Immunoenzyme determination of main allergic proteins in dairy products and control of their values in hypoallergenic food stuffs

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Introduction

Cow's milk and products derived from it are an essential component of a food ration for many people and a source of a number of valuable biologically active compounds. However, at the same time, milk is one of the most common food sources of allergens. Different processing technologies are actively developed to reduce the allergenicity of dairy products while preserving their nutritional value and functional properties. In this connection, analytical methods are required for correct assessment of the content of allergenic components in dairy products.

Aim

The study was focused on immunodetection of such main allergenic compounds of milk and dairy products as β-lactoglobulin (BLG), α-lactalbumin (ALA) and bovine serum albumin (BSA).

Materials & Methods

Enzyme-linked immunosorbent assays (ELISA) have been developed. BGL/ALA/BSA were immobilized in microplate wells. For the assays BGL/ALA/BSA were diluted to obtain solutions in the range from 5 ng/ml to 100 mkg/ml. Antibodies specific to the corresponding proteins were used for binding with allergen-containing samples. After this, incubation with peroxidase-labeled anti-species antibodies, catalytic reaction and photometric measurement were carried out.

Results & Discussion

Concentration and kinetic dependences of the analytical interactions were studied and the optimal modes of immunodetection were determined. The chosen protocols allowed carrying out all ELISA stages at room temperature. Advantages of competitive assay format for the detection of allergenic structures in partially hydrolyzed proteins have been confirmed in comparison with sandwich assay format. The developed ELISAs were characterized by detection limits of 10, 4.5, and 13.4 ng/mL for BLG, ALA and BSA, respectively. The BLG ELISA was used for testing milk and dairy products, including products with reduced allergenicity that were obtained by enzymatic hydrolysis of raw milk.

Conclusion

The developed methods allow controlling both intermediate technological products in dairy industry and final food stuffs. Their use will provide efficient protection of sensitive consumers from milk allergens.

Acknowledgements

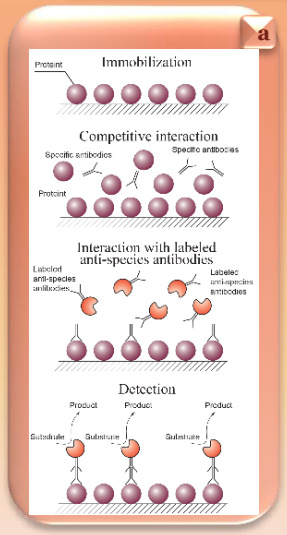
This investigation was supported by the Russian Science Foundation (grant 14-16-00149).

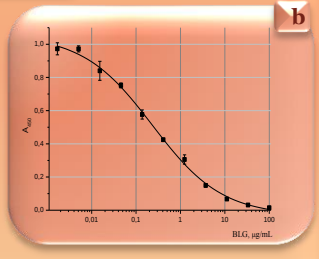
Table

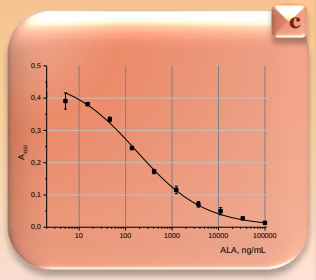
Influence of the competitive stage duration on analytical characteristics of BLGELISA

Duration of the competitive stage, min	IC20, μg/mL	IC50, μg/mL	IC80, μg/mL
60	12,10	0,86	0,07
45	11,70	0,96	0,09
30	9,77	0,75	0,07
15	13,20	1,20	0,16

Graphs







a: Scheme of competitive ELISA format, b: Calibration curve for BLG, c: Calibration curve for ALA