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Obtention Of Bioactive Peptides From Porcine Liver Hydrolysates (#129)

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Introduction

Bioactive peptides are defined as short sequences of roughly 2-20 amino acids residues that are encrypted in their parent protein. Enzymatic hydrolysis is the most common way to produce biopeptides (Nafajian et al., 2014). They exhibit benefits on the human health due to their several biofunctional properties such as antimicrobial, antioxidant, anti-hypertensive, antithrombotic, antiviral and opiate among the most highlighted (Bhat et al., 2015). By-products are a good potential as valuable sources of peptides to add into functional foods and to use as natural preservatives in foodstuffs. Likewise, they are capable of inhibiting against microorganism like fungi, viruses and bacteria (Lafarga et al., 2017). Natural antioxidants are widely used to retard or decrease the extent of oxidative deterioration (Lorenzo et al., 2018a). They are important in the prevention of human diseases and in the inhibition of lipid oxidation in foods (Lorenzo et al., 2018b). Therefore, the objective of this study was to determine the antimicrobial and antioxidant capacities of liver hydrolysates extract obtained from three different proteases.

Methods

Fresh porcine livers were diced and frozen. Then they were homogenized with ice (1:1 w/w) in a cutter. Three different enzymes (BIOCON, Spain) were used under following conditions: papain (6000 USP): 37 ºC and pH=6; bromelain 2000: 40 ºC and pH=6, and alcalase (Bioproteasa LA 660): 50 ºC and pH=8. The enzyme-substrate ratio was 1:100 (w/w). Hydrolysis took place in an orbital shaker-incubator (125 rpm) at specific temperature of each enzyme, keeping constant to the corresponding pH by adding of HCl or NaOH 1N for 10 hours. Enzymes were heat inactivated at 95 ºC for 3 minutes. Afterwards, mixtures were centrifugated at 4000 rpm for 5 min. The hydrolysates were fractionated using regenerated cellulose membranes with a molecular weight cut-off of 5 KD MWCO (Millipore, Germany). Four antioxidant methods were determined ORAC, FRAP and ABTS and DPPH radical scavenging activity (Huang et al., 2002; Benzie et al., 1996; Re et al., 1999; Brand-Williams et al., 1995, respectively). Total phenolic content (TPC) was measured according to the method described by Medina-Remón et al. (2009). Antimicrobial activity from liver hydrolysates obtained was tested using an agar well diffusion modified method (Ramirez et al., 2012) to examine the effect on the growth of Gram-negative bacteria (E. *coli*, *Salmonella enteric*, P. *aeruginosa*) and Gram-positive bacteria (*Brochothrix thermosphacta,* S. *aureus,* L. *monocytogenes*). Wells of 6 mm diameter were made using a sterile cork-borer. Positive controls consisted of gentamicin and nisin for Gram-negative and Gram-positive bacteria and sterile water was the negative control for both. The effect of type of enzyme on antioxidant and antimicrobial capacity was examined using a one-way ANOVA, with IBM SPSS Statistics 23 software package.

Results

Concerning to antibacterial activities, liver hydrolysates only inhibited *Brochothrix thermosphacta* growth. The strongest activity was achieved with papain since the percentage of inhibition was of 90%, followed by alcalase and bromelain with 71% and 60% of inhibition, respectively. This finding is in agreement with data reported by Verma et al. (2017) who observed that trypsin and papain hydrolysates from porcine liver presented higher inhibited against *L. monocytogenes, E.coli* and *S. aureus* growth compared to those form alcalase hydrolysates extracts. The antibacterial efficacy of porcine liver hydrolysate might be due to cationic property and hydrophobicity of peptides (Verma et al., 2017).

In terms of antioxidant capacity, statistical analysis showed significant ($P<0.05$) differences in all assays studied (TPC, DPPH, ABTS, ORAC and FRAP). Extracts obtained with alcalase exhibited the highest DPPH, ABTS and FRAP values, whereas between papain and bromelain no notable differences were found. This finding is in agreement with data reported by Verma et al. (2017) who observed that trypsin and papain hydrolysates from porcine liver presented higher ABTS values compared to those form alcalase hydrolysates extracts. The activity of hydrolysates to scavenge ABTS radicals depend on various factors such as the type of enzyme, degree of hydrolysis, solubility of hydrolysates, class of peptides and existence of free amino acids (Phanturat et al., 2010). Regarding the TPC and ORAC assays, hydrolysates from papain presented the lowest values, whereas the highest levels were found in extracts obtained from bromelain.

Conclusion

It can be concluded from the present study that antioxidant (TPC, DPPH, ABTS, FRAP and ORAC assay) and antimicrobial capacity of porcine liver hydrolysates was affected by type of enzyme used. The best results were observed in alcalase hydrolysates extracts.

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Table 1. Antioxidant capacity of porcine liver hydrolysates at 10 hours, 5KDa.

	10 Hours 5 KDa				
	Papain	**Alcalase**	**Bromelain**	**SEM**	**Sig.**
TPC (mg gallic acid/100g)	156.60^{a}	213.33^{b}	214.34^{b}	9.648	***
DPPH (µg trolox /g)	189.90^{a}	448.35^{b}	198.07^{a}	42.488	***
ABTS (mg ascorbic acid/100g)	291.38^{a}	712.27^{b}	301.54^{a}	69.560	***
FRAP (µmol Fe^{+2}/100g)	36.97^{a}	46.33^{b}	36.79^{a}	1.797	*
ORAC (mg trolox /g)	24.33^{a}	40.32^{b}	46.94^{c}	3.412	***

SEM. standard error of the mean; Sig. significance; *** (P<0.001), * (P<0.05). $^{a-c}$ Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly (P<0.05).

Table 1.

Notes