

# Interlaboratory validation of two multiplex quantitative real-time PCR methods to determine species DNA of cow, sheep and goat as a measure of milk proportions in cheese

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**Abstract** Milk products like yogurt, flavoured milk-drinks, curd and cheese may be composed of milk different from cow, namely of ruminant species like sheep and goat. Such products experience an increasing demand in Europe and are recognised as healthy and naturally finished specialities. To verify declared milk compositions in these dairy products, two different quantitative multiplex PCR systems have been evaluated in a comparison test with eleven participating laboratories employing two unknown, traditionally manufactured cheeses with different degrees of ripening to determine milk fractions from cow, ewe and goat. Precision and accuracy was investigated by calibration to dilutions of DNA mixtures and to homologous matrix-adapted reference cheeses, respectively. As expected, independent of the particular method, best inter- and intra-laboratory accuracy has been achieved through the use of homologous reference cheese standards. Furthermore, it has been shown that cheese ripening and the concomitant DNA degradation exert an inverse effect on the method's sensitivity and performance characteristics. Additionally, a broad market survey of different milk

products demonstrated its applicability as an efficient analytical tool for food control laboratories to challenge the authenticity of milk and its products from small ruminants.

**Keywords** Multiplex · Real-time quantitative PCR · Cow · Ewe · Goat · Water buffalo · Species determination · Extraneous milk · Cheese adulteration

## Introduction

Authentication of food stuff represents a steady challenge in the frame of modern quality assurance of any food chemist. Especially species differentiation has become an important tool for authenticity testing in animal-derived food. Such kind of protective measures against substitution with or admixture of cow milk in dairy products of ewe's and goat's milk is of particular importance for the industry's credibility to alleviate the suspicious fact of economical fraud, to decrease the risk of species selective food allergenicity and to comply with legal regulations. Several systematic investigations of official control laboratories or of private initiative for authenticity testing of ewe's and goat's cheeses from Germany, Turkey and Portugal have revealed non-declared, extraneous milk additions [1–3]. To prevent unfair competition and to guarantee transparent food declaration, accurate analytical methods are crucial to the dairy industries and to food control authorities to unveil such adulterations. The most popular approach to differentiate milk of different species is based on the discriminative molecular structure of homologous milk-proteins applying analytical methods such as electrophoresis, chromatography or immunochemistry. Isoelectric focusing (IEF) has been favoured in the past because of its superior separation power of complex protein mixtures. Determinations of cow

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