A molecular genetic approach for traceability of the source milk in cheese

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RIASSUNTO – Un approccio molecolare per la tracciabilità del latte di origine nel formaggio – *La "tracciabilità" del prodotto primario, in particolare della specie e della razza d'origine, attraverso l'uso di marcatori biologici costituisce un importante obiettivo per la valorizzazione dei prodotti caseari nazionali. Il presente lavoro riporta i primi risultati di uno studio sull'applicabilità di metodologie molecolari per l'analisi di formaggi caprini. Sono stati utilizzati diversi metodi di estrazione del DNA da alimenti e sostanze organiche. Il DNA è stato efficacemente amplificato mediante* primer *specifici per l'analisi del polimorfismo ai* loci α_{S1}*-caseina e k-caseina, e in seguito analizzato con successo mediante differenti tecniche molecolari (PCR-SSCP, PCR-RFLP, AS-PCR).*

KEY WORDS: Cheese, goat milk, traceability, molecular analysis, caseins

INTRODUCTION – The valorisation of typical cheeses meets the needs of preserving the local country culture and tradition as well of guaranteeing consumer health by the control of all the steps of production (herd, milk quality, cheese making technology). Among the variability factors significantly affecting cheese peculiarity, biodiversity plays an important role (Gandini *et al.*, 1996; Pieragostini *et al.*, 2002). The possibility of identifying or tracing the primary product, mainly the origin breed, by the use of biologic markers, is an important goal for the safeguard and valorisation of national goat cheese. In this field great interest is paid to milk protein genetic polymorphism. A first study was carried out in order to investigate the possibility of tracing the source milk in dairy products. In particular, the use of molecular techniques for the detection of casein polymorphisms in goat cheese was preliminarily investigated. It is well known, in fact, that differences occur in the frequency of casein alleles among the goat breeds (Grosclaude *et al.*, 1994; Caroli *et al.*, 2001). Moreover, in Montefalcone goat, a breed specific allele was found at k-casein locus (Angiolillo *et al.*, 2001). The research will aim, in the future, to identify alleles characterising, for particular frequencies, the local breeds of interest for the safeguard of Italian animal genetic resources and typical dairy products.

MATERIAL AND METHODS – Cheeses (n = 12) made from goat milk of different breeds and from mixed cow and goat milk, were collected and analysed by molecular techniques. DNA was extracted by using commercial kits for food (Invisorb®, Spin Food Kit 1 Invitek GmbH – Berlin) and organic substances (Invisorb®, Spin Stool DNA Kit Invitek GmbH – Berlin). The extracted DNA was successively amplified by specific primers for α_{S1}-casein *(CSN1S1)* and k-casein *(CSN3)* (Table 1). Amplification was verified by electrophoresis onto 2% (w/v) agarose gel. The PCR products were successively analysed by different molecular techniques allowing the detection of specific genetic variants (Table 1).

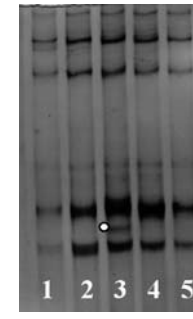
Table 1. Used methods for casein *loci* analyses.

Locus	Identifiable alleles	Species	Technique	Reference
CSN1S1	F, A/0, B/E	Caprine	PCR-RFLP	Ramunno *et al.* 2000
CSN1S1	B, C	Bovine	PCR-SSCP	Jann *et al.* 2002
CSN1S1	0, non 0	Caprine	AS-PCR	Cosenza *et al.* 2001
CSN3	A, B	Caprine	PCR-SSCP	Caroli *et al.* 2001

RESULTS AND CONLUSIONS – The commercial kit for organic substances was particularly useful, allowing the extraction of a sufficient DNA amount from goat cheese. In fact, an average of 15 μg of good quality DNA was obtained starting from about 300 mg of cheese. Amplification was efficient, and PCR products could be easily verified by migration onto agarose gel. Amplification was obtained by all the used primers, and in all the cheese samples analysed, also in successive trials. The molecular technologies successively used in order to identify the different casein alleles, gave a good resolution of the different patterns. As an example, Figure 1 shows the electrophoretic migration patterns obtained by the SSCP analysis (Single Strand Conformation Polymorphism) of five *CSN3* PCR products from different cheeses.

Figure 1. Analysis SSCP of k-casein *locus* on the DNA extracted from cheeses shown in the annexed table. The white dot indicates the band relative to the goat k-casein B allele.

Sample number	Milk origin
1	Saanen goat
2	Cow + Alpine goat
3	Ionica goat
4	Goat crosses
5	Cow+ goat crosses



The method allows the identification of goat *CSN3*A* and *CSN3*B*. A clear difference is evident between sample 3 and the other cheeses. Sample 3 derives from a cheese properly made from Jonica goat milk. Jonica is an Apulian goat breed originating in the second half of the XX century from a substitution cross between the Maltese breed and the caprine local population (Bramante *et al.*, 2002). The band indicated by the white dot is relative to *CSN3*B*, and was identified only in the DNA extracted from cheese of Jonica breed, where the *B* variant was found with a frequency higher than 10% (Caroli *et al.* 2001). *CSN3*B* occurs in Apulian goats with frequency higher than 10% (Chessa *et al.*, 2003), while its frequency is much lower (0-0.01%) in Saanen and Camosciata breeds.

The DNA extracted from cheese was also amplified and analysed for bovine *CSN1S1* polymorphism (Table 1). The high homology between caprine and bovine sequences allows the amplification of caprine DNA by the use of specific bovine primers, and *vice versa*. The study of the different molecular behaviour of caprine, bovine and eventually ovine PCR products by SSCP technique could be useful to detect the

presence of milk of other species in caprine cheese. A similar result was successfully obtained as regards the identification of bovine DNA added to buffalo DNA (Bardin *et al.*, 1994) on the basis of molecular differences at casein *loci (CSN2, CSN3)* between the two species. More recently, a molecular approach was also used by Bottero *et al.* (2003) to identify cows', goats' and sheep's milk in dairy products by mtDNA analysis. *CSN1S1* analysis, until now, did not allow distinguishing among species, however the study of different SSCP migration patterns observed is in progress. The obtained results are the basis of a wider investigation, for the characterisation of typical national dairy products.

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