P-067 ***Vigna unguiculata* and *Phaseolus vulgaris* protein hydrolysates with antioxidant activity and potential bioencapsulation**

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INTRODUCTION AND OBJECTIVES

Vigna unguiculata (V) and *Phaseolus vulgaris* (P) are widely consumed legumes in southeastern Mexico with high protein content (21-23%) which increases up to 70% when subjected to alkaline extraction and isoelectric precipitation obtaining protein concentrates (CP) which when subjected to enzymatic hydrolysis generated hydrolysates whit proteins of low molecular weight, peptides and amino acids. Recent research has focused on the properties of food protein-derived peptides, their biological activities and potential health benefits. Many peptides and protein hydrolysates can lower the pace of lipid autoxidation process. They also play a role of the heavy metal acceptors and scavenge free radicals. Many artificial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have potential health hazards and their application must be under strict regulation. The development of natural antioxidants as alternatives is of great interest for researcher. Protein hydrolysates can be potentially applied as additives to many of food products although their darkening and low fat solubility make it impossible to apply them as the antioxidative additives to fats and oils. An alternative to using these hydrolysates in food is through the encapsulation in liposomes. The liposomes are colloidal systems formed by aggregates originated from aqueous dispersions of phospholipids, and their structural characteristics allow them to encapsulate or incorporate hydrophilic, hydrophobic or amphiphilic substances.

Taking this into account, the present study objective was to evaluate the antioxidant and chelating capacities of protein hydrolysates from *V. unguiculata* and *P. vulgaris*, with the determination of the biological activity of the hydrolysates could raise their encapsulation in lyophilized liposomes.

MATERIALS AND METHODS

Hydrolysis of the protein isolates was done using a totally randomized design. Treatments were the sequential enzymatic system applied: Alcalase® (A) 2.4L FG and Flavourzyme® (F) 500M (Novo Nordisk, Bagsvaerd, Denmark); or pepsin from porcine gastric mucosa (Sigma, P7000-100G) and pancreatin from porcine pancreas (Sigma, P3292-100G). The hydrolysis with the Alcalase®-Flavourzyme® system (AF) was done according to Pedroche et al. (2002). The hydrolysis with the sequential pepsin-pancreatin system (PP) was done according to Megías et al. (2004. The response variable was the degree of hydrolysis (DH), calculated by determining free amino groups (Nielsen et al. 2001) The reducting power was determined according to Oyaiz (1986) and the chelating capacity of Fe and Cu were determined according to Dinis et al. (1994).

RESULTS AND DISCUSSION

The protein contents of the CP were 65.6% (*V. unguiculata*) and 67.7% (*P. vulgaris*). Both enzymatic systems produced high DH (Table 1).

Table 1: Degree of hydrolysis obtained with Alcalase, Flavourzyme, AF and PP.

Enzyme system	DH (%)	
	V. unguiculata	*P. vulgaris*
Alcalase	53.0	-
Flavourzyme	58.8	-
AF	-	43.01
PP	35.7	23.61

Variation in DH values was probably the result of sequential enzymatic system hydrolytic specificity. The AF system consists of endoproteinases and exopeptidases. This system presents broad activity and hydrolyzes more peptide bonds than the highly specific PP system which exhibits lower catalytic action.

The hydrolysates exhibited reducing power that indicated its antioxidant capacity (Figure 1).

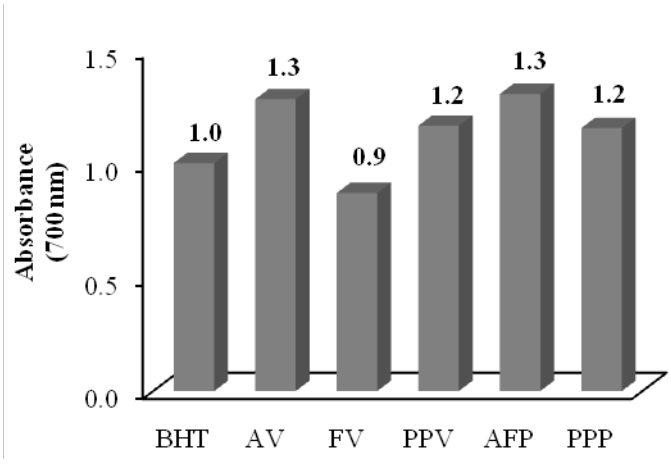

Figure 1: Reducing power of the hydrolysates obtained with Alcalase, Flavourzyme, AF and PP from *V. unguiculata* and *P. vulgaris*.

The hydrolysates showed higher reducing power than BHT except the hydrolysate obtained with Flavourzyme from *V. unguiculata*. AV, PPV, AFP and PPP had the ability to reduce free radicals by hydrogen transfer like BHT. Furthermore, FV did not show a reduction mechanism of hydrogen transfer so that this hydrolysate should reduce reactive oxygen species by the mechanism of electron transfer.

The hydrolysates exhibited ion chelating capacity (Figures 2 and 3).

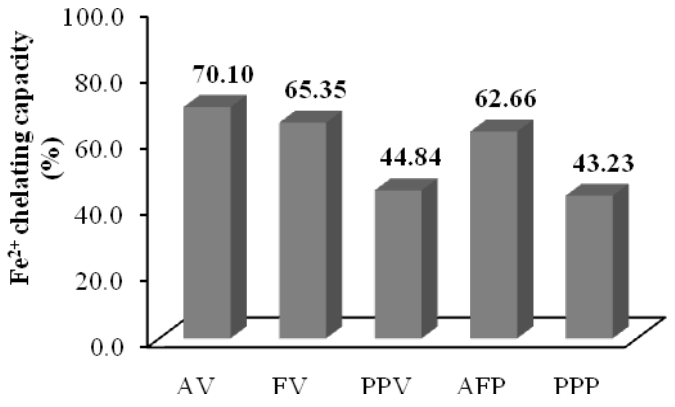

Figure 2: Fe^{2+} chelating capacity of the hydrolysates obtained with Alcalase, Flavourzyme, AF and PP from *V. unguiculata* and *P. vulgaris*.

Hydrolysates showed chelating ability, as they continued dependence between the concentration of protein and the percentage of chelation, which follows the same trend BHT used as a reference. The hydrolysates obtained with Alcalase, Flavourzyme and AF exhibited the greatest chelating capacities for both metallic ions.

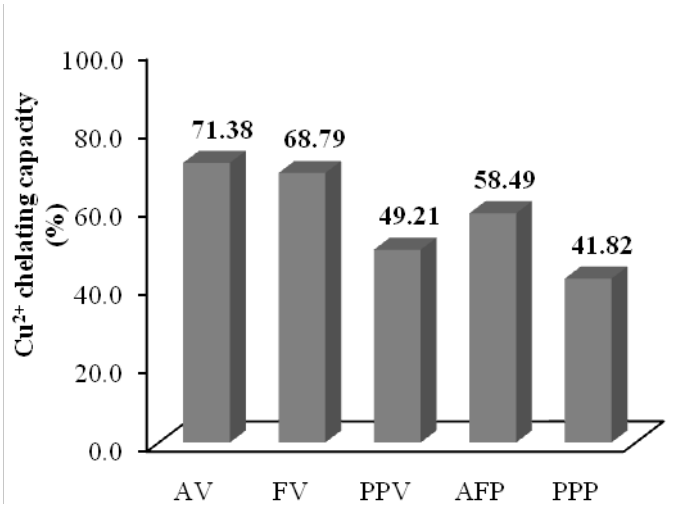

Figure 3: Cu^{2+} chelating capacity of the hydrolysates obtained with Alcalase, Flavourzyme, AF and PP from *V. unguiculata* and *P. vulgaris*.

The evaluation of the metal ion chelating capacity is critical because the metals are capable of initiating lipid peroxidation reactions of unsaturated fatty acids.

CONCLUSIONS

The results indicated that the enzyme systems used may generate peptides with biological activity, which gives to the hydrolysates a high antioxidant activity due to its ability to transfer hydrogen and chelate metal ions. However its low fat solubility makes it impossible to apply them as additives in fats and oils, so that will be encapsulated in liposomes and lyophilized at laboratory scale to establish the feasibility of incorporating them in food.

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