DETECTION OF ADULTERATION OF GOAT MILK WITH CHEESE WHEY BY WESTERN BLOT

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Introduction. In Mexico and all over the world the demand for goat milk products has increased gradually by its biological-chemical properties and health benefits. A common practice of milk producers is its adulteration with cheese whey (CW), which affects milk processing industries as it has a negative effect on yield and nutritional characteristics of the product to obtain. Therefore, it is necessary a rapid, effective, sensitive and accurate methodology to detect CW in goat milk. Immunoassays have these requirements. Glycomacropeptide (GMP) is obtained from the κ-casein during cheese making by the action of chymosin, being present in CW but not in raw milk.
In this work it was performed one immunoassay, the Western blot, to identify goat GMP (GMPc) as indicative of milk adulterated with CW, using a polyclonal anti-GMP bovine (GMPb) antibody.

Methods. GMPc extraction: Liquid samples of goat CW were mixed with a trichloroacetic acid (TCA) solution to a final concentration of 0.49 mol.L−1, in order to precipitate κ-casein which cross-reacts with GMP. To recover and concentrate GMPc, a second TCA treatment to a final concentration of 0.86 mol.L−1 was developed to the supernatant. The precipitate was dissolved in 300 µL of PBS.Protein samples were separated by 13.5% SDS-PAGE. The proteins were transferred to polyvinylidene difluoride membranes by electroblotting. GMPc detection was performed with polyclonal anti-GMPb antibody and with alkaline-phosphatase-conjugated secondary antibody.

Results. The antibody detects two protein fractions (11.26 and 9.12 kDa) corresponding to GMPc, with different molecular weights from those of commercial GMPb (13.92 kDa and 9.78 kDa) (fig 1).

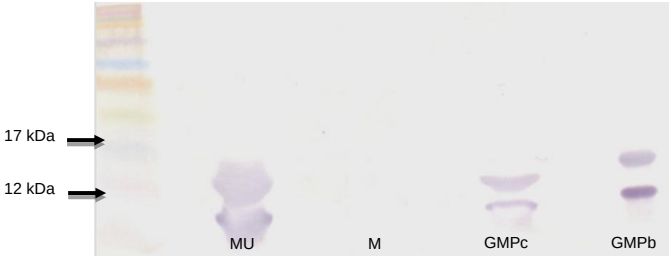

Fig.1. MU: 5 µL of unprocessed goat milk (without TCA) M: 20 µL of TCA processed goat milk. GMPc : 20 µL of TCA processed goat milk containing 20% (v/v) of cheese whey. GMPb: 20 µg of pure GMPb pure. Molecular weight markers and protein sizes (kDa) are indicated in the left side of the gel.

The effectiveness of this procedure was demonstrated by Western blot analysis of processed and unprocessed milk samples, revealed with the polyclonal anti-GMPb antibody. No bands appeared when goat milk was processed by TCA precipitation, indicating the total removal of the κ-casein from the sample and the absence of GMPc. Besides, it showed that the antibody does not cross-react with other milk components. The western blot has a sensitivity of 0.5% v/v, with a full time development of 8h.

Conclusions. Antibodies "anti-GMPb" were reactive to GMPc. This is important because with these antibodies we could develop a Western blot immunoassay to detect and quantify SQ goat milk in a quick and accurate.
This immunoassay can be used in milk industries to detect goat milk adulteration with cheese whey by western blot.

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