Production of Protein hydrolysates from tuna (*Thunus spp.)* and *Carica papaya* by-products with radical scavenging activity determined by spectrophotometry and electron paramagnetic resonance

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Introduction. Fish processing by-products are raw material to obtain protein hydrolysates with potential use in animal feed or media formulation, in addition to human consumption as nutraceuticals and food additives.[1] Protein hydrolysis takes place by endogenous fish enzymes or by addition of plant, animal or microbial proteases to obtain peptides in wide molecular weight distributions. The aim of this work was to produce protein hydrolysates from agricultural and fishery by-products by a simple processing throughout lactic acid bacteria (LAB) fermentation (LAF). Furthermore, radical scavenging property was investigated.

Methods. Fishery by-products consist in dark meat, viscera, bones and fins from tuna (*Thunnus spp*). Agricultural by-products are papaya fruit *(Carica papaya)* and molasses. Culture starter was *Lactobacillus spp.* Ingredient proportions were assayed to obtain product stability. Degrees of hydrolysis (DH) and protease activities were determined during LAF. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was monitored spectrophotometrically and the data were analyzed by Probit, in order to determine IC_{50}.[2] Radical scavenging activity was also determined by electron paramagnetic resonance (EPR). Protein hydrolysates were injected through SEP-Pack C18 cartridges for removal of interferences. Molecular weight (M_w) was determined by Tricine SDS-PAGE protocol for peptides in the fractions.[3]

Results. pH decreased and TTA increased due to LAB activity, which promotes stability. A high DH was achieved (87.7%) during LAF (120h), which might be ascribed to acidic and neutral proteases (Fig. 1). Acidic protease activities had an important contribution during first days of LAF. Later on, neutral proteolytic activities remained for the rest of fermentation time. Radical scavenging activity determined by spectrophotometry remained during the fermentation without significant changes (Fig. 2). This was confirmed with EPR assays and calculation of relative percentage (%R) (Fig. 3).
Fig.4 shows the Tricine SDS-PAGE gel corresponding to proteins of the initial and final LAF times. The protein bands in the M_w range 12-13 kDa remained in the protein lane of the final fermentation time; this might be responsible for sustained radical scavenging activity during LAF.

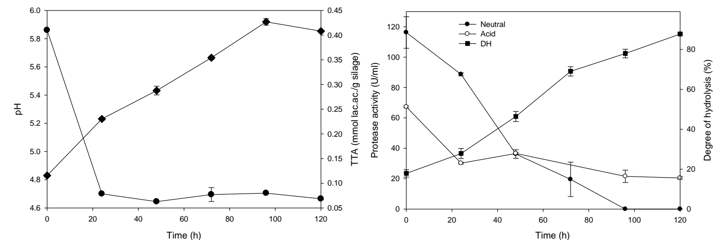
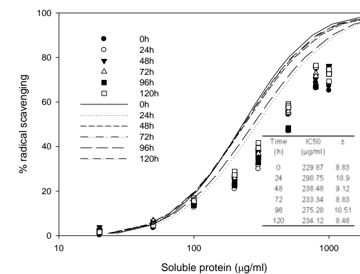
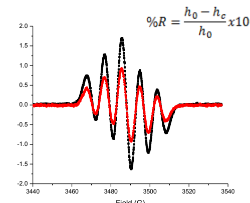


Fig. 1 Time course changes in LAF: a) pH and TTA (expressed as lactic acid); b) Degree of hydrolysis (DH), neutral (N) and acidic (A) protease activities.



Time (h)	IC50 (μg/ml)	±
0	229.87	8.83
24	290.75	10.9
48	238.48	9.12
72	233.34	8.83
96	275.28	10.51
120	234.12	8.48

Fig. 2 Probit plots of DPPH radical scavenging data of initial and hydrolysates during fermentation times. Estimated IC_{50} values.



$$\%R = \frac{h_0 - h_c}{h_0} x100$$

Fig. 3 EPR spectra for a) blank DPPH solution, b) initial fermentation time (%R=49.39% with IC_{50}=229.87μg/ml).

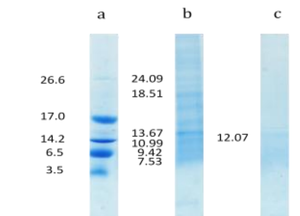


Fig.4 Tricine SDS-PAGE: a) protein low M_w standard; b) initial (0h) and c) hydrolysates (120h).

Conclusions. The LAF conducted produced stability, high degree of protein hydrolysis and peptides with antioxidant activity.

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