Investigation of milk origin in sheep and goat cheese with Real-Time PCR

Research Article

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ABSTRACT

Milk and dairy products are very important in life, because they contain important nutrients for human health. Recently, some producers have used cow's milk instead of goat and sheep's milk for producing goat and sheep cheese and sell these cheeses as sheep and goat cheese. In this study, it was aimed to determine the milk type of white cheese and tulum cheese, which are sold under the names of sheep cheese and goat cheese in the markets and supermarkets of Afyonkarahisar and Antalya. In this study, the Real-Time PCR analysis method was utilised to detect fraud in products. For the analysis, 60 goat cheese samples and 60 sheep cheese samples in total were collected from the aforementioned provinces. As a result of the analysis, among all goat cheese samples which were obtained from both provinces, only 20% were produced from goat milk, 38.33% were produced from goat-cow's milk mixtures, and 41.67% were produced using cow's milk only. In the sheep cheese samples from both provinces, only 18.33% were produced from sheep's milk, 50.00% were produced from sheep's-cow's milk mixtures, and 31.67% were produced from cow's milk only. Consequently, regular inspections and controls are required to detect counterfeit cheeses and we recommend that consumers be made aware of these cheats.

Keywords: Cheese, adulteration, imitation, Real-Time PCR

INTRODUCTION

Cheese is an important dairy product which has high nutritional value, is a concentrated form of milk and contains more nutrients than milk per volume (Walther et al., 2008). It is a 99% digestible food for all age groups (Kosikowski, 1982). There are more than 4000 varieties of cheese all around the world, and Turkey produces around 100 varieties of it (Steele and Unlu, 1992; Coskun, 2005). The highest percentage of cheese is produced by using cow's milk, while cheese can also be made using goat and sheep's milk. Sheep's and goat milk is mostly used in local and special cheese production (Guney and Kaymakci, 1997).

Quality and safe food consumption is one of the most fundamental rights and freedoms for human safety. Nowadays, with the development of technology, adulterations applied on foods are increasing, and it is very difficult to detect counterfeit products. Food authenticity and safety problems like imitation and adulteration in the agriculture and food industry are a problem not only in Turkey but also in other countries (Ertas and Topal, 2009). Reasons for practicing imitation and adulteration activities in the food sector may include the purpose of functional food production and similar purposes, while these processes may also reduce the health risks and increase the shelf life of food products (Ertas and Topal, 2009).

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Recently, food safety, quality and composition have become an important issue for consumers, and for the consumers of today, the authenticity of food has an important place. Therefore, especially in animal products, identification of animal species has gained increasing importance (Dalmasso et al., 2012). Consumers are expected to believe the vendors and what is written on the labels because the authenticity of some products is not directly observable (Mayer et al., 2012). In this study, it was aimed to determine the origins of milk used in cheese sold as goat-sheep cheese by Real-Time PCR.

MATERIAL and METHOD

Cheese Samples

In total, 120 goat cheese and sheep cheese samples were collected from markets and supermarkets in the Afyonkarahisar and Antalya provinces of Turkey between November 2018 and March 2019. Each cheese sample was bought at quantities of about 250-500 g, and 60 goat cheese samples (32 white cheeses / 28 tulum cheeses) and 60 sheep cheese samples (26 white cheeses / 34 tulum cheeses) were gathered. Until the analysis, the cheese samples were stored at -18 ± 2 °C.

DNA Isolation

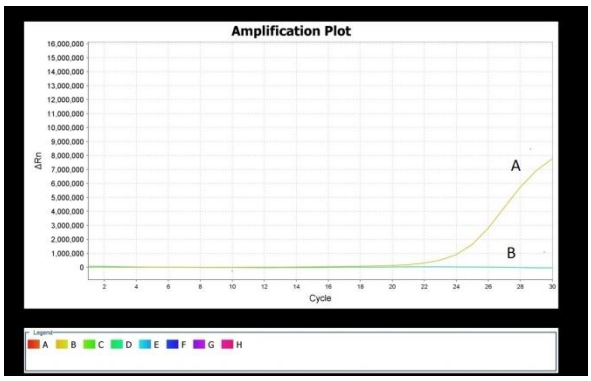
DNA isolation was performed in the cheese samples in accordance with the instructions of the manufacturer of the kit that was used (Genomic DNA Isolation kit, SNP ure Genomic DNA Extraction Kit 1806/001). The method which was applied in the analysis was the spin column procedure. 100 mg of the cheese sample, 300 µl of solution B3 and 25 µl of Proteinase K were added together for the analysis.

The mixture was mixed and incubated at 70 °C for 30 minutes. After the incubation, the product was centrifuged at 13,000 rpm for 2 minutes. At the isolation stage, approximately 400 µl was taken from the middle phase of the 3 phases that formed and transferred to a 1.5-ml tube. 250 µl of 95% alcohol was then added to the tube and mixed gently, and the contents of the tube were transferred to a new spin column. The samples were centrifuged at 11,000 rpm for 1 min. After centrifugation, the Eppendorf tube was replaced, and 500 µl of Solution WB was added to the column. This process was repeated twice, the columns were then placed in clean new tubes, and 100 µl of the elution solution (preheated) was added to these columns and centrifuged. As a result, DNA samples were obtained and kept at -20 °C.

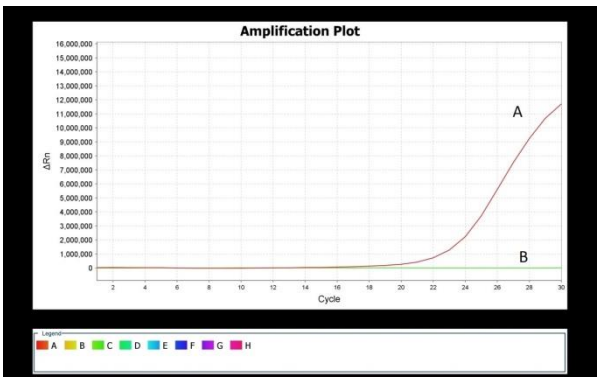
Real-Time PCR Process

Real-Time PCR was performed in the extracted DNA sample in accordance with the instructions of the manufacturer of the kit that was used (SNP Detection Real-Time PCR Kits goat milk-401R-10-01, sheep milk-402R-10-01, cow milk-403R-10-01 in Turkey). Twenty µl master mix and 5 µl (nearly 10-100 ng) of the extracted of DNA sample were added to the strips and gently mixed separately for each sample. The analysis of the prepared mixture was conducted with the program set (95°C - 5 min. Taq Activation; 95 °C - 15 sec. / 60 °C - 1min. = 30 cycles) in the Real-Time PCR (Applied Via 7, Thermo Fisher Scientific).

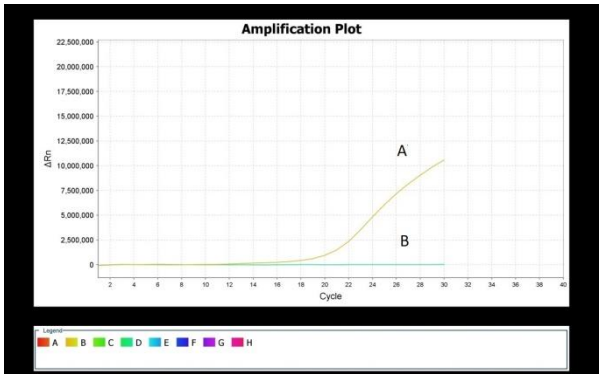
The results were evaluated by analysing the internal control peaks with the VIC-TAMRA dye and sample peaks with the FAM-TAMRA dye. Positive and negative controls were used for each species (Sheep-Goat-Cow) in the analyses, and these control peak images and internal control peak images are shown in Figure 1.



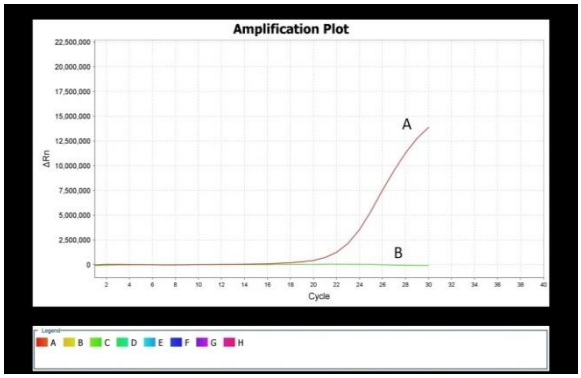
The sorts of goat positive (A) and negative (B) control peaks used goat species analysis



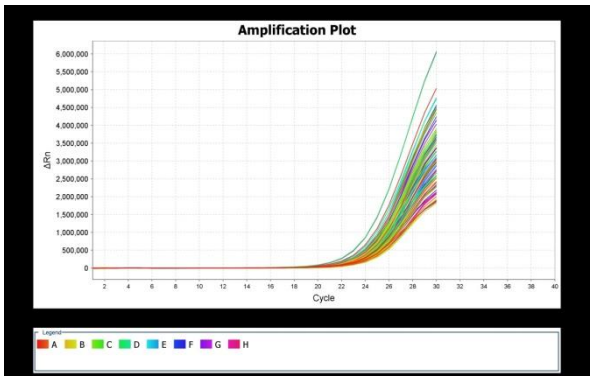
The sorts of cow positive (A) and negative (B) control peaks used goat species analysis



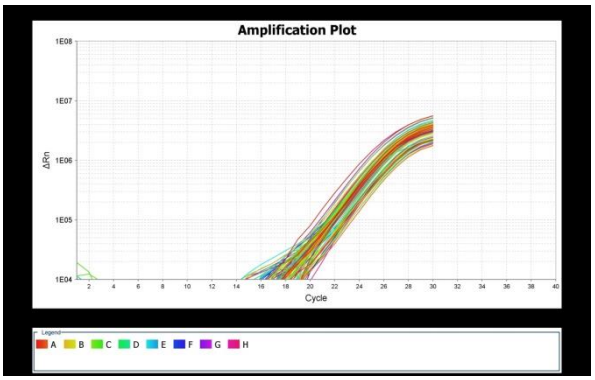
The sorts of sheep positive (A) and negative (B) control peaks used sheep species analysis



The sorts of cow positive (A) and negative (B) control peaks used sheep species analysis



İntegral control peaks in goat cheese samples



İntegral control peaks in sheep cheese samples

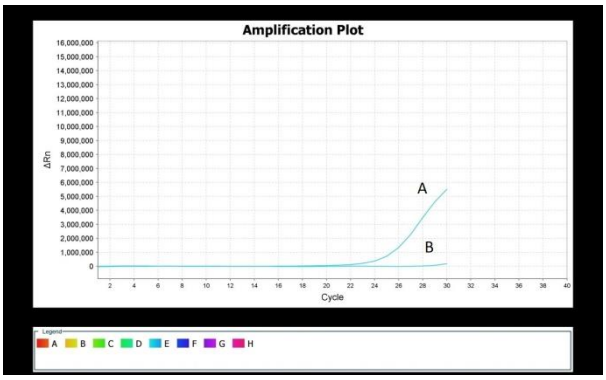
Figure 1. Positive, negative control and internal control peaks of species

RESULTS

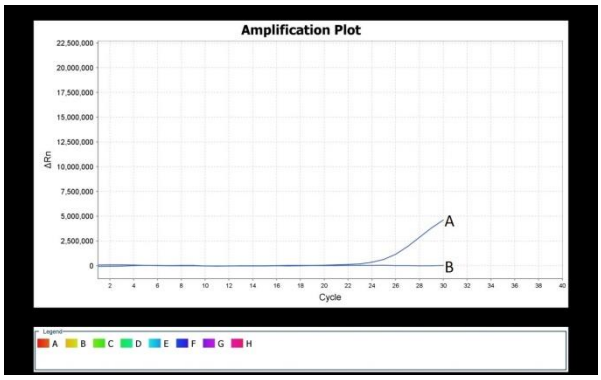
According to the results of the analysis, goat milk was identified in 12 of the 60 goat cheese samples, cow's milk and goat milk mixtures were identified in 23 samples, and only cow's milk was identified in 25 samples. For the sheep cheese samples, sheep's milk was identified in 11 of the 60 sheep cheese samples, sheep's milk and cow's milk mixtures were identified in 30 cheese samples, and only cow's milk was identified in 19 cheese samples. The details of the results of the analysis conducted on the goat and sheep cheese samples are shown in Table 1. The Real-Time PCR image result of the analysed goat and sheep cheese samples is presented in Figure 2.

Table 1. The analysis results of goat and sheep cheeses samples

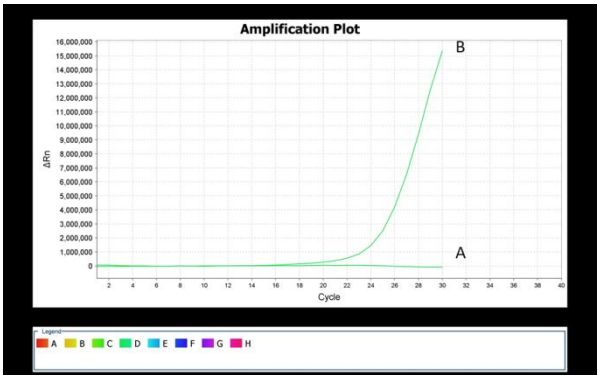
Samples		n	Original (Goat-Sheep) n (%)	Mix n (%)	Cow n (%)
Goat Cheeses	White Cheese	32	10 (31.25)	7 (21.88)	15 (46.88)
	Tulum Cheese	28	2 (7.14)	16 (57.14)	10 (35.71)
	Goat Cheese Total	**60**	**12 (20.00)**	**23 (38.33)**	**25 (41.67)**
Sheep Cheeses	White Cheese	26	4 (15.38)	12 (46.15)	10 (38.46)
	Tulum Cheese	34	7 (20.29)	18 (52.94)	9 (26.47)
	Sheep Cheese Total	**60**	**11 (18.33)**	**30 (50.00)**	**19 (31.67)**
TOTAL		**120**	**23 (19.17)**	**53 (44.57)**	**44 (36.67)**



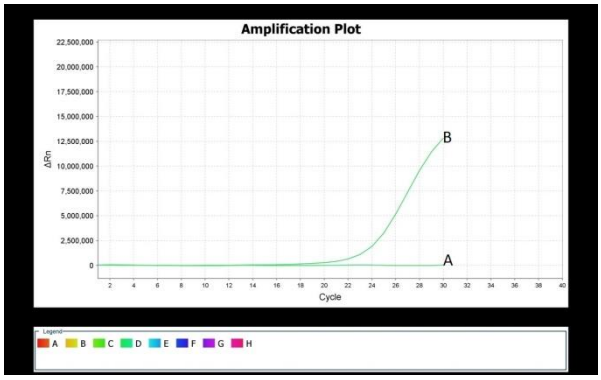
Goat type positive (A) and cow type negative (B) in the goat cheese sample



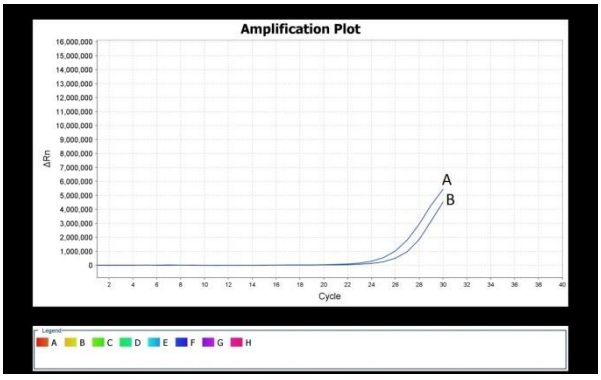
Sheep type positive (A) and cow type negative (B) in sheep cheese sample



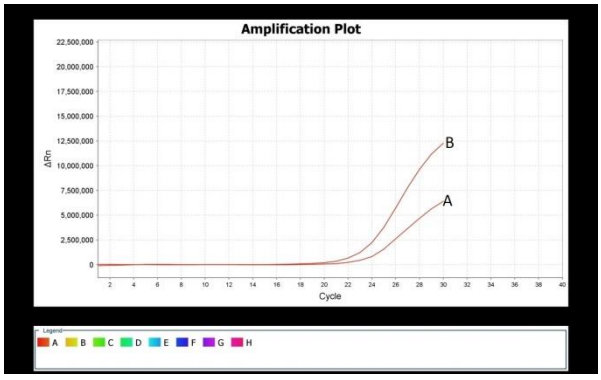
Goat type negative (A) and cow type positive (B) in the goat cheese sample



Sheep type negative (A) and cow type positive (B) in sheep cheese sample



Goat (A) and cow (B) type positive in the goat cheese sample



Sheep (A) and cow (B) type positive in sheep cheese sample

Figure 2. The result of goat and sheep cheese samples in Real Time PCR

DISCUSSION

There are many similar studies about determining the presence of various milk types in dairy products using various methods. In their study conducted in the Czech Republic, Maskova et al. (2006) determined that only 3 of 17 cheese samples sold as goat cheeses were actually goat cheeses, and only 1 of 7 cheese samples sold as sheep cheeses were actually sheep cheeses by using the PCR method. Kara et al. (2016) reported in the province of Afyonkarahisar in Turkey that, based on their Real-Time PCR results, among 100 cream samples sold as buffalo cream, only 28% were produced from buffalo-cattle milk, and 59% were produced by from bovine milk. In their study conducted in Italy, Bottero et al. (2003) used the multiplex PCR method and found cow's milk in 21% of 19 dairy products sold as goat dairy products.

Colak et al. (2005) analysed 100 cheese samples sold as sheep cheeses, and 48% of the samples were found to contain mixtures of cow's milk and sheep's milk, and 52% of the samples contained sheep's milk, according to the researchers' immunochromatographic test conducted in Istanbul.

Darwish et al. (2009) determined by using the PCR method that 47.62% of 21 samples of milk sold as water buffalo milk contained water buffalo milk, 14.29% contained cow's milk, and 38% contained water buffalo-bovine milk. These milk samples were bought in local Egyptian markets. Khanzadi et al. (2013) detected the undeclared presence of cow's milk in 31.5% (33) and goat milk in 65% (68) of 105 dairy product samples sold as sheep's milk products using the PCR method. These 105 sheep milk and dairy products were bought from markets in the Mashhad city of Iran. Zachar et al. (2011) analysed 30 sheep cheese samples in Slovakia and found that 12 samples contained mixtures including cow's milk.

According to Di Pinto et al. (2004), among 30 mozzarella cheese samples sold as buffalo mozzarella, cow milk was detected in 22 (%73.3) samples. The cheese samples were analysed with the PCR method and had been collected from different regional producers in Southern Italy.

Cow's milk is used as a adulteration method in other dairy products as it is more accessible and affordable. Moreover, if different dairy products from different milks are produced in the same production machine, different mixtures of milk types may be detected in analyses on these dairy products. In the literature, different studies have reported the presence of milk from animals other than what was specified by the vendor or the product label in different dairy products that were claimed to be authentic (Ertas and Topal, 2009). Researchers have developed analytical methods especially for detecting the origins of milk and dairy products (Mayer et al., 2012).

In general, immunological, electrophoretic, chromatographic, ELISA and PCR methods are used to determine the origin of meat and dairy products (Zachar et al., 2011). The most common and sensitive method, PCR was used to determine the origin of dairy products in this study. In this study, cow's milk was found in dairy products sold as goat and sheep cheese, and this showed that considerable numbers of these cheeses were imitated and adulterated.

CONCLUSION

Cow's milk DNA was detected in 45% of the 120 cheese samples that were investigated by PCR test in this study. At the same time, as a result of the analysis, mixtures of goat and cow DNA were identified in 38.33% (23) of the goat cheese samples, and mixtures of sheep and cow DNA were identified in 50% (30) of the sheep cheese samples. Accordingly, it was concluded

that cow milk was used in the production of some goat and sheep cheese products. Problems arising in the supply of goat-sheep's milk should be eliminated at first. Especially for the production of authentic and traditional sheep-goat cheese, cow's milk should not be used. In the production processes of dairy products which include different animal sources, the tools, equipment and processes must be separated. If goat, sheep's and cow's milk is used in cheese production, this information should be included on the product label, and consumers should be informed by the vendor about unlabelled products. Inspections and controls on food products should be carried out regularly, and sensitive analyses that provide results in a short time are recommended determining the origins of products.

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This study is summarized from the Master's Thesis (2019/050) with the same name.

This study was presented as a summary report at Turkey 13. Food Congress; (21-23 October 2020, Çanakkale).

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

Conflict of interest: The author declares no potential conflict of interest.

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