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Evaluation Of Antioxidant And Antimicrobial Activities Of Peptides Obtained From Porcine Liver Using Flavourzyme (#266)

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Introduction

Porcine liver is a by-product generated by meat industry during slaughtering. It is an extraordinary source of vitamins, among we can highlight the riboflavin, retinol, niacin, folacin, ascorbic acid and vitamins B6 and B12 (Das et al., 2018). It contains great amounts minerals such as iron and manganese (Liu, 2002). In addition, it is also characterized by its high cholesterol and PUFA content and by its low levels of MUFA (Jayathilakan et al., 2012). It is been employed to produce bioactive peptides with diverse functionalities such as antioxidant and antimicrobial (Lorenzo et al., 2018). Bioactivity and functionality of peptides depend mainly on the hydrolysis conditions: pH, time, type of protease and ratio of substrate/enzyme used (Verma et al., 2017). Due to its beneficial properties, the synthesis of hydrolysates rich in peptides is gaining more importance in wide range of applications such as pharmacy, cosmetics and food. The aim of this study was to assess the antioxidant and antimicrobial activities of peptides obtained from porcine liver using flavourzyme.

Methods

Raw porcine livers were diced and frozen. Then they were homogenized with ice (1:1 w/w) in a cutter. Hydrolysis were carried out using flavourzyme (1000L, Novozymes, Denmark) with enzyme substrate ratio 1:100 (E:S ratio w/w) under orbital shaker-incubator (125 rpm) at 50 ºC, keeping constant pH of 5.5 with HCl 1N for 4, 6, 8 and 10 hours. Enzyme was deactivated heating at 95 ºC for 3 minutes. Afterwards, mixtures were centrifugated at 4000 rpm for 5 min. Ultrafiltration was the purification method utilized regenerated cellulose membrane with a molecular weight cut-off of 10KDa MWCO (Millipore, Germany) was employed. 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 for Gram-negative bacteria and nisin for Gram-positive bacteria and sterile water was the negative control for both. Total phenolic content (TPC) was assessed according to the method described by Medina-Remón et al. (2009). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) were also performed for determining the antioxidant capacity (Brand-Williams et al.,1995, Re et al., 1999, Huang et al., 2002, Benzie et al., 1996, respectively). The influence of incubate time on antioxidant and antimicrobial capacity was examined using a one-way ANOVA, with IBM SPSS Statistics 23 software package.

Results

Statistical analysis did not show significant differences ($P>0.05$) on TPC, ABTS and ORAC assays. Conversely, DPPH and FRAP values decreased significantly with higher hydrolysis times. Between 6 and 8 hours no differences were found. These findings are in agreement with data reported by Yu et al. (2017) who found that DPPH and FRAP values declined with the hydrolysis time using papain in the same matrix. However, they observed an inversed effect using pepsin and alcalase enzymes. In addition, Verma et al. (2017) noticed that antioxidant activities were better with higher hydrolysis times using papain and alcalase. On the other hand, Wang et al. (2018a) and Wang et al. (2018b) showed the FRAP and DDPH values increased during the first hour and then dropped till 3 hours of hydrolysis using flavourzyme in poultry meat.

Regarding the antimicrobial activity, none of the samples inhibited Gram-negative bacteria nor S. aureus. These results could be due to that Gram-negative bacteria are more resistant than Gram-positive (Chakka et al., 2015). In our study, hydrolysates at 4 (14 mm), 8 (9 mm) and 10 (18 mm) hours inhibited *Brochothrix* growth, whereas the zone of inhibition was of 16 mm and 6 mm, for positive and negative control, respectively. Moreover, L. *monocytogenes* was inhibited with the same strength as the positive control (nisin) at 10 hours (10 mm), whereas the negative control also was of 6 mm. The antimicrobial effect increased in accordance with the diameter of the inhibition zone formed. As we could see flavourzyme hydrolysates at 10 hours exhibited strongest antibacterial activity against *Brochothrix*. In the middle hours the activity was not so evident since it was not even detected at 6 hours.

Conclusion

It can be concluded that after 4 hours of incubation, we can obtain extract from porcine liver with the highest antioxidant capacity using flavourzyme. Regarding antimicrobial activity, our flavourzyme extracts only showed inhibition against to *Brochothrix* and *L. monocytogenes.*

Notes

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References

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25-30.

Chakka, A. K., Elias, M., Jini, R., Sakhare, P. Z., & Bhaskar, N. (2015). In-vitro antioxidant and antibacterial properties of fermentatively and enzymatically prepared chicken liver protein hydrolysates. *Journal of Food Science and Technology*, *52*(12), 8059-8067.

Das, A., Satyaprakash, K. (2018). Animal Byproducts - Boon to Human Health: A review. *International Journal of Livestock Research*, 8(11), 38-49

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry*, *50*(16), 4437-4444.

Jayathilakan, K., Sultana, K., Radhakrishna, K., & Bawa, A. S. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*, *49(3)*, 278-293.

Liu, D. C. (2002). Better utilization of by-products from the meat industry 2002-10-01. *Extension Bulletins. Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC publication database)*.

Lorenzo, J. M., Munekata, P. E., Gomez, B., Barba, F. J., Mora, L., Perez-Santaescolastica, C., & Toldra, F. (2018). Bioactive peptides as natural antioxidants in food products–A review. *Trends in Food Science & Technology*, *79*, 136-147.

Medina-Remón, A., Barrionuevo-González, A., Zamora-Ros, R., Andres-Lacueva, C., Estruch, R., Martínez-González, M. Á., ... & Lamuela-Raventos, R. M. (2009). Rapid Folin–Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Analytica Chimica Acta*, *634*(1), 54-60.

Ramírez Aristizabal, L. S., & Marín, D. (2012). Evaluación de la actividad antibacteriana de aceites esenciales y extractos etanólicos utilizando métodos de difusión en agar y dilución en pozo. *Scientia Et Technica*, *17*(50).

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237.

Verma, A. K., Chatli, M. K., Kumar, P. A. V. A. N., & Mehta, N. (2017). Antioxidant and antimicrobial activity of protein hydrolysate extracted from porcine liver. *The Indian Journal of Animal Sciences*, *87*, 711-717.

Wang, D., & Shahidi, F. (2018a). Protein hydrolysate from turkey meat and optimization of its antioxidant potential by response surface methodology. *Poultry Science*, *97*(5), 1824-1831.

Wang, D., Zhang, M., Zou, Y., Sun, Z., & Xu, W. (2018b). Optimization of Flavourzyme Hydrolysis Condition for the Preparation of Antioxidant Peptides from Duck Meat using Response Surface Methodology. *The Journal of Poultry Science*, 0160155.

Yu, H. C., Hsu, J. L., Chang, C. I., & Tan, F. J. (2017). Antioxidant properties of porcine liver proteins hydrolyzed using Monascus purpureus. *Food Science and Biotechnology*, *26*(5), 1217-1225.

Table 1. Antioxidant capacity of porcine liver hydrolysates extracts.

	Flavourzyme 10 KDa					
	4 h	**6 h**	**8 h**	**10 h**	**SEM**	**Sig.**
Antioxidant capacity						
TPC (mg gallic acid /100g)	153.65^{a}	162.22^{a}	155.77^{a}	152.18^{a}	1,.703	n.s.
DPPH (µg trolox /g)	446.87^{c}	430.53^{b}	426.07^{b}	397.85^{a}	5.487	***
ABTS (mg ascorbic acid /100g)	399.07^{a}	407.53^{a}	410.92^{a}	410.07^{a}	3.598	n.s.
FRAP (µmol Fe^{+2}/100g)	40.97^{c}	38.34^{b}	39.29^{b}	36.03^{a}	0.572	***
ORAC (mg trolox /g)	18.40^{ab}	21.72^{b}	9.80^{a}	18.06^{ab}	1.808	n.s.

SEM. standard error of the mean; Sig. significance; *** (P<0.001). n.s. (not significant). $^{a-c}$ 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