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## Evaluation of allergenic potential of protein ingredients through *in vitro* methods

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Protein hydrolysates are used in special infant formulas for dietary treatment of allergy to cow's milk protein in infants<sup>(1)</sup>. The aim of this study was to characterise two different milk casein hydrolysate (HCN-1 and HCN-2) and one whey protein hydrolysate (HWP), and to evaluate their potential for allergenicity in *in vitro* studies. The IgE reactivity was evaluated using sera of patients with clinically demonstrated allergy to cow's milk proteins that contains  $\geq 20$  KU/l of specific IgE antibodies towards milk proteins, measured using FEIA-CAP System (Pharmacia diagnostics, Uppsala, Sweden).

IgE binding was measured using an indirect ELISA coupled to a signal amplification system (ELAST ELISA amplification System, Perkin Elmer Life Sciences, Waltham, MA, USA<sup>(2)</sup>). Each sample was also fractionated into three different fractions using Centriprep columns (Amicon): L-Fraction ( $>10$  kDa), M-Fraction ( $<10$  kDa–3 kDa) and S-Fraction ( $<3$  kDa) and IgE reactivity was measured as previously described. In addition, reactivity against several commercial antibodies anti-casein, anti- $\beta$ -lactoglobulin and anti- $\alpha$ -lactalbumin was measured in each product.

Hydrolysates presented no reactivity against IgE in serum of allergic patients when they were used at concentrations similar to native protein (0.0025 mg/ml). However, when they were used in concentrated form (1000  $\times$ ), HCN-2 showed the lower reactivity, whereas HCN-1 and HWP showed moderate reactivity (Fig. 1). Tests with protein fractions showed that reactivity in the low molecular weight fractions ( $<3000$  Da) was lower than reactivity in the other two fractions. Again HCN-2 fractions also showed the lower IgE reactivity compared to CN-1 and HWP.

HCN-1 showed reactivity against anti-caseins antibodies and HWP also showed reactivity against antibodies anti- $\beta$ -lactoglobulin and anti- $\alpha$ -lactalbumin and to anti-caseins, whereas HCN-2 showed very low reactivity against anti-caseins. In conclusion, we have thus identified a casein hydrolysate that shows a very low *in vitro* reactivity (HCN-2). Even though clinical evidence is still a must, *in vitro* allergenic approach may help to predict reactivity in allergic individuals.

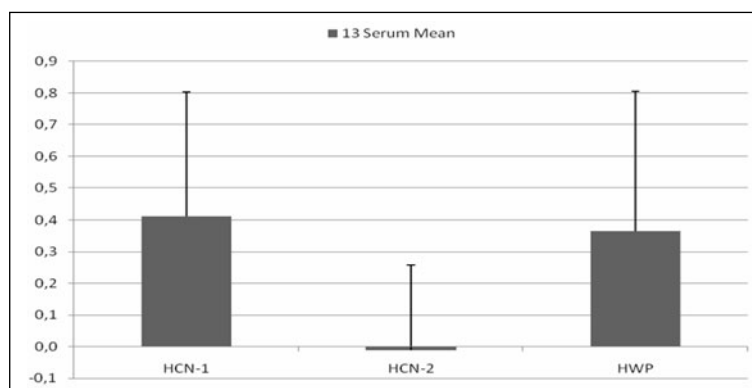


Fig. 1. IgE reactivity.

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- Chicón R, López-Fandiño R, Alonso E *et al.* (2008) *J Dairy Sci* **91**, 928–938.