Evaluation of allergenic potential of protein ingredients through \textit{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\textsuperscript{(1)}. 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 \textit{in vitro} studies. The IgE reactivity was evaluated using sera of patients with clinically demonstrated allergy to cow’s milk proteins that contains \textgtr; 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\textsuperscript{(2)}. 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 \textit{in vitro} reactivity (HCN-2). Even though clinical evidence is still a must, \textit{in vitro} allergenic approach may help to predict reactivity in allergic individuals.

\begin{figure}[h]
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\includegraphics[width=0.5\textwidth]{Fig_1.png}
\caption{IgE reactivity.}
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