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Milk protein and cheese yield in buffalo species

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ABSTRACT – Buffalo milk samples differing significantly for cheese yield values were analysed by 2D electrophoresis in order to outline a protein profile, with specific regards to k-casein fractions. Four buffaloes, two of which showing high cheese yield and two with low cheese yield selected from a group of 135 subjects were chosen for the proteomic analyses. Six main spots in 2D gels were recognized as αs1-, αs2-, β- and k-casein, α-lactoalbumin, β-lactoglobulin. The main visible differences in the 2D gels between buffaloes with high vs. low cheese yield were found in the appearance of the four k-casein spots (spots numbers:20, 19, 16, 18) which differ in the number of phosphorilation and glycosilation. The area and the intensity of the four spots were calculated by using Melanie II (Bio-Rad) software. Samples with high cheese yield showed higher value of the by-products: area x intensity of spot 16, correspondent to k-casein with one phosphorilation site, and lower values of spots 19 and 20, of k-casein with more than one phosphorilation site and glycosilated.

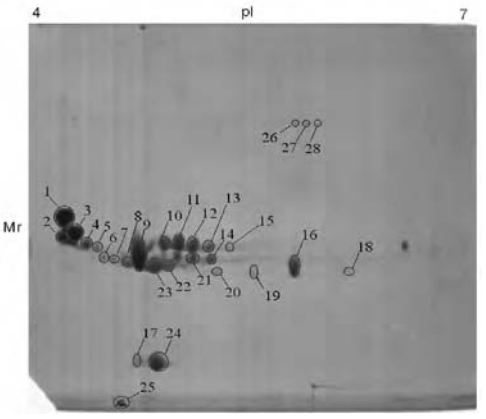
Key words: Buffalo milk, K-casein, Cheese yield.

Introduction – In previous studies, individual buffalo milk samples showed cheese yield values not fully explained by their chemical composition (Zicarelli *et al.*, 2001; Zicarelli, 2004) with differences in curd yield/milk protein (g/g) ratio of by about 4g. A different milk protein aptitude to cheese making could be hypothesized in buffalo in analogy to what found in other dairy species (Di Stasio *et al.*, 2000; Formaggioni *et al.*, 1999). Aim of this study was to outline a protein profile in buffalo milk samples differing significantly in cheese yield values.

Material and methods – Milk samples were collected from four out of 135 buffaloes subjected to monthly samplings to determine milk chemical composition and cheese yield, as reported in a previous note (Zicarelli *et al.*, 2001). Data were analysed between buffaloes in order to define two groups of subjects significantly different for cheese yield but with a similar milk fat and protein concentration. From each group, two subjects were selected for the proteomic analyses: number 2 and 4 with high cheese yield and number 5 and 11 with low cheese yield. Milk samples were centrifuged at 10,000 rpm for 10' in order to separate the lipid fraction. 40 µL of free fatty milk samples were mixed in 600 µl of urea chaps (Sigma-Aldrich). 320µL of milk in urea/chaps were suspended in dithiothreitol (DTT, Sigma-Aldrich) 65mM, IPG (anfoline carrier) biolyte pH 4-7 (Bio-Rad) and few drops of brome/phenol blue in a saturated solution. For the first dimension electrophoreses by isoelectric focusing, strips (ReadyStrip IPG) of 17 cm with a pH 4-7 in a linear gradient were utilised. Strips were re-hydrated for 12 hours at 50V and 20°C, and then electrophoreses were performed in Protean IEF cell (Bio-Rad) in three steps: 1) 250 V for 15'; 2) from 250 to 10,000V in a linear voltage increase for 3 h 3) the run was stopped when the end point of 60,000 V was reached (current limit in gel, 50 µA). Disulphide bonds of proteins in the gel were reduced by using a solution containing Tris 50 mM pH 8.8, 6 M urea, glycerol 30%, SDS 2%, dithiotrheitol 1% and brome/phenol blue for 10'. The alkylation of free sulphydryl

was obtained by incubating the gel for 10' with a solution containing Tris 50 mM pH 8.8, urea 6M, glycerol 30%, SDS 2%, iodoacetamide 2,5% and brome phenol blue.The second dimension electrophoresis was carried out by Protean II xi 2-D Electrophoresis Cell (Bio-Rad). Each equilibrated strip was loaded on top of a 12.5% polyacrylamide gel (20x20cm x 1mm) and run at 1W for 2h and then at 5W until the end of the of the run. Gel staining was performed by Blue Comassie for 3h and then destaining was carried out overnight. Bidimensional gels were acquired using a Fluor-s scanner (Bio-Rad) and analysed by Melanie II - Bio-Rad software.

Figure 1. 2D electrophoresis of free fatty buffalo.milk.



Results and conclusions – Bidimensional electrophoresis showed 35 spots of protein and 28 of them were identified (Figure 1). In Table 1 all the recognized spots were reported each one with the type of identified protein, the relative p.I. and molecular weights and the score, that is the reliability percentage of the identification with those reported for *Bubalus bubalis* species.

The main spots were recognized as αs1- (spots 1-7), αs2- (spots 10-15, 21-23), β- (spots 8-9) and k-casein (16, 18-20), α-lactoalbumin (spots 17, 25), β-lactoglobulin (spot 24). Several different spots were identified as by products of casein degradation and proteins with carrier function i.e.:abumin (spots 26-28). Gels obtained in this study were comparable to what found by D'Ambrosio *et al.* (2008a, b) and in analogy with their study, we were able to ascribe each casein fraction to a specific glyco- phosphopeptides. The main visible differences in the 2D gels between buffaloes with high *vs*. low cheese yield were found in the appearance of k-casein spots which differ, as reported by Ambrosio *et al.* (2008a, b) in the number of phosphorilation and glycosilation. In fact, the spot identified in our study as 18 has been recognized by Ambrosio *et al.* (2008a, b) as k-casein without phosphorilation, spot 16 is relative to k-casein with only one phosphorilation site and spots 19 and 20 resulted to be relative to the k-casein more phosphorilated and glycosilated. In order to quantify in a

Figure 2. Intensity of spots 20, 19, 16, 18 in the four milk samples (2 and 4: high cheese yield; 5 and 11: low cheese yield).

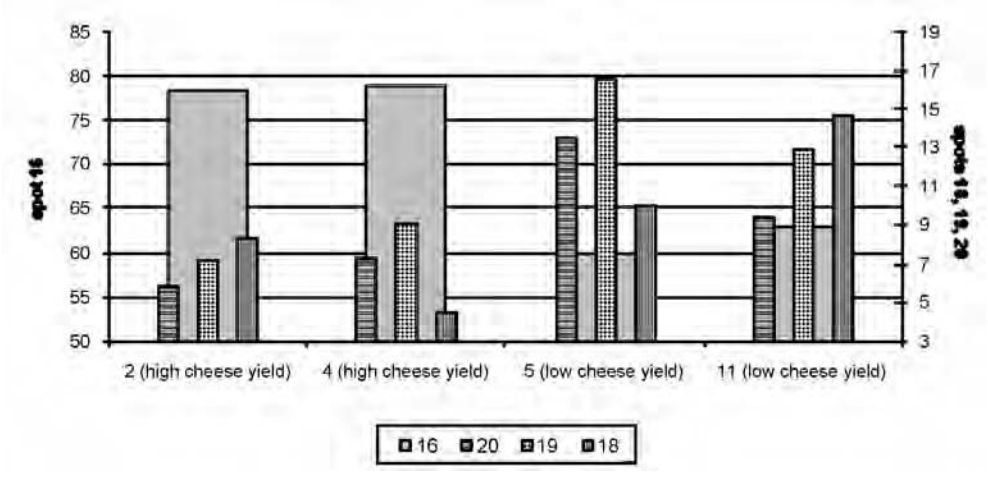


Table 1. Identification of the spots by ESI-TRAP analysis of the 2D proteomic map of free fatty buffalo milk.

Spot	Protein	p.I.	Mw	Score	Spot	Protein	p.I.	Mw	Score
1	αs1- casein	4.80	24440	80	15	αs2- casein	7.17	24798	91
2	αs1- casein	4.80	24440	71	16	k- casein	6.84	24498	83
3	αs1- casein	4.85	24368	43	17	α-lactoalbumin	4.70	14691	78
4	αs1- casein	4.85	25508	82	18	k- casein	6.84	21384	68
5	αs1- casein	4.85	27204	75	19	k- casein	6.84	21498	62
6	αs1- casein	4.85	26971	86	20	k- casein	6.84	19320	79
7	αs1- casein	4.85	23918	88	21	αs2- casein	4.86	26283	82
8	β- casein	5.13	25197	49	22	αs2- casein	7.17	26283	61
9	β - casein	5.26	1857	42	23	αs2- casein	6.54	26270	60
10	αs2- casein	6.54	262853	53	24	β-lactoglobulin	4.83	18255	72
11	αs2- casein	7.16	26283	61	25	α-lactoalbumin	4.83	16692	63
12	αs2- casein	7.16	24798	53	26	Seric albumin	5.78	71221	79
13	αs2- casein	7.17	26270	78	27	Seric albumin	5.78	71221	65
14	αs2- casein	7.17	26283	89	28	Seric albumin	5.78	71221	76

preliminary way a real differences in concentration of the different k-casein fractions between the samples with different cheese yield, the area and the intensity of the four spots correspondent to the k-casein fractions (spots numbers:20, 19, 16, 18) were calculated by using Melanie II (Bio-Rad) software. The value of the mean intensity of each spot multiplied by the respective area is reported in Figure 2. Samples with high cheese yield (n. 2 and 4) showed higher value in correspondence of spot 16 and lower values of spots 18, 19, 20. Other studies has been undertaken in order to better clarify the level of significance of the k-casein differences between buffaloes with high *vs.* low cheese yield of milk, as well as to reveal further differences in other casein fraction.

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