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DNA-based detection of milk adulteration in dairy products from the Greek market

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ABSTRACT

Milk adulteration, particularly the undeclared addition of cow's milk to higher-value sheep and goat dairy products, poses significant health, economic, and regulatory challenges. Given the central role of small-ruminant dairy production in Greece—one of Europe's largest producers of sheep and goat milk—monitoring authenticity is critical for protecting both consumers and the national agri-food economy. In response to growing global demand and increasing incidents of fraud, this study conducted the first comprehensive survey of milk authenticity in the Greek market using an optimized and validated TD-PCR protocol. Three DNA extraction kits were evaluated using spiked cheese and yogurt samples, with the automated Maxwell RSC system (Promega) showing the highest recovery efficiency and minimal contamination risk. The TD-PCR method achieved high sensitivity, with a limit of detection as low as 1 % cow DNA in yogurt and up to 5 % in cheese matrices. Analysis of 74 commercial dairy products revealed widespread adulteration, particularly in goat yogurts (40 %) and cheeses (40 %), as well as in three kefirs and several mixed and whey-based cheeses. Notably, only 7 out of 17 feta samples contained detectable goat DNA, suggesting possible mislabeling. Overall, the developed approach provides a robust and scalable molecular tool for routine authenticity testing, supporting regulatory enforcement, fair trade, and consumer confidence in Greek dairy products.

1. Introduction

Demand for milk and dairy products has risen due to their nutritional benefits, according to a recent review by the Food and Agriculture Organization (FAO). Key drivers of this demand include rising incomes, population growth, urbanization, and evolving dietary preferences. Nevertheless, the increasing production costs and the impacts of climate change have contributed to an anticipated decline in dairy production across Europe (FAO, 2022). Studies indicate that milk is the second most commonly adulterated food product, surpassed only by olive oil (Ionescu et al., 2023). The most prevalent form of adulteration involves mixing cheaper cow's milk with more expensive and higher-quality milks from buffalo, sheep and goat (Giglioti et al., 2022).

Milk adulteration has significant economic consequences, including unfair competition and mislabeling, while also poses health risks to consumers particularly those with allergies to specific milk proteins (Ortega et al., 2016). Furthermore, it undermines the integrity and reputation of traditional cheeses with Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI), as defined by Commission Regulation (EC) 1151/2012, which specifies the required animal species and/or quantities (Kritikou et al., 2022; Tsakali et al., 2019).

Dairy production from small ruminants constitutes a key pillar of the Greek agri-food sector and rural economy. Greece ranks among the top producers of sheep and goat milk in the European Union, contributing approximately 20 % and 25 % of the total EU output, respectively (Eurostat, 2024). Feta cheese, protected under PDO status, accounts for more than 70 % of the country's total cheese production, with an estimated annual market value exceeding €400 million and substantial export activity. The high economic value and strong international demand for authentic Greek sheep and goat dairy products make them particularly vulnerable to adulteration with cheaper cow's milk. Consequently, ensuring product authenticity is essential not only for consumer protection but also for safeguarding the integrity, reputation,

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and sustainability of one of Greece's most economically significant food sectors (Katsouri et al., 2020).

A variety of analytical methods have been developed to verify the authenticity of milk and dairy products, largely driven by regulatory demands. Traditional techniques—such as electrophoresis, immunoassays, chromatography, and mass spectrometry—have been widely used. Notably, isoelectric focusing is adopted by the European Community (EC Regulation 1081/96) as the reference method for detecting bovine milk in non-bovine products. While immunological methods (e.g., ELISA) and advanced mass spectrometric techniques (e.g., LC-ESI-MS/MS, MALDI-TOF MS/MS) offer improved sensitivity, they are often time-consuming, poorly suited to complex food matrices and may lack the resolution needed to distinguish between closely related animal species (Azad & Ahmed, 2016; Dalmasso et al., 2011; De Pascale et al., 2025; Li et al., 2023; Lopez-Calleja et al., 2004).

In this context, DNA-based molecular techniques have become key tools for species identification in dairy products due to DNA's high stability during food processing, including thermal treatments (Liao et al., 2017). Polymerase chain reaction (PCR) methods, in particular, are widely used for dairy species authentication. Classical PCR with gel electrophoresis enables species-specific detection but lacks quantitative capability (Bottero et al., 2003; Deng et al., 2020; Tsirigoti et al., 2020). This limitation is addressed by real-time PCR (qPCR), which uses SYBR Green or TaqMan probes for multiplex detection and quantification (Agrimonti et al., 2015; Guo et al., 2018; Hai et al., 2020). High-resolution melting (HRM) analysis improves species discrimination, while droplet digital PCR (ddPCR) offers precise absolute quantification (Agrimonti et al., 2015; Cutarelli et al., 2021).

Recently, our group developed and analytically validated a qualitative touchdown (TD) PCR method for detecting the undeclared addition of cow's milk in goat and sheep milk (Kourkouli et al., 2024). The method relies on analyzing the peak areas of melting curves following the modification of bovine-specific primers (Kourkouli et al., 2024). This technique offers distinct advantages in overcoming the challenges posed by high annealing temperatures required for certain primer-template combinations. Additionally, it is particularly effective for amplifying difficult templates, such as those with extensive secondary structures or high GC content (Korbie & Mattick, 2008).

The objective of this study was twofold: first, to expand the applicability of our novel TD-PCR assay to a range of dairy products—including cheese, yogurt, and kefir—and second, to conduct a comprehensive survey of the Greek dairy market to evaluate compliance with current legislation and labeling requirements. Accurate detection of milk adulteration safeguards consumer health, ensures food authenticity, and mitigates economic fraud, while extending the TD-PCR assay to ferment and complex dairy matrices enhances its value as a robust tool in food diagnostics. Moreover, the market survey offers valuable insights into the real-world implementation of food labeling legislation within the EU framework. Collectively, these efforts contribute to the development of more transparent, traceable, and trustworthy food systems.

2. Materials and methods

2.1. Synthetic controls and sample collection

Synthetic DNA oligonucleotides corresponding to mitochondrial gene of cytochrome *c* oxidase subunit I (*cox1* gene) of goat and sheep assay were employed for the development and validation of each assay (Table 1). In addition, a synthetic DNA oligonucleotide was used as an external control to assess DNA loss during the extraction protocol (% Recovery) (Table 1). Finally, a total of 74 commercial dairy products of cow, sheep and goat origin were collected during the winter months from Greek supermarkets and analyzed to verify their animal origin (Table 2). In more detail, this included 15 different commercial brands of goat yoghurt, 7 brands of sheep yoghurts and 3 brands of goat kefir.

Table 1
List of synthetic oligonucleotides controls.

Species	Sequence (5'→3')
Cow (*Bos taurus*)	CGA AGT CTA TAT TTT AAT CTT ACC TGG GTT TGG AAT AAT CTC TCA TAT CGT GAC CTA CTA CTC AGG AAA AAA AGA ACC ATT CGG ATA TAT GGG AAT AGT TTG GGC TAT AAT GTC AAT CGG ATT TCT AGG TTT CAT CGT ATG AGC CCA CCA
Goat(*Capra hircus*)	GAC ACC CTG AAG TAT ATA TTC TTA TTT TAC CTG GAT TTG GAA TAA TCT CTC ACA TCG TAA CCT ACT ACT CAG GGA AAA AAG AAC CAT TCG GGT ACA TAG GAA TAG TGT GAG CCA TAA TAT CAA TCG GGT TTC TAG GAT TTA TTG TAT GAG CCC ACC ATA T
Sheep (*Ovis aries*)	ATA TTC TTA TTT TAC CTG GGT TTG GGA TAA TCT CCC ATA TTG TGA CCT ACT ATT CAG GAA AAA AAG AAC CAT TCG GAT ATA TAG GAA TAG TAT GAG CCA TAA TAT CAA TTG GGT TCC TAG GAT TCA TTG TAT GAG
External Control (EC)	GTT GAC CTT AAA AGT TTC AAA TCT AGG TTA TGT TAG CAA CTC TTC AAG TTC CCT GTC TCT TGG GGG GAG GCA TTG GCT GAG GCA TGT CAT AGC AGG TGA GGT ACA TGG CTG TCC TTG CTC ACC ATC CTC CTG AGA CTT TGT TCC AGC CCT ACC TGC CTC AGA GGC TCC GGC TTC TCT TAG AGA CCA AGA G

Regarding the cheeses, samples of feta cheese and sheep-goat cheese from 16 to 11 different geographical origins were purchased, respectively. In addition, 7 brands of goat cheese were analyzed. Specifically, DNA was extracted from 42 cheese samples, 29 yoghurts samples and 3 kefir products.

2.2. DNA extraction

For the evaluation of the efficacy and analytical performance, three different commercially available DNA isolation protocols were evaluated, namely a) the DNEasy Blood and Tissue (Qiagen, Hilden, Germany), b) the Food DNA Isolation kit (NorgenBiotek, Thorold, ON, Canada) and finally c) the automated Maxwell RSC (Rapid Sample Concentrator) System (Promega Corporation, Madison, WI, USA). For this purpose, DNA was isolated from 3 representative samples - 1 sheep and goat, 1 cow and 1 mixture of both – per dairy product category (brine cheese, yellow cheese and yoghurt). Each sample was spiked with 10^6 copies of external control at the first step of the extraction protocol. Thereafter, isolation of DNA was performed according to the manufacturer's instructions for each of the examined extraction protocols. All experiments were performed in triplicate across the entire analytical procedure.

2.3. PCR assays

For the detection of cow milk adulteration in goat and sheep dairy products, the TD-PCR assays previously described by Kourkouli et al. (2024) were applied to the isolated DNA samples. In addition, DNA recovery efficiency was assessed by PCR amplification of an external DNA control with specific primers (Kourkouli et al., 2024). All samples were subjected to TD-PCR, with positive controls prepared using synthetic DNA specific to each animal species. Specifically, for the cow–goat and cow–sheep detection protocols, synthetic mixtures were generated containing 10^4 copies of goat DNA and 10^4 copies of sheep DNA, respectively, together with samples representing 100 % cow milk DNA.

2.4. Validation of TD-PCR assay in processed dairy products

Although the TD-PCR assay's sensitivity for detecting cow DNA in milk was previously established, its performance in processed dairy products required further validation. To address this, cheese and yogurt mixtures containing goat or sheep milk adulterated with 1 %, 5 %, 15 % and 30 % cow milk were prepared and analyzed. The LOD was defined as the lowest level of adulteration that produced at least 95 % positive replicates. Synthetic mixtures at 1 % and 5 % cow milk adulteration were tested in 10 independent replicates. A range of representative dairy

Table 2
List of commercial dairy products.

Sample name	Sample type	Label	Identified species	Adulteration
CC1	Cow's white cheese	Cow	Cow	NO
CC2	Gouda	Cow	Cow	NO
CY1	Cow yoghurt	Cow	Cow	NO
SY1	Sheep yoghurt	Sheep	Sheep	NO
SY2	Sheep yoghurt	Sheep	Sheep	NO
SY3	Sheep yoghurt	Sheep	Sheep	NO
SY4	Sheep yoghurt	Sheep	Sheep	NO
SY5	Sheep yoghurt	Sheep	Sheep	NO
SY6	Sheep yoghurt	Sheep	Sheep	NO
SY7	Sheep yoghurt	Sheep	Sheep	NO
SY8	Sheep yoghurt with clotted cream	Sheep	Sheep, Cow	YES
GC1	Goat white cheese	Goat	Cow	YES
GC2	Goat yellow cheese	Goat	Goat	NO
GC3	Goat barrel cheese	Goat	Cow	YES
GC4	Goat cheese in brine	Goat	Goat, Cow (<5 %)	NO (<10 %)
GC5	Goat semi'hard cheese	Goat	Goat	NO
GC6	Gruyere goat cheese	Goat	Goat	NO
GC7	Goat cheese in brine	Goat	Goat	NO
GC8	Goat cheese in brine	Goat	Goat	NO
GC9	Goat cheese in slices	Goat	Goat	NO
GY1	Goat yoghurt	Goat	Goat	NO
GY2	Goat yoghurt	Goat	Goat	NO
GY3	Goat strained yoghurt	Goat	Goat	NO
GY4	Goat yoghurt	Goat	Goat	NO
GY5	Goat strained yoghurt	Goat	Cow	YES
GY6	Goat yoghurt	Goat	Goat, Cow	YES
GY7	Goat yoghurt	Goat	Cow	YES
GY8	Goat yoghurt	Goat	Goat, Cow	YES
GY9	Goat yoghurt	Goat	Goat, Cow	YES
GY10	Goat yoghurt	Goat	Goat	NO
GY11	Goat yoghurt	Goat	Goat, Cow	YES
GY12	Goat yoghurt	Goat	Goat	NO
GY13	Goat yoghurt	Goat	Goat	NO
GY14	Goat yoghurt	Goat	Goat	NO
GY15	Goat yoghurt bio	Goat	Goat, Cow	YES
GY16	Goat yoghurt	Goat	Goat	NO
GY17	Goat yoghurt	Goat	Goat	NO
GY18	Goat yoghurt	Goat	Goat	NO
GY19	Goat yoghurt	Goat	Goat	NO
GY20	Goat yoghurt	Goat	Goat, Cow	YES
GK1	Goat kefir	Goat	Goat	NO
GK2	Goat kefir	Goat	Goat	NO
GK3	Goat kefir	Goat	Goat	NO
SGC1	Sheep & Goat white brine cheese	Sheep & Goat	Sheep, Goat	NO
SGC2	Barrel cheese	Sheep & Goat	Cow	YES
SGC3	Anthotyro dry	Sheep & Goat	Sheep	NO
SGC4	Pecorino Amfilochia PDO	Sheep & Goat	Sheep	NO
SGC5	Graviera Crete	Sheep & Goat	Sheep	NO
SGC6	Kaseri PDO Mytilini	Sheep & Goat	Sheep	NO
SGC7	Kefalograviera PDO Amfilochia	Sheep & Goat	Sheep	NO
SGC8	Mizithra	Sheep & Goat	Cow	YES
SGC9	Mytilene Graviera	Sheep & Goat	Sheep	NO
SGC10	Sfela cheese PDO	Sheep & Goat	Sheep	NO
SGC11	Kalathaki Limnou cheese	Sheep & Goat	Sheep	NO
SGC12	Graviera Crete PDO	Sheep & Goat	Cow	YES
SGC13	Kaseri Mytilene PDO	Sheep & Goat	Sheep	NO
SGC14	Manouri Turnavou PDO	Sheep & Goat	Goat, Sheep, Cow	YES
F1	Feta cheese	Sheep & Goat	Sheep, Goat	NO
F2	Feta cheese	Sheep & Goat	Sheep, Goat	NO
F3	Feta cheese	Sheep & Goat	Sheep	NO
F4	Feta cheese	Sheep & Goat	Sheep, Goat	NO
F5	Feta cheese	Sheep & Goat	Sheep, Goat	NO
F6	Feta cheese	Sheep & Goat	Sheep, Goat	NO
F7	Feta cheese	Sheep & Goat	Sheep	NO
F8	Feta cheese	Sheep & Goat	Sheep	NO
F9	Feta cheese	Sheep & Goat	Sheep	NO
F10	Feta cheese	Sheep & Goat	Sheep	NO
F11	Feta cheese	Sheep & Goat	Sheep, Goat	NO
F12	Feta cheese	Sheep & Goat	Sheep	NO
F13	Feta cheese	Sheep & Goat	Sheep	NO
F14	Feta cheese	Sheep & Goat	Sheep	NO
F15	Feta cheese	Sheep & Goat	Sheep	NO
F16	Feta cheese	Sheep & Goat	Sheep	NO
F17	Feta Kalavriton PDO	Sheep & Goat	Sheep, Goat	NO

products—including Feta PDO, Gouda, Mytilene Gruyere, and various yogurts—was used to assess the method's applicability across diverse matrices. In the last step, commercial dairy samples of sheep and goat milk origin were tested for authenticity assessment. Signals corresponding to concentrations below 1 % were interpreted as potential accidental cross-contamination rather than intentional adulteration, in line with the thresholds established in the legislation (EU, 2008; L 88:1).

3. Results and discussion

3.1. Analytical evaluation of three commercial DNA extraction protocols for dairy products

To compare the efficacy and analytical performance of the three commercially available DNA isolation kits, we evaluated recovery (% yield) based on external control, cost per reaction, and hands-on time. As shown in Table 3, all tested kits demonstrated high efficiency using different matrices, with recovery ranging from 88.72 % to 102.62 %, 90.11 % to 96.24 & and 74.11 %–100.00 % for white brine, yellow cheese and yoghurt respectively. However, Promega's RSC PureFood GMO and Authentication Kit was selected as the most suitable for the analysis due to its automation, minimal hands-on time, and reduced risk of human error. Although it has a higher cost per reaction, the analyst's active involvement is limited to just 7 min, with the remainder of the process completed automatically in under an hour per sixteen samples.

3.2. Limit of detection on the TD-PCR assay in dairy products

The TD-PCR assay demonstrated a limit of detection (LOD) for cow DNA at 5 % in goat white cheese (Fig. 1a) and sheep white cheese (Fig. 1b). Increased sensitivity was observed in goat yoghurt (Fig. 1e) and sheep yoghurt (Fig. 1f), where cow DNA was reliably detected at concentrations as low as 1 %. In contrast, the assay showed reduced

Table 3
Percentage of recovery of the different DNA extraction methods in dairy products.

Sample	DNA extraction method	% Revocery yoghurt	% Revocery yellow cheese	% Revocery white brine cheese
Cow	DNEasy Blood and Tissue	99.05	90.41	102.62
	Food DNA Isolation kit	94.24	93.00	90.15
	RSC Maxwell System	94.37	93.34	93.41
Mix Cow-Sheep-Goat	DNEasy Blood and Tissue	98.86	91.06	102.31
	Food DNA Isolation kit	74.11	92.93	90.41
	RSC Maxwell System	95.98	96.24	88.72
Mix Sheep & Goat	DNEasy Blood and Tissue	100.00	90.11	101.43
	Food DNA Isolation kit	86.19	91.74	90.25
	RSC Maxwell System	96.83	94.26	93.90

sensitivity in both goat and sheep yellow cheeses, with successful detection only at 30 % and 15 % levels of cow milk adulteration (Fig. 1c and d). This reduced sensitivity is likely attributable to inadequate mixing of the yellow cheese samples, as the goat cheese was significantly harder in texture compared to the softer cow cheese, making homogeneous blending difficult. Moreover, several processing-related factors may account for the reduced sensitivity of the TD-PCR assay in yellow cheeses. Fermentation, high salt concentrations, and thermal treatments typically applied during cheese manufacture can contribute to extensive DNA degradation, thereby limiting the availability of amplifiable templates (Bickley et al., 1996; Li et al., 2023). Additionally, the marked reduction in somatic cell content during cheese and whey processing decreases the overall DNA yield extracted from these matrices (Jiménez-Montenegro, 2022). Yellow cheeses may also contain higher levels of PCR inhibitors, such as lipids or fermentation-derived metabolites, which can further compromise amplification efficiency. These effects, combined with the practical difficulty of achieving homogeneous mixing in hard cheese matrices, likely explain the higher LOD observed for yellow cheeses compared with yoghurt and white brined cheeses.

3.3. Application of the TD-PCR assays in commercial products

During this study, a total of 74 dairy samples derived from sheep,

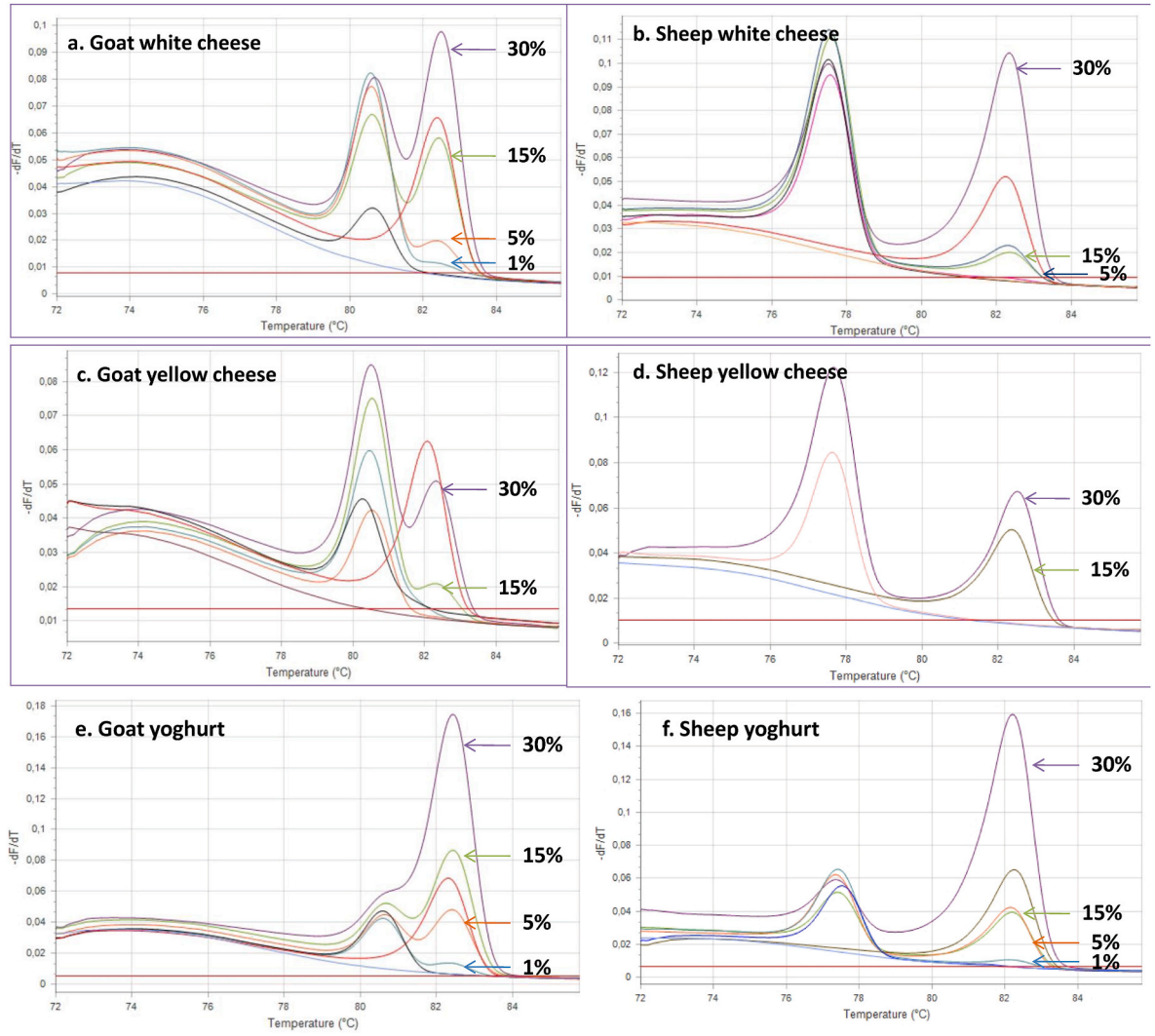

Fig. 1. Determination of Limit of Detection of cow DNA in (a) Goat white cheese, (b) Sheep white cheese, (c) Goat yellow cheese, (d) Sheep yellow cheese, (e) Goat yoghurt and (f) Sheep yoghurt.

goat and cow were obtained from the Greek market and analyzed. All the results are presented in Table 2. The analysis revealed evidence of adulteration in several products (Fig. 2). Specifically, 8 out of 20 (40 %) goat yoghurts, 1 out of 8 (12.5 %) sheep yoghurts sample (SY8), 2 out of 9 (22.2 %) goat cheeses, 1 barrel-aged mixed sheep and goat cheese, 1 mixed sheep and goat Mizithra sample (SGC8), 1 mixed sheep and goat Manouri Turnavou (SGC14) and 1 Graviera Crete cheese were found to contain undeclared cow DNA. None of the tested kefir samples were found to be adulterated with cow or sheep milk.

Sample SY8 was the only sheep yogurt in which cow DNA was detected (Fig. 2a). One possible explanation may involve the inclusion of clotted cream, as the label did not specify the animal source of this ingredient. However, the distinct peak observed at Tm = 81.95 °C was substantially higher than the reference profile, and when considered alongside the product labeling, this sample was classified as adulterated.

Furthermore, the analysis revealed that the Mizithra cheese sample (SGC8), which was marketed as a mixture of goat and sheep milk, exhibited a peak at Tm = 82.20 °C—a value consistent with the presence of cow DNA (Fig. 2b)—while no peaks corresponding to goat or sheep DNA were detected. This indicates that the sample was produced exclusively from cow's milk, in contradiction to its declared composition. The relatively low peak intensity observed for sample SGC8 may be attributed either to inefficient DNA extraction or to a low DNA concentration. It is well documented that the intense thermal processing involved in cheese and whey production markedly reduces somatic cell content, thereby lowering the yield of extractable DNA (Bobbo et al., 2016).

Interestingly, in the goat white brine cheese (GC4) the analysis revealed two distinct peaks, with Tm = 80.49 °C, corresponding to goat DNA, and Tm = 82.20 °C, indicating the presence of cow DNA (Fig. 2c). However, the intensity of the cow DNA peak closely resembled that observed in the 1 % cow DNA synthetic mixture. This suggests that the presence of cow DNA at such a low concentration may not be indicative of intentional adulteration but could instead result from cross-contamination. Such contamination may occur during milk transport or storage in inadequately cleaned tanks, or during processing stages within the cheese production facility.

Additionally, PCR screening was performed on feta samples to assess the presence of goat DNA, as these products are traditionally produced from at least 70 % sheep's milk and up to 30 % goat's milk, although it can also be made from 100 % sheep's milk. In this study, 17 Feta cheese samples were analyzed using TD PCR with the cow-goat protocol, with the aim of detecting the presence of goat DNA, as indicated on the product labels, which claimed a mixture of goat and sheep milk. The analysis revealed that only 7 out of 17 (41.2 %) samples contained detectable amounts of goat milk, despite all products being labeled as containing both goat and sheep milk. As expected, the goat DNA signal was generally of low intensity, consistent with the expected range of 0 %–30 % goat milk content in authentic feta.

The analysis revealed that at least 31 % of all goat samples were adulterated, indicating that goat dairy products are more prone to adulteration than sheep products—likely due to the higher cost and limited availability of goat milk. Previous studies in Greece and neighboring countries have reported similarly high rates: Tsakali et al. (2019) detected cow's milk in 90 % of commercial goat products in Greece, Tuncay and Sancak (2024) reported 76 % adulteration while Zengin and Kara (2022) found 45 % of goat and sheep cheeses tested adulterated in Turkey, and Pinto et al. (2017) found 80 % in Italy (Di Pinto et al., 2017; Tsakali et al., 2019; Tuncay & Sancak, 2024; Zengin & Kara, 2022). Furthermore, Rodrigues et al. (2012) indicated that 41.2 % of the goat milk presented to market was positive for bovine milk in northeastern Brazil (Rodrigues et al., 2012). Meanwhile, Khanzadi et al. (2013) detected the undeclared presence of cow's milk in 31.5 % of dairy product samples sold as sheep's milk in Iran (Khanzadi et al., 2013). Such evidence-based surveys are crucial for guiding inspections and policy development and for strengthening consumer trust. In line with these findings, the Greek Ministry of Rural Development and Food announced intensified dairy adulteration inspections in January 2024 (Greek Ministry of Rural Development and Food, 2024).

4. Conclusions

Food production frequently faces the widespread issue of raw materials being adulterated for commercial gain through illegal practices.

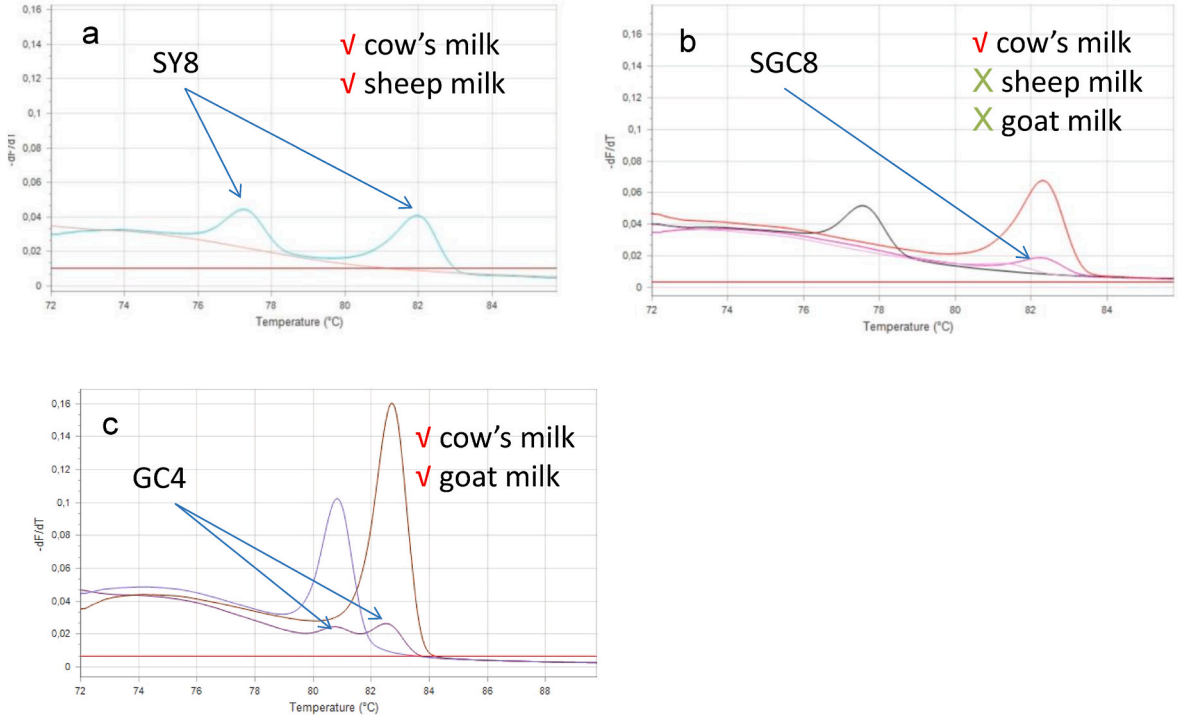

Fig. 2. Representative graphs of adulterated and none adulterated samples.

The "Farm to Fork" approach underscores the need for careful traceability and verification at every stage of a food product's journey, from its initial production to the final consumer (Tuncay & Sancak, 2024). In this context, accurate food labeling is essential for enabling consumers to make informed choices (Herman, 2001). As food adulteration and mislabeling continue to pose global challenges, they highlight the critical importance of ensuring ingredient quality and safety (Di Pinto et al., 2017).

Compared to classic PCR, the use of touchdown PCR in this study provided substantially higher specificity and sensitivity, particularly important given the high sequence homology among bovine, caprine and ovine DNA. Initial trials under standard PCR conditions produced overlapping melting curves and non-specific signals, whereas the optimized touchdown protocol—with progressively decreasing annealing temperatures—enabled stringent early-cycle primer binding and eliminated non-specific products, resulting in clear species-specific melting peaks (Kourkouli et al., 2024). This improvement was essential for achieving reliable discrimination at low levels of adulteration. In contrast to qPCR, which offers quantification but whose performance depends heavily on primer/probe design and may be challenged by closely related templates, the developed TD-PCR method demonstrated robust qualitative detection down to 1 % adulteration, aligning with regulatory thresholds for intentional fraud. While qPCR can help distinguish contamination from economically motivated adulteration in some contexts, our results show that TD-PCR provides a cost-effective, rapid, and highly specific alternative for routine screening, especially when clear identification rather than quantification is required. This positions the method as a practical and reliable tool for authenticity testing in dairy products.

This study demonstrated the successful application of a validated TD-PCR assay for detecting cow milk adulteration in a variety of dairy matrices, including cheese, yoghurt, and kefir. The method proved sensitive, robust, and suitable for routine authenticity screening. The analysis of samples from the Greek market revealed several instances of non-compliance, particularly among goat dairy products; however, these findings should not be interpreted as representative of the entire national market, as the sampling was limited in size and geography. Instead, the results highlight that adulteration can occur and underline the value of continuous monitoring. Strengthening routine authenticity testing—together with transparent labeling practices—can contribute to safeguarding consumers, supporting fair trade, and protecting the reputation of traditional Greek dairy products.

CRediT authorship contribution statement

Foteini Roumani: Writing – original draft, Methodology. **Maria-Christina Serdari:** Methodology. **Nikolaos S. Thomaidis:** Writing – review & editing, Resources. **Marilena Dasenaki:** Writing – original draft, Resources, Conceptualization. **Athina Markou:** Writing – original draft, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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