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Bioactive proteins against pathogenic and spoilage bacteria

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ABSTRACT

Background: It is likely that both human nutrition and the nutrition of livestock are benefited by the presence of bioactive proteins within their respective diet regimes. Bioactive proteins have been defined as specific protein fragments that positively impact bodily functions or conditions and may, ultimately, influence overall human health. The ingestion of bioactive proteins may have an effect on the major body systems—namely, the cardiovascular, digestive, immune and nervous systems. According to their functional properties, bioactive proteins may be classified as antimicrobial, antithrombotic, antihypertensive, opioid, immune-modulatory, mineral binding and anti-oxidative. There are many examples of biologically active food proteins and active peptides that can be obtained from various food protein sources. They have a physiological significance beyond the pure nutritional requirements; in other wordsthey have the acquisition of nitrogen for normal growth and maintenance.

Objective: This study aims to specify and characterize the extent and mode of action of bioactive proteins in their native form, (glycinin, glycinin basic sub-unit and β-conglycinin) against specific main pathogens (*Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella enterica serovar Enteritidis*). We will be using standard media while identifying the main constituents responsible for this action.

Methods: Glycinin, basic sub-unit and β-conglycinin were isolated from soybean protein and tested for their antimicrobial action against pathogenic and spoilage bacteria, They were thencompared to the properties of penicillin. Methylated soybean protein and also methylated chickpea protein (MSP and MCP), with isoelectric points around pI 8, were prepared by esterifying. 83 % of their free carboxyl groups and their interactions with Gram positive and Gram negative bacteria were examined.

Results: The three divisions of cationic proteins exhibited antibacterial activities equivalent to or higher than the activity of penicillin, with the basic sub-unit exhibiting the highest activity,

followed by glycinin.; β-conglycinin exhibited the lowest level of activity with a MIC of 50, 100 and 1000 μg/mL, respectively. The $IC_{50\%}$ values of the basic subunit, glycinin and β-conglycinin, against *Listeria monocytogenes,* were 15, 16 and 695 μg/mL; against *Bacillus subtilis* the values were 17, 20, and 612 μg/mL; and against *Salmonella Enteritidis* the values were 18, 21 and 526 μg/mL, respectively. Transmission electron microscopy images of *L. monocytogenes* and *S. Enteritidis* exhibited an increase in cell size and a separation of the cell wall from the cell membrane when treated with glycinin or basic sub-unit. The scanning electron microscopy of *B. subtilis* indicated signs of an irregular, wrinkled outer surface as well as the fragmentation, adhesion, and aggregation of damaged cells or cellular debris when treated with glycinin or the basic subunits; however not with penicillin. The proliferation of *L. monocytogenes*, *S. Enteritidis* and *Escherichia coli* O157:H7-when artificially inoculated in raw milk ,stored at 4 or 25 °C) was significantly ($P<0·05$) reduced by the glycinin sub-unit and nisin (0·5% w/v); but they were only slightly reduced by β-conglycinin and moderately reduced by lysozyme. The two substances (MSP and MCP) exhibited a concentration-dependent inhibitory action against two of the studied bacteria with a minimum inhibitory concentration of approximately 100 μg/mL. The supplementation of raw milk with esterified legume proteins (MSP and MCP) has significantly ($p < 0.05$) reduced the levels of TBC, PBC and PSC in raw milk stored at a temperature of 4 °C. This potentially will delaythe onset of spoilage of by four days.

Conclusion: Both glycinin and the basic sub-unit have a more swift antimicrobial action than that of penicillin. Basic sub-units exhibited the highest efficiency at killing bacterial cells, followed by glycinin, penicillin and β-conglycinin-with the lowest effect; while the bacteria most susceptible to the antimicrobial agents were shown to be *L. monocytogenes*, followed by *B. Subtilis* and *S. Enteritidis*- with the lowest susceptibility. The antibacterial action of glycinin was similar to the effects exerted by nisin, and was much more effective than lysozyme. The modified legume proteins have general antibacterial properties against both spoilage and pathogenic bacteria in raw milk preserved under refrigeration or at room temperature.

Keywords: bioactive proteins, pathogenic and spoilage bacteria,