



## ANTIMICROBIAL PEPTIDE FRACTIONS OF *Phaseolus lunatus* PROTEIN

Segura-Campos, M. (1); Ruiz-Ruiz, J. (2); Bojórquez-Balam, E.G. (1); Chel-Guerrero, L. (1); Betancur-Ancona, D. (1).

<sup>1</sup>Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Periférico Norte. Km. 33.5, Tablaje catastral 13615, Col. Chuburná de Hidalgo Inn, Mérida, Yucatán CP 97203, México. e-mail: bancona@uady.mx

<sup>2</sup>Departamento de Ingeniería Química-Bioquímica, Instituto Tecnológico de Mérida, Av. Tecnológico km. 4.5 S/N, 97118 Mérida, Yucatán, México.

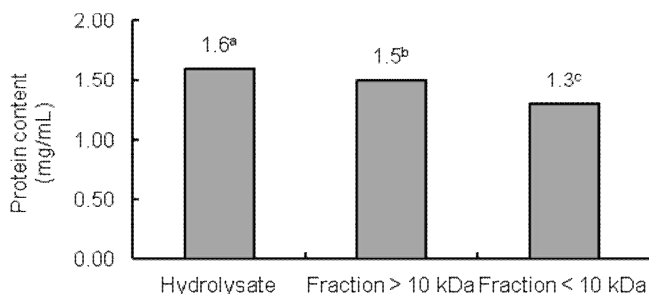
*Key words: Phaseolus lunatus, peptide fractions, antimicrobial activity.*

**Introduction.** Although potent antibiotics are available for the treatment of microbial infections, antimicrobial peptides derived from protein hydrolysates from animal and vegetal sources show advantages of being able to kill target cells rapidly, while having a broad spectrum of activity. In addition, as the rate of killing by peptides is higher than the rate of bacterial multiplication, the problem of drug resistance may be overcome (1). *Phaseolus lunatus* bean is a tropical legume which is rich in protein (29%) and has a nutritional quality comparable to that of other pulses like soybean.

The objective of this study was to evaluate the antimicrobial properties of hydrolysates and peptide fractions produced from enzymatic hydrolysates of *Phaseolus lunatus* bean protein concentrate.

**Methods.** Hydrolysis of the protein concentrates was done with the enzymes pepsin, pancreatin and the sequential system pepsin-pancreatin. Degree of hydrolysis was calculated by determining free amino groups. Hydrolysate was fractionated by ultrafiltration to obtain the peptide fractions. Peptide fraction protein content was measured using a dye-binding colorimetric assay. The antimicrobial activity against *Staphylococcus aureus* and *Shigella flexneri* was determined by the minimal inhibition concentration (MIC).

**Results.** The individual enzymes and the sequential system produced low degrees of hydrolysis: pepsin (5.5%), pancreatin (8.3%), and pepsin-pancreatin (7.8%). According to Denisson et al. (2), with a lesser degree of hydrolysis usually is obtained a greater antimicrobial activity. Hydrolysate obtained with pepsin was selected to obtain peptide fraction by ultrafiltration (Figure 1).



**Fig. 1** Protein content (mg/mL) of peptide fractions. Different superscript letters indicate statistical difference ( $P < 0.05$ ).

The protein content decreased proportionally according to the molecular cut-off weight membranes employed, showing the lower amount of protein in fraction  $< 10$  kDa. The antimicrobial activity of the peptide fractions was determined against strains of human-pathogenic bacteria. As shown in Table 1, the peptide fraction  $< 10$  kDa possessed antimicrobial activities against both gram-negative (*S. flexneri*) and gram-positive (*S. aureus*) bacteria, with the most potent inhibitory potency to *S. aureus*.

**Table 1.** MIC values of the peptide fraction ( $< 10$  kDa) derived from protein hydrolysate of *P. lunatus* to human-pathogenic bacteria.

| Human pathogenic bacterial strains | Inhibition (%) | MIC ( $\mu\text{g/mL}$ ) |
|------------------------------------|----------------|--------------------------|
| <i>Staphylococcus aureus</i>       | 100            | 392.04                   |
| <i>Shigella flexneri</i>           | 100            | 993.17                   |

**Conclusions.** This work is the first report of antimicrobial peptide derived from the legume *Phaseolus lunatus*. Pepsin hydrolysis made up peptide fragments but only peptides of mass  $< 10$  kDa had the potent inhibitory effect to the growth of both strains *S. aureus* and *S. flexneri*. The results suggested that the peptide derived from the legume *P. lunatus* may be used as food additive with antimicrobial activity or as therapeutic agent for treatment of the infectious diseases caused by different strains. Further research should be done to identify the amino acids sequence of the antimicrobial peptides and clarify its mechanism of action.

**Acknowledgements.** This research was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT) de México. Proyecto de ciencia básica "Actividad biológica de fracciones peptídicas derivadas de la hidrolisis enzimática de proteínas de frijoles lima (*Phaseolus lunatus*) y caupí (*Vigna unguiculata*)". Con número de convenio 153012.

### References

- McCann, K.B., Shiell, M.W.P., Lee, A., Wan, J., Roginski, H., Coventry, M.J. (2006). Int. Dairy Journal, 16, 316-323.
- Denisson, S., Wallace, J., Harris, F., Phoenix, D. (2005). Protein and Peptide Lett., 12(1): 31-39.