# Libro de Resúmenes

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## 050- COMPARATIVE STUDY OF THE ANTIMICROBIAL ACTIVITY OF KEFIR GROWN UNDER DIFFERENT SUBSTRATE

*Amieva MI[1,1,2], Delfini CD[1], Quiroga E[2,3], Villegas LB[1,2]*
*[1]Instituto de Química de San Luis (INQUISAL). CONICET-UNSL San Luis.*
*[2]Área de Bromatología. Departamento de Farmacia. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis.*
*[3]Instituto de Instituto de Física Aplicada (INFAP) - CONICET-UNSL San Luis.* ***tati.amieva.81@gmail.com***

Kefir is an ancient beverage, slightly acidic and alcoholic fermented that originated in the Caucasian region of Asia. Kefir is a natural fermented product comprised of a probiotic bacteria and yeast complex that coexist in symbiotic association. Kefir consumption has been associated with many advantageous properties to general health, including as an antioxidative, anti-obesity, anti-inflammatory, anti-microbial, and anti-tumor moiety. Generally, kefir may be identified depending on the type of substrate used for fermentation, which are dairy and non-dairy kefir. The different manufacturing conditions of kefir (agitation; the inoculum concentration; as well as the fermentation time and temperature) may alter the original characteristics of the microbial composition, hence affecting their health-giving properties. Therefore, this study aims to comparative the antimicrobial activity of kefir grown under different substrate.
Kefir drinks were prepared from three different substrates: 0% milk fat (4.9 g% carbohydrates), water (4.8g% Muscovado sugar) and LB lactose (4.5g% lactose). A total of 3 g of kefir grains were inoculated in 30 mL of each substrate (10% w/v). Erlenmeyer flasks were incubated at 28°C and 100 rpm. Samples were taken at 24 (T1), 48 (T2), 72 (T3), 120 (T4) and 168 h (T5). The supernatants were obtained by centrifugation at 10,000 xg for 10 minutes. Antimicrobial activity was determined by diffusion in agar on Petri dishes containing the LB for bacteria and potato-glucose agar for fungi. The target strains used were: *E. coli*, *Staphylococcus aureus*, *Fusarium sp.*, *Aspergillus sp*. and a fungus isolated from bread without identified yet.
The antimicrobial activity varied according to the type of kefir and the fermentation time, and was found after T3. The supernatants of water kefir presented the best results of microbial activity, followed by the milk kefir, while the kefir that grew in LB-lactose did not show activity. The water kefir supernatant inhibited four of the five target strains. The most sensitive microorganisms to it were *E. coli*, followed by *Aspergillus* sp., the mold isolated from bread and *Fusarium* sp. *Staphylococcus* was not inhibited by any of the supernatants. The pH of the three types of kefirs decreased as the incubation time increased. The antimicrobial activity of milk and water artificially acidified with acetic acid and lactic acid was evaluated as control but no inhibitory activity was obtained for any of the target strains. Therefore, supernatants from kefir could be attributable to antimicrobial metabolites in supernatants rather than the low pH. Further research is necessary to study the compounds responsible for these functional properties and their stability for its use as food additive.

## 051- OPTIMIZATION OF PURIFICATION AND IDENTIFICATION METHODS OF IGY FROM HEN EGGS

*Poggio L[1], Isaquirre A[1], Dávila S[1], Mattar Domínguez MA[1]*
*[1]Área Microbiología e Inmunología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. E-mail:* ***mattardominguezmariaaida@gmail.com***

Egg yolk immunoglobulin (IgY) biotechnology has many advantages over mammalian antibodies and is used in Veterinary Medicine for passive immunization against bacterial infectious diseases. The method of obtaining IgY guarantees animal welfare and produces a high concentration of antibodies. Like IgG, IgY is a compound antibody with two heavy chains of between 67-70 kDa and two light chains of 25 kDa, linked by disulfide bridges; with a molecular weight of ~150kDa. IgY does not activate complement, does not bind to proteins A and G, does not bind to mammalian antibodies, reducing the risk of obtaining false-positive reactions in immunoassays, and does not bind to the cell surface of the Fc receptor. All these differences have allowed the application of IgY in different methods in research areas such as diagnosis, medicine, and biotechnology. The aim of this work was to optimize the protocols for the separation, purification, and identification of IgY in our laboratory. The high lipid content of egg yolk interferes with purification steps. It is for this reason that IgY purification requires an initial delipidation by centrifugation at 8000 x *g* for 12 min at 4 °C. The lipid-free supernatant was stored at -20 overnight to eliminate proteins sensitive to freezing. The fractionation was carried out with ammonium sulphate. The sample was separated by chromatography using ion exchange columns (HiTrapDEAE FF) and Tris buffer solutions (pH=8) with different NaCl molarities (0 mM to 250 mM). The proteins collected were subjected to 10% and 8% polyacrylamide gel electrophoresis under non-reducing and reducing conditions. For identification, ELISA and Western Blot assays were performed. For ELISA the plates were coated with the different eluids of IgY and subsequently, they were confronted with different dilutions of a conjugated specific anti-IgY rabbit antibody. For WB analysis, protein samples of electrophoresis were transferred to nitrocellulose membranes and subsequently, they were marked with different dilutions of a conjugated specific for subsequent determination by chemiluminescence. In SDS-PAGE, the bands corresponding to both whole molecule-IgY (141-148 kDa) and their heavy (64-73 kDa) and light (23-29 kDa) chains were obtained. The presence of IgY could be determined by ELISA and identified by WB in the eluted obtained at the different NaCl molarities tested. The eluted at the different molarities gave a significant difference compared to the negative controls in an order of $10^{-6}$ with the 1/5.000 conjugate, by ELISA. In WB, better results were obtained with the 1/20.000 conjugate at the different molarities. The three techniques