

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Abstract: In this study, it was aimed to determine certain microbiological characteristics, mineral contents heavy metal levels in 16 Aho cheeses of Sürmene and Araklı districts of Trabzon and to determine the suitability of the Turkish Food Codex. Mineral ingredient and heavy component contents have been identified based on ICP-OES implementation and in mg kg^{-1} unit type. In Aho cheese samples the average levels of the mineral contents and heavy metals in the Aho cheeses were determined as; calcium 3628.30±355.07 mg kg^{-1}, potassium 461.20±33.48 mg kg^{-1}, sodium 8463.75±658.97 mg kg^{-1}, magnesium 163.42±10.96 mg kg^{-1}, iron 5.46±1.29 mg kg^{-1}, zinc 27.04±2.62 mg kg^{-1}, copper 1.358±0.13 mg kg^{-1}, manganese 0.31±0.17 mg kg^{-1}, phosphorus 4187.05±327.60 mg kg^{-1}, lead 0.174±0.04 mg kg^{-1} and cadmium 0.028±0.04 mg kg^{-1}. The microbiological values obtained were as follows; yeast and moulds 4.12±0.09 log cfu g^{-1}, total aerobic mesophilic bacteria 6.72±0.11 log cfu g^{-1}, Lactobacilli bacteria counts in the MRS agar 6.42±0.05 log cfu g^{-1}. Coliforms and *Staphylococcus aureus* were <10 in all samples. As a result, it was determined that the mineral contents and heavy metal levels of the cheese samples were within the limits determined by the Turkish Food Codex for some foods.

Keywords: Aho cheese, Mineral content, Heavy metal, ICP-OES.

INTRODUCTION

Milk and dairy products in terms of their nutritional content and nutritional value are indispensible nutrients for the mankind. Mineral substances in milk in terms of their quantities are diversified into two groups as macro and trace elements. Calcium, magnesium, chlorine and sodium are placed into macro elements group, iron, zinc, copper, chrome; aluminium and manganese are placed into trace elements group [1]. Mineral components in milk have significant functions pertain to physical and chemical properties, nutrient value and technology of milk.

Cheese is one of the most privileged products of the dairy industry both in terms of its ingredients' indisputable importance for the human nutrition and its economic impact [2]. Because of its geographical location and a cradle of civilization our country has a rich diversity in dairy products especially in cheese types. In our country, depending on local conditions, especially cultural habits, differences in animal species and breeds various regional cheeses are produced with different construction techniques. Depending on the source of the milk and the technical process used in region and production there are more than 200 cheeses which have their own chemical and sensory qualities, especially flavour and texture. Some of these cheeses are regional cheeses [3, 4].

In many countries there are some studies about the transmission of the production techniques of traditional products to medium and small- scale industry to contribute the regional economy and increase the product diversity and safety in recent years [5]. Black Sea Region is very rich in terms of regional dairy products. Some part of the local products in the region pass into oblivion and some part are produced in houses, small family type enterprises and sold in the cheese passages and in the district markets of the region. Aho cheese is one of the regional cheeses in the Black Sea Region and it takes its name from a village of Araklı district Ayvadere (Aho) village in Trabzon province. This cheese has a very salty and bitter taste and its commercial production is made in Bayburt, Gümüşhane, a highland of Trabzon Sürmene (Taşlı Yaylası), Çaykara and some villages of Araklı [6]. As in many food sectors, heavy metal contamination can also occur in the dairy sector during milk production and product processing. In milk-producing enterprises, contamination can usually transmit from environmental sources (e.g. soil and water) and feed. Also contamination can

result from the machines and equipment used in milking, storage and processing [7]. Because the risk of dissolving metals in the composition of instruments and equipment used in the milking or production of acidic foods such as milk and cheese may be easier than in other foods [8, 9]. The main elements of metal contaminants originating from metal containers used in the preservation of milk and dairy products during technological processing copper, zinc, iron, tin, lead, cadmium and arsenic [1].

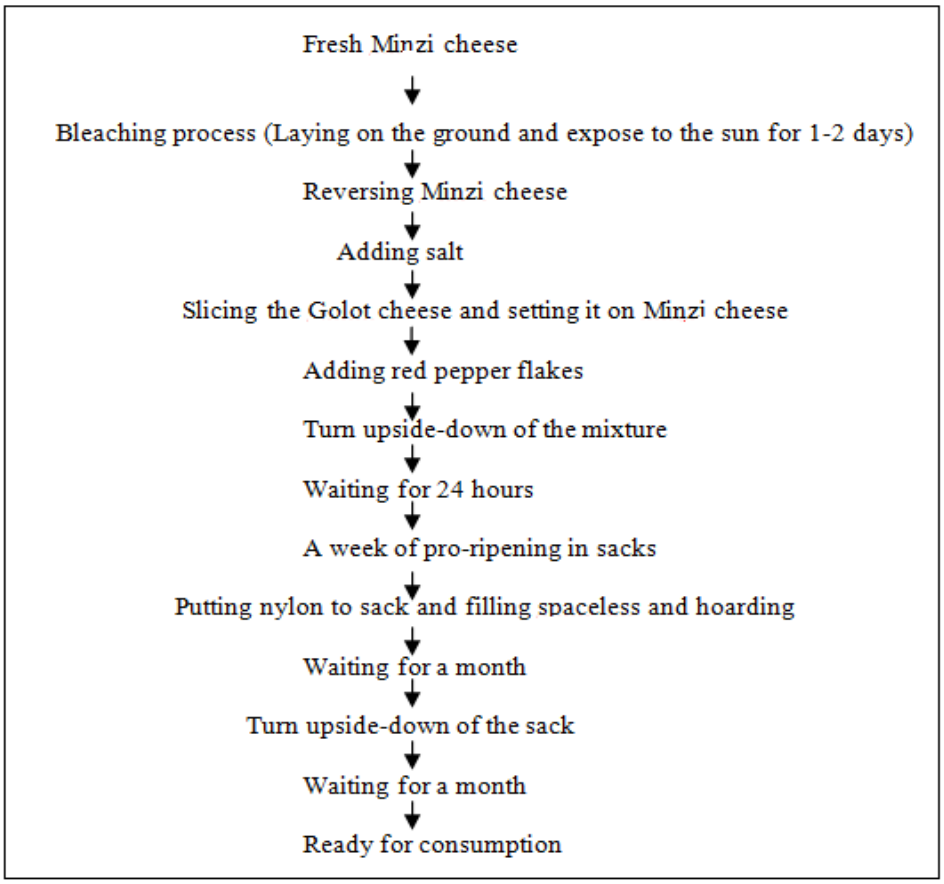
As a result of the negative consequences of developing industries and efforts to provide a more modern life contamination of food with high levels of heavy metals is one of the most important problems that threaten public health today. Some of the heavy metals (such as iron, zinc and copper) are essential for life and are essential for the function of important enzyme systems. Some other heavy metals' functions in human physiology have not been determined yet. However, some heavy metals (e.g. lead and mercury) can potentially have toxic effects even at low levels. Metals tend to accumulate in the human body especially in certain tissues and organs (e.g. liver, bone and kidney). The rate of accumulation of heavy metals taken with food in the body can vary according to the chemical form of the metal, the age and nutritional status of the person [10].

Although there are studies on the physical, chemical and microbiological properties of Aho cheese, studies on mineral content and heavy metal contamination were not found. It was aimed to determine the levels of calcium, potassium, sodium, magnesium, manganese, iron, zinc, copper, nickel, lead and cadmium in the Aho cheeses consumed in the Sürmene and Araklı districts of Trabzon.

MATERIAL AND METHODS

Sample collection

16 Aho cheese samples, consumed in Sürmene and Araklı districts of Trabzon, were obtained from cheese passages and district markets. The samples were transported to the laboratory using cold chain and stored at 4±1 °C until the analysis.



Fig−1: The production stages of Aho Cheese [6].

Methods

Heavy metal and mineral analysis

Sample preparation

After being kept in a 1:1 HNO_3 solution in glass jars overnight, the samples were washed first in distilled then in bidistilled water and finally dried. In order to eliminate the organic compounds and release the inorganic compounds in a soluble phase, 1 g cheese samples were placed in Teflon containers to which a combination of 5 ml of 65% nitric acid (Merck, 1.00452) and 5 ml of 30% hydrogen peroxide (Merck, 1.08597) was added. The teflon containers were capped and the samples were burned in a Mars-5 microwave oven (Cem Corporation) using a two-stage procedure. First, the samples were washed in bidistilled water and complemented to 50 ml with bidistilled water. In the second stage, the samples were filtered using S&S blue tape filter papers. A blank solution was prepared in the same way. In order to avoid metal contamination, materials used in the dissolution of the samples were rinsed a couple of times with 1+9 (v/v) HNO_3 ultra-distilled water. Then, the materials were thoroughly washed and rinsed with ultra-distilled water and dried in the stove. The levels of mineral substances and heavy metals in the samples were measured using the ICP-OES (Thermo scientific ICAP 7400) device [11].

MICROBIOLOGICAL ANALYSIS

16 samples of Aho cheese taken from the cheese passages and neighbourhood markets of Trabzon's Sürmene and Araklı districts were delivered to the laboratory by preserving the cold chain and analysed. 25 g of cheese samples taken from the packaging material with sterile pens and spatulate aseptic conditions were transferred to sterile Stomacher pouches and homogenized in Stomacher for 1 minute with the addition of 225 ml annular water. Sterilised pipets were used to prepare the other dilution liquids and 1 ml was taken from the first tube and transferred to sterilised tubes which have 9 ml of dilution liquid. The incubation was implemented and generated colonies were counted for the total aerobic mesophilic bacteria counting in the cheese samples Plate Count Agar (PCA) and at 30±1°C for 48 hours (Özdemir & Sert, [12] for lactic acid bacteria counting (MRS Agar) at 30±1°C for 48 hours [13] for the group of coliform bacteria counting Violet Red Bile Agar (VRBA) and at 35°C for 48 hours Özdemir & Sert, [12] and counting of yeast and mould Potato Dextrose Agar (PDA) and at 25°C for 5-7 days [14], for the *Staphylococcus aureus* bacteria counting Baird Parker Agar was used and waited at 37°C for 24 hours [14].

STATISTICAL ANALYSIS

The results obtained from the cheese samples were analysed using the SPSS package program to determine the minimum, maximum, average values and standard deviations.

RESULTS AND DISCUSSIONS

The microbiological analyses of Aho cheese are given in Table 1. The mineral substances and heavy metal amounts of the cheese samples are given in Table 2. Considering to the analyses results Coliform and *Staphylococcus aureu*s bacteria counts are stated as < 10.

The average number of aerobic mesophilic bacteria number of examined Aho cheese samples are 6.72 log cfu g^1 in average. In another Aho cheese study, the average number of aerobic mesophilic bacteria was 6.82 log cfu g^{-1} in average [6]. These results show parallelism with the results of our study but lower than the value of the study on Minzi cheese, stated as 8.39 log cfu g^{-1}, which was conducted by Şanlıdere *et al.* [15].

In this study the Lactobacilli bacteria number is stated as 6.42 log cfu g^{-1} in average. The number of Lactobacilli bacteria (6.42 log cfu g^{-1} were lower than (7.39 log cfu g^{-1}) determined by Dinkçi *et al*. [16] in Kargı Tulum cheese and similar to the values determined by Kara & Akkaya, [17] in Afyon Tulum cheese 6.36 log cfu g^{-1} .

The average number of yeast and mould numbers of Aho cheese samples are 4.12 log cfu g^{-1} in average. Kılıç [6] was found the yeast and mould number as 6.27 log cfu g^{-1}. The value we stated in cheese samples is lower than Kılıç's value. It is indicated in the Turkish Food Codex Microbiological Criteria Notification [18] that the yeast and mould number in cheeses cannot be higher than $1x10^2$-$1x10^3$ cfu g^{-1}. The results in our study are higher than the value specified in the standard. High numbers of yeast and moulds; as a raw material milk used in the production of Aho cheese are not pasteurized, the required healthy conditions cannot be achieved at every stage of production and the hygienic conditions are not met in the cheeses offered for sale after maturing, usage of different types of fresh cheeses especially collected from bazaars are risky for human health.

Coliform and *Staphylococcus aureus* bacteria were not detected in Aho cheese samples. As seen from Table 2, in Aho cheese samples the calcium values are found lower than Mendil [19] studies conducted in Erzincan Tulum cheese (4416.00 µg g^{-1}) and higher than the study of Mendil [19] Kayseri Çömlek cheese (3473 µg g^{-1}) and Kırdar *et al*. [20] conducted in Burdur (2842.24 mg kg^{-1}) determined .

Sodium contents are found higher than the rates determined by [19] 3957 µg g^{-1} and by [21] 11.73 mg g^{-1} found. In association with saturation humidity loss in cheese especially in sodium rate an increase may be expected proportionally. Usually, excessive Na ratio is subject to salt usage in manufacturing process. Potassium content are found higher than the rates determined by Mendil [19] 3957 µg g^{-1} and found lower than the rates determined by Öksüztepe *et al*. [22] 1200.4 mg kg^{-1}. Average magnesium content in Aho cheese samples are considerably over the rates determined by Kırdar *et al*. [20] 49.62 mg kg^{-1} and Mendil [19] 101.00 µg g^{-1} and determined by [23] 27.42-60.51 mg $100g^{-1}$.

Average phosphorus rate are found higher than the rates in the studies incorporated by Kılıç *et al*. [24] 413.10 mg $100g^{-1}$ and found lower than the rates determined by Öksüztepe *et al*. [22] 5016.97 mg kg^{-1}. These dispersions in the mineral component levels in Aho cheeses; result from the type of animal, content of the fed milk, genetic features, lactation period, nutrition and seasonal factors, maturation level and different cheese production methodologies. It has been reported that metals such as nickel and manganese which are present in the composition of steel containers used in the heating and boiling stages of cheese production [25].

In Aho cheese iron content detected very low compare to the ratios confirmed by Mendil [19] (8.90 µg g^{-1}) and Kırdar *et al*. [20] 12.62 mg kg^{-1}) and Oksüztepe *et al*. [22] 10.46 mg kg^{-1}. As per Turkish Food Codex [26, 27] in milk and dairy products pertaining to the iron content even though no presentation was detected rates described for multiple food types varied in the range of 0.2-25 mg kg^{-1}. Accordingly, cheese samples are found to remain under consumable limits.

Zinc content remain too higher than the ratios determined by Mendil [19] collected in Kayseri Çömlek cheese 10.1 µg g^{-1} and by Yalçın & Tekinşen, [28] in Konya 15.96 mg kg^{-1} and detected the ratios set forth in the study employed by [21] 0.01 mg g^{-1}. In miscellaneous studies dispersions detected in zinc content of cheeses have been thought to be related to milk used in cheese production and devices and tools applied in production phase. That circumstance also pointed out by Yalçın &Tekinşen [28]. Furthermore, in milk 85% of zinc content has been reported to be associated with casein micelles and in acidic pH ratios converting into free form reported to be eliminated from clot Fresno *et al*. [29].

In the study, mean cupper rate in Aho cheese samples found to be lower than the rates provided by [20] 3.36 mg kg^{-1} and found higher than the rates determined by Mendil [19] 0.191 µg g^{-1} found. Copper level detected in cheeses forces us to consider the fact that it is related to the dissolution of cupper following reaction held by milk which is preserved in the components of the metal containers where milk is preserved or may have been increased owing to contamination of feeds which is used for feeding the animals with the agricultural pharmacological agents containing excessive magnitude cupper, in spite of this, subject to multiple phases during cheese production and despite longer time length for cheese preservation compare to milk giving lower quantities in cheese, also possible to attribute to the fact that cupper's dissolution and penetration into whey and during maturation and preservation may be related to penetration into brine. That circumstance also has been proved by Temurci & Güner, [30].

Mean lead volume in Aho cheeses diagnosed as 0.174 mg kg^{-1}. Lead levels set forth by Kurt *et al*. [31] as 0.21-0.77.08 µg kg^{-1} and by Mendil [19] 0.63 µg g^{-1} higher than the ratio found in the present study. Dispersions were concluded to be referred to distances of milk production farms, cheese operation businesses and sales points to industrial enterprises and highways.

Average cadmium levels in Aho cheese samples were calculated as 0.028 mg kg^{-1}. While associated with the cadmium level reported by Engin *et al.* [32] as 0.02-2.05 mg kg^{-1} found lower than the levels identified by [30] as 0.073 mg kg^{-1}. In the study calculated average mangan contents 0.313 mg kg^{-1} was found higher than manganese levels identified in the study executed by Işleten *et al*. [23] 0-0.063 mg $100g^{-1}$ reported to be lower than the ratio indicated by [31] corresponding to 1.12-1.83 µg kg^{-1} and by [19] 0.90 µg g^{-1}. Thus, many researchers emphasised that the cheese production techniques and environmental conditions may cause differences in mineral substance contents of produced cheeses. Preserved in the composition of the steel containers used in the heating and boiling phases of cheese production metals such as manganese and nickel have been reported to be potential to penetrate into the product [25].

Average contents of the mineral contents and heavy metals found in the milk composition were stated as calcium 1221-1259 mg L^{-1}, potassium 1424-1550 mg L^{-1}, sodium 310-523 mg L^{-1}, magnesium 89-228 mg L^{-1}, iron 0.70-2.40 mg L^{-1}, zinc 0.96-4.84 mg L^{-1}, copper 0.19-0.29 mg L^{-1}, manganese 0.02-0.038 mg L^{-1}, nickel 0-0.730 mg L^{-1}, lead 4-100 mg L^{-1} and arsenic 30-100 mg L^{-1} [1, 33]. Likewise mineral materials heavy metals may penetrate into milk definite proportions as well [33]. With that object in mind, heavy metal level in Aho cheese may vary in accordance with heavy metal contamination of milk which is used for cheese production. Although Turkish Food Codex has not maximum limits of cheese in the notification of Specification of Maximum Limits of Foodstuff Contaminants, the acceptable values of heavy metals in some foods were stated. In compliance with Turkish Food Codex Provision Specification of Maximum limits of Specific Contaminants in Food Components published in the year 2002 in the relevant Provision highest ratios are depicted as follows; for aluminium 2-15 mg kg^{-1}, for cadmium 0.01-1 mg kg^{-1}, for cupper 0.05-50 mg kg^{-1}, for lead 0.02-2 mg kg^{-1}, for chrome no value was indicated [27]. Whereas in the relevant directive issued in the year 2008 in some of the foods lead, cadmium, zinc and tin excluded maximum limits of other heavy metals were underestimated, ideal cadmium limits were reported to be as 0.05-1 mg kg^{-1}, lead limits were reported to be as 0.02-1.5 mg kg^{-1} [26, 27]. Even though allowed iron levels in milk and cheese are underestimated for plenty of foods identified values varied in the range of 0.2-25 mg kg^{-1}. In cheese samples heavy metal levels was found in the constraints stated out by Food Codex for some of the food materials.

Table –1: Microbiological counting results of analysed AHO cheese samples (log CFU g^{-1})

Microorganism	n	Mean±S.D	Min.	Max
Total aerobic mesophilic bacteria	16	6.72±0.11	6.38	7.93
Lactobacilli	16	6.42±0.05	5.95	6.94
yeast and mold	16	4.12±0.09	3.49	4.83
Coliform	16	<1	-	-
Staphylococcus aureus	16	<1	-	-

Table –2: Mineral content and heavy metal content in Aho Cheese (mg kg^{-1})

Matter	n	Mean±S.D	Min.	Max
Calcium (Ca)	16	3628.30±355.076	2213	5320
Potassium (K)	16	461.20±33.48	280.13	583
Magnesium (Mg)	16	163.42±19.96	111.25	212.65
Sodium(Na)	16	8463.75±658.97	4853.2	10470
Phosporus(P)	16	4187.05±327.60	2412.5	5465
Cadmium (Cd)	16	0.028±0.004	0.02	0.35
Copper (Cu)	16	1.350±0.13	0.90	2.02
Manganese(Mn)	16	0.31±0.17	-	0.90
Lead(Pb)	16	0.12±0.06	-	0.73
Zinc (Zn)	16	27.04±2.62	11.88	36.83
Iron(Fe)	16	5.46±1.29	-	22.50

CONCLUSION

The production of Aho cheese with its own distinctive taste and aroma needs to be made under modern and healthy conditions in small-scale or large enterprises, and the sensory, chemical and microbiological standards should be built in production. The fact that, milk as a raw material used in the production of Aho cheese are not pasteurized, the required healthy conditions cannot be achieved at every stage of production and the hygienic conditions are not met in the cheeses offered for sale after maturing, does not provide a certain standard in the properties of the cheese. It is thought that the use of fresh cheeses collected from very different sources in Aho cheese production and especially from the markets can create a very serious risk for human health. For this reason it can be suggested that the production of Aho cheese, especially the production of Golot and Minzi cheese used in Aho cheese making in modern hygienic establishments, the prevention of contact with the materials causing contamination, the training which is necessary for raising awareness of producers and the public, the production of raw milk with good microbiological quality in cheese production or pasteurized milk, the production of cheeses in hygienic conditions in modern enterprises, the prevention of uncontrolled sales of cheese outdoor, the prolonged use of cheeses according to the required ripening time and conditions. Also these heavy metals which may be responsible for some serious health problems corresponding to desired levels although is a blessing case; production of safe and efficient products sustainability must be intended

predominantly. With that object in mind, standardization of cheese production methodologies, at the production and preservation phase applied devices and tools in convenience with standards must be enabled to be kept far away from production locations and must be eliminated from chemical residues and from locations where environmental pollution is possible, in the preparation for livestock seeds in isolation from chemical content nutrients are recommended concerns attentively.

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