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Research article

Detection of adulteration of goat milk sold in the Turkish market by real-time Polymerase Chain reaction

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Abstract

Detection of milk adulteration is important to maintaining the quality of milk and milk products regarding pricing and consumer protection. Thus, in the current study, we investigated the adulteration of goat milk sold in the market and labeled it as "100% Goat Milk" with cows and sheep milk by real-Time PCR reaction (RT-PCR). This study is the first investigation on goat milk in Turkey and has particular importance as it was carried out using TaqMan probe RT-PCR. In total, 60 milk samples sold in the market and labeled as "100% Goat Milk" were collected from 12 different provinces of Turkey. The RT-PCR TaqMan probe detected the adulteration of goat milk with cow and sheep milk. In the study, 18 (30%) samples were found to be compatible with the label. It was determined that 42 (70%) samples did not comply with the "100% Goat Milk" statement on the label. It was determined that 6 (10%) of the samples contained only sheep milk, 18 (30%) contained only cow milk, another 6 (10%) contained goat and cow milk, 12 (20%) contained sheep and cow milk, and 36 (60%) did not contain any goat milk. The results of this study revealed high levels of adulteration in goat milk products. Therefore, careful continuous monitoring of these products' production and sales is necessary regarding deception of consumers and public health.

Keywords: Adulteration, Goat milk, Cow milk, Sheep milk, RT-PCR

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Introduction

Healthy, adequate, and balanced nutrition; refers to the intake of nutritional elements such as protein, fat, carbohydrates, vitamins, and minerals in amounts that meet the body's daily needs for body cells to function normally (Yangilar, 2013). The protection of human health, improvement, and maintaining a quality life is possible with adequate and balanced nutrition. Essential food groups for adequate and balanced nutrition are meat and meat products, milk and dairy products, cereal group products, vegetables, and fruits (Çom, 2008).

Milk and dairy products are considered among the basic food components for humans. Recently, with the discovery of its benefits to human health, interest in goat milk and its products has been increasing rapidly worldwide. With its unique taste, goat milk differs from other milk due to its low content of αs1-casein, small diameter of fat molecules, and low lactose content. Small fat molecules increase the digestibility and absorption of goat milk. It is also a substitute for people with cow's milk allergy and lactose intolerance (Haenlein, 2004; Yaralı et al., 2013; García et al., 2014; Altun and Sarıcı, 2017). Goat milk is preferred more than milk from other animal species due to its superior nutritional properties, being rich in proteins, vitamins, and minerals, and at the same time having fewer fat molecules (Golinelli et al., 2014). Therefore, goat milk is more expensive than the milk of other livestock species.

Goat milk has smaller fat globules than cow milk. The small fat globules in goat milk make it easier to digest. Racial differences are the most important factor affecting fat composition. However, the quality and quantity of feed, genetics, season, lactation stage, etc., affect the fat percentage in the milk. In terms of cholesterol, goat milk provides a specific distinction compared to cow's milk. Cow's milk usually contains about 14 to 17 mg of cholesterol per 100 g of milk, while in goat's milk, this ratio is generally recorded as 11 to

25 mg per 100 g of milk; however, goat milk consumption has a lower effect on cholesterol (Auld et al., 2000; Alférez et al., 2001; Malau-Aduli et al., 2001; Tomotake et al., 2006).

The enzymes of goat's milk are similar to those of cow's milk, although there are some specific differences. The alkaline phosphatase level in goat's milk is slightly lower than in cow's milk, but the enzyme is equally heat sensitive. It has therefore been shown to serve equally well as a pasteurization marker (Lorenzen et al., 2010). The peroxidase activity in the milk of both species is identical in all respects, the xanthine oxidase level being lower in the goat's milk. Higher activity levels are observed for both ribonuclease and lysozyme (Bruhn and Schutz, 1999). Sheep milk has higher dry matter, casein, and fat content than goat and cow milk. For this reason, it is mainly used for making yogurt, cheese, and butter (Cheng et al., 2006; Şebnem, 2019).

The fact that goat milk is produced in specific seasons and by small-scale farmers, especially in developing countries, makes goat milk and products more expensive than cow and sheep milk. As demand for these products has increased, the possibility of mixing different types of animal milk, especially cow's milk, which is cheaper and more abundant, has been raised. However, due to similarities in appearance and composition, it is difficult to distinguish goats' milk from cows' milk. This poses a significant risk, especially for people with cow's milk allergy, consuming goat's milk adulterated with cow's milk (Cheng et al., 2006; Dias et al., 2009; Golinelli et al., 2014).

Identifying species in milk and dairy products is important for public health, labeling regulations, and consumer rights (Di Pinto et al., 2004; López-Calleja et al., 2004). In many European countries, laws have stated that manufacturers of cheese and dairy products must specify the type of cheese milk they use in production (Calvo et al., 2002). The European Union food safety policy aims to protect customers from food pathogens and fraudulent species substitutions. The key priorities for these purposes are to ensure correct labeling of food and traceability of food and to fulfill the requirements of European Commission Regulation 178/2002, commissioning scientific studies if necessary (EC, 2002). In Turkey, the fact that the products in the food are not clearly stated in the label regulation constitutes adulteration, and legal action is taken against the identified companies (TFC, 2017).

The problem of adulteration is widespread for raw materials used in the commercial preparation of food. The "Farm to Fork" concept is being implemented to overcome this problem. This concept refers to the traceability and originality of a product's production and other stages from its raw material state until it is ready for consumption. The RT-PCR method is one of the most widely used molecular techniques in foods to determine the origin of species (Ghovvati et al., 2009; Rodríguez et al., 2004; Kesmen et al., 2007). In dairy products, such studies are more limited (Agrimonti et al., 2015; Di Pinto et al., 2017; Tuncay and Sancak, 2022). The definitions and information on food labels should be accurate for consumers to make informed choices (Herman, 2001). The study aimed to detect the presence of sheep and cow milk in milk labeled as "100% Goat Milk" by the RT-PCR method.

Materials and methods

Ethics committee

Approval was obtained from the Van Yuzuncu Yil University (Turkey) Animal Researches Local Ethic Committee with the letter No: 2022/12-07 dated 01.12.2022.

Milk samples and reference DNA

Sixty milk samples with different production dates and batch numbers labeled "100% Goat Milk" were collected from markets. Twelve samples originated in Van province, and 48 samples were from other various provinces of Turkey through the virtual market, i.e., Ankara (n=8), Antalya (n=8), Izmir (n=7), Canakkale (n=5), Balikesir (n=5), Hatay (n=4), Konya (n=3), Hakkari (n=2), Erzurum (n=2), Kırklareli (n=2), and Siirt (n=2). The pure reference goat, sheep, and cow DNA used in the study were obtained from DIAGEN (Turkey).

DNA extraction

DNA Purification kit (GeneMATRIX FOOD-EXTRACT DNA Purification Kit, Poland) was used to extract DNA from the milk according to the manufacturer's recommendation. A 50 ml milk sample was taken in a falcon tube and centrifuged at 5000 ×g for 15 min. After centrifugation, 400 μL of lysis buffer was added to the pellet at the bottom, vortexed, transferred to a 1.5 ml Eppendorf tube, and then 25 μL of Proteinase K was added. The tubes to which proteinase K was added were incubated at 60℃ for 45 min and then centrifuged at 11000 ×g for 1 min. 400 μL supernatant was transferred to another tube, and 200 μL binding buffer was added. After vortexing, it was transferred to a spin column and centrifuged at 11000 ×g for 1 min. The collecting tube was changed, 650 μL of wash buffer 1 was added, then centrifuged at 11000 ×g for 1 min, and the collection tube was changed, and wash buffer 2 was added. After centrifugation at 11000 ×g for 5 min, it was transferred to Eppendorf, and elution buffer heated at 60℃ was added in a volume of 50 μL milk and centrifuged. The obtained DNAs were stored at -20℃ until the RT-PCR process.

RT-PCR reaction

RT-PCR TaqMan Probe commercial kits (DIAGEN, Turkey) that detects the NADH dehydrogenase (ND5) for cattle & sheep and the rRNA-ribosomal RNA for goat. The kit's sensitivity rate (0.1%) was determined in a previous study (Tuncay and Sancak, 2022). The RT-PCR TaqMan probe method in the kit qualitatively detects the species-specific (goat, sheep, cow) region in mitochondrial DNA and distinguishes at the species level. PCR mixtures consisting of 10 μL mix A, 5 μL mix B, and 5 μL DNA of each species were prepared separately according to the manufacturer's (DIAGEN,

Turkey) recommendations. The PCR mixture was subjected to pre-denaturation at 95℃ for 5 min, and a total of 35 cycles of 95℃ for 10 s denaturation, 59℃ for 30 s annealing, 72℃ for 5 s extension, and 25℃ for 1 min final extension protocol was applied during the amplification phase.

Statistical analysis

Statistical analysis of the findings obtained in the study was carried out using the SPSS 13.0 package program (SPSS, 2006).

Results and discussion

The results of 60 RT-PCR analyses of milk samples are given in Table 1. In the study, 18 (30%) samples were found to comply with the label. It was determined that 36 of the samples (60%) did not contain goat milk in any form.

Although milk is an easily accessible food, milk from some animal species can be difficult to access. Goats' milk is very nutritious. It is sold as a nutraceutical food for consumers and costs more than cow's milk. In particular, the use of species-specific milk can result in an economic burden for producers and a lower quality product and health hazard for consumers. Due to their similarity in appearance and composition, it is not easy to distinguish goat's milk from cow's milk. An example is a product sold with the label of pure goat's milk on the market but actually contains cow's milk and may cause serious harm if consumed by a person with a cow's milk allergy. For these reasons, species determination in milk is of considerable importance (Bottero et al., 2003; Cheng et al., 2006; Pesic et al., 2011).

Optimized, sensitive (0.1%), specific, and reproducible RT-PCR assay kits were used in our study to distinguish between cow and sheep milk in goat milk and dairy products (Tuncay and Sancak, 2022). It was determined that 42 (70%) of the 60 goat milk samples investigated did not comply with the "100% Goat Milk" statement on the label. It was determined that 6 (10%) of the samples contained only sheep milk, 18 (30%) contained only cow milk, another 6 (10%) contained goat and cow milk, and 12 (20%) contained sheep and cow milk. It was observed that 36 samples (60%) did not contain any goat's milk. There are various studies on species determination in goat milk (Khanzadi et al., 2014; Di Pinto et al., 2017; Tsakali et al., 2019).

According to studies in European countries, a paper on dairy products in Italy determined that 5 out of 19 cheese samples were unsuitable for the label (Bottero et al., 2003). In 2005 and 2009, cow DNA was searched in a total of 48 Ultra High-Temperature (UHT) goat milk samples in Poland. In 2005, they reported collecting 26 UHT goat milk samples and detecting cow DNA in all of them. They estimated the addition of cow's milk to be around 1% in nine samples, between 2-5% in 10 samples, and about 5-10% in seven samples. In 2009, they reported that they detected cow DNA in 11 of 22 UHT goat milk samples, nine of these samples contained less than 1%, one sample between 5-10%, and one contained between 10-20% cow milk (Dabrowska et al., 2010). It was determined by PCR that 12 out of 96 sheep, goat, buffalo, and cow milk and dairy products, i.e., one buffalo butter, two buffalo cheese, one cow cottage cheese, 43 cow cheese, one cow cream cheese, three cow UHT milk, two cow milk powder, two cow+goat cheese, seven cow+sheep cheese, six cow+sheep+goat cheese, five goat cheese, one goat UHT milk, one goat yogurt, 17 sheep cheese, two sheep cottage cheese, one fresh sheep milk, one sheep yogurt, were not suitable for the label in Portugal (Gonçalves et al., 2012).

Forty milk and dairy products, including 15 goat milk products, 15 goat cheese, and 10 goat milk yogurt in Greece in 2019, were examined, and 90% (36 pieces) of the products were mixed with cow's milk. They stated that all 15 goat milk products and 10 yogurts (100%) were mixed with cow's milk, and 11 (73%) of 15 goat kinds of cheese were mixed with cow's milk (Tsakali et al., 2019). It was shared that 2 of 6 Halloumi cheese samples, 1 of 4 yogurt samples, and 5 samples in total were found non-compliance with the label in Cyprus (Kastanos et al., 2022). Forty cheese and yogurt samples from local markets in Greece in 2020 were analyzed. It was determined that a total of 33 samples (15 cheese and 18 yogurt samples) were not suitable for the label (Tsirigoti et al., 2020).

The situation in some countries in Asia is similar; for example, eighty goat milk powder samples and 24 goat milk tablets were investigated by PCR in Taiwan. It was shared that cow's milk or cow's milk powder was detected in 25% of the goat's milk powder samples and 50% of the goat's milk tablets (Cheng et al., 2006). The presence of goat and cow milk in sheep milk in Iran was analyzed by multiplex PCR method, and it was determined that only 21 of 105 sheep milk and products were labeled, and 84 were off-label (Khanzadi et al., 2014). Fifty samples were analyzed from buffalo milk, yogurt, and cheese sold in Iran in 2016. As a result, they determined that 15 (30%) of 50 buffalo milk, 13 (26%) of 50 buffalo yogurt, and 17 (34%) of 50 buffalo cheese were suitable with the label. They found that 35 (70%), 26 (52%), and 32 (64%) of the milk, cheese, and yogurt samples, respectively, were a mixture of buffalo and cow's milk, while 5 (10%) of the yogurts and 7 (14%) of the cheeses were made only from cow's milk (Zarei et al., 2016). In some other countries, the report made on 160 fresh goat milk samples in Brazil in 2012 determined that 41.2% of them were bovine milk (Rodrigues et al., 2012). They reported that in Egypt in 2018, about 90% of 50 raw buffalo milk samples were mixed with cow's milk, and only 10% were unmixed. As a result, they concluded that the product sold as raw buffalo milk in Assiut city was fraudulent and could pose public health hazards (Ewida and El-Magiud, 2018).

In our country in 2016, it was determined that 13% of 100 Afyon creams were obtained from buffalo milk, 59% from cow's milk, and 28% from a mixture of buffalo and cow's milk (Kara and Demirel, 2016). In the literature research, there are studies similar to our

Table 1: Real-time PCR analysis results of milk samples labeled "100% Goat Milk".

Province	No. of samples	Pure goat milk No. (%)	Number of adulterated goat milk samples (%)				
			With cow milk No. (%)	With sheep milk No. (%)	Pure cow milk No. (%)	Pure sheep milk No. (%)	Only sheep and cow milk No. (%)
Van	12	4 (33.33)	1 (8.33)	-	5 (41.67)	1 (8.33)	1 (8.33)
Hakkari	2	1 (50)	-	-	-	-	1 (50)
Erzurum	2	-	-	-	2 (100)	-	-
Canakkale	5	1 (20)	1 (20)	- 1 (20)	1 (20)	1 (20)	
Konya	3	1 (33.33)	-	-	1 (33.33)	-	1 (33.33)
Ankara	8	3 (37.5)	1 (12.5)	-	3 (37.5)	1 (12.5)	-
Antalya	8	2 (25)	-	-	-	1 (12.5)	5 (62.5)
Kirklareli	2	1 (50)	-	-	1 (50)	-	-
Izmir	7	2 (28.57)	1 (14.29)	-	2 (28.57)	1 (14.29)	1 (14.29)
Balikesir	5	2 (40)	1 (20)	-	1 (20)	-	1 (20)
Siirt	2	-	-	-	1 (50)	-	1 (50)
Hatay	4	1 (25)	1 (25)	-	1 (25)	1 (25)	-
TOTAL	60	18 (30)	6 (10)	-	18 (30)	6 (10)	12 (20)

study. It was determined that 70% of the samples used in our study were incompatible with the label. While the rate determined in our study may be higher than the rate determined by some literature (Bottero et al., 2003; Cheng et al., 2006; Rodrigues et al., 2012; Tsirigoti et al., 2020; Kastanos et al., 2022), while the rate is lower than others (Dabrowska et al., 2010; Khanzadi et al., 2014; Kara and Demirel, 2016; Ewida and El-Magiud, 2018; Tsakali et al., 2019). It was found to be compatible with the ratio determined by Zarei et al. (2016). It is thought that these differences between the studies are due to the differences in the samples collected from the market, the analysis method, and the sensitivity of this method.

Conclusions

This study revealed high levels of adulteration in goat milk products. Therefore, the production and sales of goat milk and products must be carefully and continuously monitored. Detailed monitoring requires a fast and accurate diagnostic technique. The RT-PCR method was preferred in this study because it can detect the presence of sheep and cow milk in sheep milk and cheeses, even in low quantities, and it is a convenient and simple method. Consequently, it may be suggested that regulatory agencies use the RT-PCR method. It is also essential that regulatory agencies increase their supervision to prevent unfair competition and ensure consumers that product labels are accurate. Meanwhile, a stronger approach is to avoid situations such as these that could cause serious health problems for consumers allergic to cow's milk. To our knowledge, the present study is the first to detect goat milk adulteration in Turkey. Additionally, the study has particular significance due to its use of the TaqMan probe RT-PCR analysis method for goat milk analysis.

Article Information

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Conflict of Interest. The authors have no conflict of interest to declare.

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