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Donkey's milk caseins characterization

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RIASSUNTO – Caratterizzazione caseinica del latte di asina. *Il latte vaccino può causare nei neonati gravi allergie. In questi casi la normale terapia si basa sull'utilizzo del latte di soia o di un latte in cui le proteine siano state idrolizzate rendendolo così ipoallergenico. Una valida alternativa è data dal latte d'asina che presenta delle caratteristiche organolettiche simili a quelle del latte umano e può costituire quindi il trattamento d'elezione per bambini che presentano allergie alimentari nei primi mesi di vita. Il latte d'asina ha una composizione simile al latte umano soprattutto per quanto riguarda la frazione proteica (basso contenuto di caseine e β-lattoglobuline) e la frazione lipidica. In questo studio abbiamo purificato e caratterizzato alcune caseine da latte di asina per confronto con sequenze di caseine già identificate in nostri precedenti studi al fine di approfondire le conoscenze nutrizionali sul prodotto "latte d'asina".*

Key words: donkey's milk, caseins, purification, sequence.

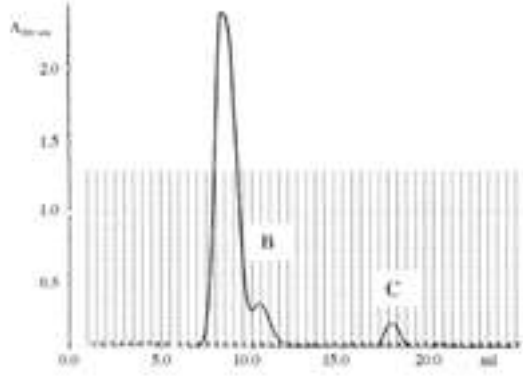
INTRODUCTION – In the recent years the interest around donkey's milk had a marked increase since it has been demonstrated that this milk can be used for feeding of infants affected by dairy cow's milk protein intolerance (Businco *et al.*, 2000; Iacono *et al.*, 1992). Donkey's milk composition is very similar to the human milk especially concerning the protein fraction, in fact it is characterized by a low casein and β-lactoglobulin contents, and by an high content of lisozyme, important to preserve child from intestinal infections. (Fantuz *et al.*, 2001). Also the donkey's milk lipid fraction is comparable to human milk since it is characterised by high levels of linoleic and linolenic acid (Salimei *et al.*, 2004). Casein fraction of donkey's milk has not been deeply investigated yet; preliminary studies have regarded a partial purification and characterization of αs_1 and β-caseins from this milk (Vincenzetti *et al.*, 2005). In this work we focused our attention on a more specific purification of caseinic fraction based on their different isoelectric point.

MATERIAL AND METHODS – Two asses Ragusana breed were used to provide milk samples. The animals were machine milked as described by Salimei *et al.* (2004). Skim milk was obtained from 20 ml of fresh milk by centrifugation at 2250 g for 10 min. The isoelectrically precipitated caseins were obtained by adding 10% (v/v) acetic acid to the skim milk until a pH value of 4.6, followed by a centrifugation at 2250 g for 10 min. The precipitated caseins were resuspended in 20 ml of 50 mM ammonium acetate buffer pH 5.5 containing 8M urea (TAA-urea buffer). The whole casein were subjected to a gel filtration analysis on HPLC using a Superdex 75 HR 10/30 (Amersham Biosciences). The column was equilibrated and eluted by TAA-urea buffer at a flow rate of 0.5 ml/min. The effluent was monitored at 280 nm and collected in 0.5 ml fractions. From the gel filtration three pools (pool A, pool B and pool C) were obtained and were subjected to 13% SDS-PAGE analysis. An aliquot of each pool A and pool B was further subjected to a chromatofocusing analysis on HPLC (MONO-P HR 5/5, Amersham Biosciences). The column was equilibrated with the start buffer (Bis-tris 25 mM, pH 8.0 containing 8M urea) and eluted by a linear gradient with the eluant buffer (100 ml containing 3 ml Polybuffer

96, 7 ml Polybuffer 74, 8M urea pH 4.0). The casein fractions obtained from the chromatofocusing analysis were subjected to 13% SDS-PAGE and the molecular weight were calculated. The protein concentration was determined by the method described by Bradford (1976). The standard used in the electrophoretic course were low molecular weight standard (Bio-Rad).

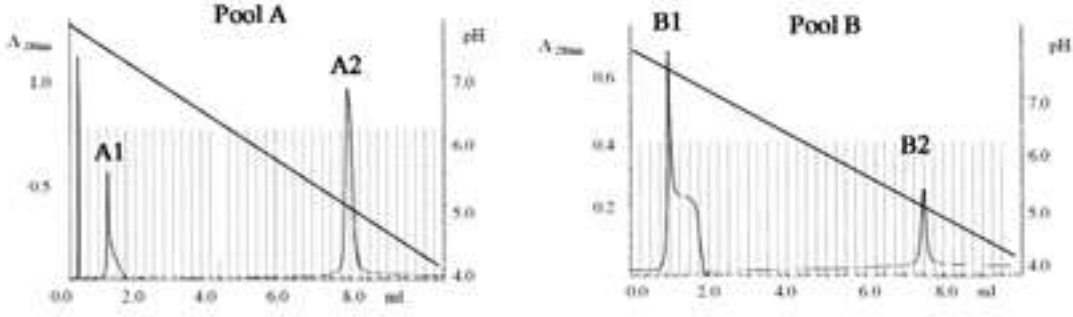
RESULTS AND CONCLUSIONS – Previous studies on ass's milk proteins (Vincenzetti *et al.*, 2005), allowed to the identification of αs_1-caseins having N-terminal sequence RPKLPHRXPE with a Mr of 30.9 and 33.0 kDa, and β-caseins, N-terminal sequence REKEELNVS with a Mr of 34 and 35.4 kDa. In this work we were looking for other type of caseins like γ- and κ- that were found in mare's milk (Egito *et al.*, 2002). For this purpose, in order to obtain a better separation of the caseinic fraction, we set up a new purification protocol based on the different isoelectric point and different molecular weight of caseins. The gel filtration analysis of whole caseins performed in HPLC resulted in the presence of 3 peak proteins (see Figure 1) named pool A, pool B and pool C.

Figure 1. Gel filtration analysis of whole caseins performed as described under "Materials and Methods" session.



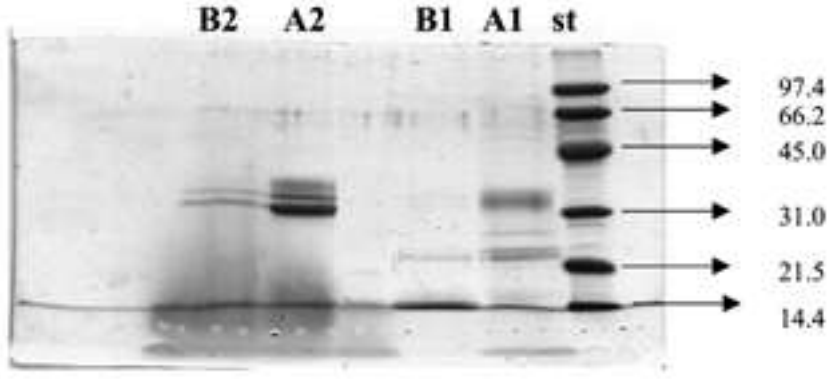
Protein determination and 13% SDS-PAGE analysis on each pool eluted from gel filtration showed a low protein content in pool C. Therefore only pool A and pool B were subjected to a chromatophocusing performed in the pH range between 4 and 8. In Figure 2 is shown that after MONO P analysis the pool A and the pool B could be further distinguished in four peaks named A1, A2 and B1, B2 respectively.

Figure 2. Mono P analysis of Pool A and Pool B eluted from gel filtration.



Using this new protocol, after a 13% SDS-PAGE analysis, we were able to separate different classes of donkey's milk proteins (see Figure 3). Pool A1 revealed four bands: a diffuse one having a Mr of 33.0 kDa, probably corresponding to a α_{s1}-casein, and other three interesting electrophoretic bands (Mr = 25.7 kDa; 23.4 kDa; 22.3 kDa), that will be then subjected to N-terminal analysis for a better identification. Pool A2 showed a band having a Mr of 36.3 kDa and another band of 32.4 kDa, actually not completely identified. Finally pool B2 showed the presence of two bands with a Mr of 38.0 kDa and another one of 35.5 kDa (β-casein). Further studies are actually in progress, in order to deepen the knowledge about "donkey's milk" as hypoallergenic food.

Figure 3. 13% SDS-PAGE of each peak eluted from chromatofocusing st: low molecular weight standard (Bio-Rad).



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