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Real-time PCR is a potential tool to determine the origin of milk used in cheese production

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ABSTRACT

Traceability of foods has become very important problem respect to food quality and typicalness of foods. Cheeses' milk origin cannot be identified by the consumer and they are sold at different prices under various product names. Recently, this has caused the problem of 'adulteration'. The aim of this study is to determine the amount and origin of milk used in cheese production by using real-time PCR which has been used for identification of animal species in dairy products. In this study, 90 different cheeses offered for sale in Turkey of 30 brands from various sources such as cow, sheep and goat have been studied. At the end of the study, only 36.67% of samples were determined to be produced from 100% cow milk. In the remaining 13.33%, a mixture of goat and sheep milk have been found. Only one sample of sheep cheese was produced from 100% sheep milk. In goat cheese samples, 16.67% of cheese was produced from 100% sheep milk and the origin of only 10% was identified as 100% cow milk. No linear relationship could be determined between chemical composition, fatty acids ratios and amount and origin of cheeses ($P < 0.05$).

1. Introduction

Today it is estimated that there are thousands of kinds of cheese in the world. However up to 18 typical cheeses have great importance worldwide. They can be named according to their origin and production method like in this alphabetical order; Brick, Camembert, Cheddar, Cottage, Cream, Edam, Gouda, Hand, Limburger, Neufchatel, Parmesan, Romano, Roquefort, the Sapsag the Swiss, Trappist, Whey Cheese (Demirci & Şimşek, 2004).

vThe total annual production of cheese in the world is estimated to be about 20 million tons. Cow milk cheese produced in milk processing plants (industrial cheeses) constitutes more than 80% of the global cheese production. The rest of the cheese is produced in farms from other milk origins (sheep, goats and buffalo) (USDA, 2014). About 200 varieties of cheese are produced in Turkey. Some of these, such as; white cheese, kashar cheese, tulum cheese, plaited cheese, cottage cheese, mihalic cheese are nationally popular and have economic value (Celik & Uysal, 2009). White cheese is produced from cow, goat, sheep's milk or a mixture thereof. Dairy field is subjected to growing number of frauds. In particular, milk and cheese are expensive foodstuffs, and their consumption is spread among the population because of their high nutritional value; for this reason, dishonest producers, who are tempted by the possibility to enlarge their profits, commit different frauds (Calvano, Ceglie, Monopolia, & Zambonin, 2012; Cozzolino et al., 2001; Mayer, Bürger, & Kaar, 2012). Common adulterations of dairy products are the substitution of higher value milk (sheep's and goat's) with milk of lower value (cow's milk). Thus, the detection of milk species is important in cheese producing branch, especially those made from one pure species such as pure cow, sheep or goat cheeses (Bottero, Civera, Anastasio, Turi, & Rosati, 2002; Zachar, Šoltés, & Kasarda, 2011). In the market, milk origin cannot be identified by consumers looking at the name of this cheese and the varieties of white cheese are sold at different prices. Recently, this has caused the problem of 'adulteration'. Adulteration is delibrate addition of low quality raw materials to increase the quantity of the cheese in raw form (Sincer, Şenyuva, & Gilbert, 2010). This illegal practice has an economic advantage form ilk producers and can lead to unfair competition by reducing product quality. Moreover it can seriously threaten human health (Ertaş & Topal, 2009).

In 2014, Ministry of Food, Agriculture and Livestock, has

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announced that 25 out of 32 food producers of dairy products, meat and meat products, honey imitate and adultrate. According to a statement on the website of the Ministry, 14 food companies have been found to imitate and adultrate in 19 party products (8 cheese, 6 yogurt, 3 butter, 2 cream). Some of the most adulterated food in Turkey are dairy products. Some adulterate and cheat in milk and milk products; vegetable oil is added to butter, yogurt and cheese (Anonymous, 2016).

All foods must be adequately labelled according to European Regulation regarding to procedures in matters of food safety (Regulation (EC) No 178/2002). Therefore, the origin of the milk used in cheese production must be on the product label information. If a mixture of different types of milk is used in cheese production, the rates of different types of milk must be on the label of the product. To avoid the possible fraudulent substitution of goat and sheep milk with cow's milk, some analytical methods have been developed to detect such frauds and protect the consumers from misleading labelling (De La Fuente & Juárez, 2005). Among these methods, chromatographic (Enne et al., 2005; Bernardi et al., 2015), electrophoretical (Mayer, 2005; Molina, Martín-Álvarez, & Ramos, 1999), immunological (Xue, Hu, Son, Han, & Yang, 2010), fourier transform infrared (FT-IR) spectroscopy combined with chemometric (Nicolaou, Xu, & Goodacre, 2010), direct matrix-assisted laser desorption/ionization–time-of-flight (MALDI–TOF) MS (Calvano et al., 2012) have been described as rapid methods for adultration detection.

In relatively years molecular biology techniques have been developed for identification of animal species in dairy products. Enzyme technology and DNA-based techniques are widely used among other techniques because of more pratical, sensitive and robust (Cuollo et al., 2010; De Noni, Tirelli, & Masotti, 1996; Ferreira & Caçote, 2003).

These techniques take advantage of the high specificity and sensitivity of polymerase chain reaction (PCR)-based methods to detect very low amounts of cow milk (Feligini et al., 2005; Lopez-Calleja et al., 2005). Geographical origin of food stuff can be achived by determining the food microbial populations diversity using genetic fingerprinting techniques such as Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) (El Sheikha, Condur, M, é, tayer, , Nguyen, Loiseau, & Montet, 2009; El Sheikha, 2010; El Sheikha, 2015). This method has been used for determination of cheese origin by characterizing microbial populations of samples. Arcuri, Sheikha, Rychlik, Piro-Métayer, and Montet (2013), studied on determination of Minas cheese orgin by using 16 S rDNA fingerprinting of bacteria communities by PCR-DGGE. Ercolini, Mauriello, Blaiotta, Moschetti, and Coppola (2004) studied on the starter effectiveness recording a series of fingerprints of the microbial diversity occuring at different steps of mozzarella cheese by using PCR-DGGE.

In the PCR technique together with the work of determining the origin of milk or cheese product mix, it was concluded that it would be useful for food inspections (Lopez-Calleja, 2004; Guarino et al., 2010). Real-time polymerase chain reaction (RT-PCR) method has become the most reliable method of reseach on adulteration, because by using this method the kind of precision upto 0.1% can be able to identify different content in cheese (Gonza'les, Lopez-Calleja, & Fajardo, 2007). Compared to other techniques, RT-PCR method is more sensitive and rapid (Brahma & De, 2010). So, this method is accepted as the present and future method used in determining content (Ertaş & Topal, 2009; Settanni & Corsetti, 2007). In our study, Real-time polymerase chain reaction method was used to determine the origin and quantity of the milk types used in the manufacturing of cheeses which are sold under different names.

2. Material and method

2.1. Material

In our study, a total of 90 samples of cheese including 30 different brands sold as cow cheese, 30 different brands sold as sheep cheese and 30 different brands sold as goat cheese were obtained. After the samples were purchased to be analyzed they were vacuumed, packed and transferred to the laboratory (0–4 °C). The samples were stored in the refrigerator until analyzed. All samples were collected from aegean region of Turkey between march and november in 2015.

2.2. Method

2.2.1. Chemical analysis

Determination of dry matter, fat content, water content in lean cheese mass, protein level, acidity and salt assay analyses were performed according to James (1995) and pH determination was performed with a pH meter (HANNA Instruments HI 221, Canada).

2.2.2. Determination of fatty acids

Oil extraction of cheese was performed according to Renner (1993). Fatty acids were converted into methyl esters according to AOCS. (1997, pp. 1–2) method. Methyl esters were injected to gas chromatography (GC) under the following conditions. Fatty acid compositions of samples were determined using retention times.

Working conditions.

GC model; Hewlett-Packard (5890, Avondale, PA, USA).

Column; Supelco SP-2380 silica capillary (100 m × 0,25 mm i.d. 0,2 μm film thickness, Supelco INC. Bellefonte, USA).

Sensor; Flame ionization detector.

Injection; 2 μl.

Temperature program; From 100 °C to 220 °C 4 °C/min.

Injection and detector temperature; 300 °C.

Transporter gases; Nitrogen 1 ml/min.

Split; 20: 1.

2.2.3. Detection of milk origin and quantification by RT-PCR

Cheese samples were primarily subjected to DNA isolation process for the RT-PCR device. After isolation, DNA concentration was measured in the NanoDrop device. RT-PCR primers and probes were diluted by using guides and intermediate stocks created for each parameter (cow, goat, sheep). So, certain amount of water, enzymes and Bos taurus (cow), Capra hircus (goat), Ovis aries (sheep) are used. Common gene was prepared using special primer probes. Each mix was prepared in 7 μL to be installed into plate (Lightcycler multiwell 480) and then the isolated DNA were added in 3 μL over them and the mixture was centrifuged at 2060 rpm 30 for seconds.

Then the plate 480 II RT-PCR Light Cycler (Roche) was placed into the device. After starting the first 10 min, temperature reached to 100 °C. Then primers, probes in the mix were bound to the region we wanted them to and they were paint there and replicated that part of the DNA. This form was completed by continuing analysis. Obtained results for the relative quantification were examined (Delta-Delta CT method).

The amplification efficiencies of the primers were analysed by testing the DNA. R^2 values were 0,97.

2.2.4. Statistical analyses

One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used for statistical analysis of the data collected. Means were accepted to be significantly different if $P < 0.05$. SPSS (version 9.0) commercial statistical package was used for all statistical analyses.