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Development and characterization of protein hydrolysates originated from animal agro industrial byproducts

Abstract

This study aimed at developing different enzymatic protein hydrolysates from animal-origin byproducts; pig liver (PL) and tilapia (*Oreochromisniloticus*) processing residue (TR), to be used in animal feed. The experimental design was completely randomized, 2x2 factorial, the first factor being the raw materials, TR and PL, and the second, the Alcalase® and Brauzyn® enzymes, with three replications. The temperature-controlled hydrolysis used an enzyme: substrate ratio of 1:200w/w. The products hydrolyzed with Alcalase® had higher levels of free amino acids compared to the other hydrolysates, regardless of the feedstock used. The TR hydrolysates had higher mean lipid contents especially monounsaturated fatty acids. Among the poly-unsaturated fatty acids, those of the n-6 series were predominant. The processing of PL and TR byproducts in the presence of Alcalase® and Brauzyn® resulted in products with potential for use in animal feed as flavoring and/or source of essential nutrients.

Keywords: biotechnology, enzyme complex, hydrolysate, industrialization, animal nutrition

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Abbreviations: PL, pig liver; TR, tilapia *(Oreochromisniloticus)* processing residue; A, alcalase®; B, brauzin®; CRD, completely randomized design; ChS, chemical score; RM, raw materials; E, enzyme; MM, mineral matter; PLA, pig liver hydrolyzed with alcalase®; PLB, pig liver hydrolyzed with brauzyn®; TRA, tilapia processing residue hydrolyzed with alcalase®; TRPB, tilapia processing residue hydrolyzed with brauzyn®

Introduction

The large volume of byproducts generated in the form of organic residues has led to the need of developing alternative uses; possibly creating new sources of revenue and sustainable use for industrial waste. In general, hydrolysis is an efficient process used to solubilize proteins, broadening their application range either as an ingredient or a new product.[1]

Protein hydrolysates are products that result from the action of proteolytic enzymes or chemical agents. These hydrolysates consist of protein fragments: peptides of different sizes; poly-, tri- and dipeptides and free amino acids, which greatly influence the taste of food.[2] The protein hydrolyzing process, by enzymatic route, has advantages related to the intrinsic characteristics of enzymes such as selectivity to substrates and processes carried out in milder controllable thermal conditions. These conditions minimize the development of secondary undesirable reactions, e.g., the formation of toxic compounds, ensuring the maintenance of the nutritional value of the product.[3]

The nutritional quality of the substrate is preserved when enzymatic hydrolysis is conducted under controlled conditions. In general, fish protein hydrolysates contain essential amino acids, in levels similar or superior to that of the reference protein of FAO/WHO.[4] In addition to the amino acids content, these byproducts also provide long chain fatty acids (C20 and C22) that are hardly found in tissues of terrestrial organisms.[5]

Among the proteases, Alcalase® (Subtilisin Carlsberg) is a serine protease widely used to produce enzymatic hydrolysates from various food-grade protein sources,[6] which cleaves into many types of peptide bonds, preferably those with hydrophobic side chain in the C-terminal portion.[7] Papain differentiates itself by being a cysteine protease, which acts on ester bonds and amide groups, hydrolyzing preferentially the bonds adjacent to the amino acids phenylalanine, valine and leucine.[8]

In order to develop an alternative use for animal origin agro-industrial byproducts, this study aims at producing, in industrial-scale, hydrolysates from pig liver (PL) and tilapia *(Oreochromisniloticus)* processing residue (TR) using the enzymes Alcalase® (A) and Brauzin® (B).

Materials and methods

Materials

The byproducts pig liver (PL) and tilapia processing residue (TR), consisting of heads and carcasses, were purchased refrigerated from the agro-industries located in Toledo, PR, Brazil. The raw materials were collected at the end of the slaughtering day, transported to the processing plant and kept under refrigeration until use. The enzymes used in the process were Alcalase® 2.4Lmanufactured by Novozymes Latino Americana Ltd., Paraná, Brazil and Brauzyn® 100 (papain), by Prozyn BioSolutions, Sao Paulo, Brazil.

The industrial process

The industrial process consisted of producing four hydrolysates on an industrial scale using pig liver (PL) and tilapia residue (TR) in the presence of the enzymes, Alcalase® and Brauzyn®. To this end, it was used a completely randomized design (CRD), 2x2 factorial, with the byproducts (PL and TR) as the first factor and the enzymes (Alcalase® and Brauzyn®) as the second factor, and three replications, totaling 12 plots, with fixed enzyme substrate ratio and process temperature.

Processing of the hydrolysates

The crushed (5.0mm) and homogenized raw materials were sent to an industrial jacketed stainless steel reactor with a total capacity of two tons and electric heating. Water and enzyme 100:15 (w/w) were added to the reactor under constant stirring. The enzyme/substrate ratio used was 1:200 (w/w) and temperatures of 60 and 65°C for Alcalase® and Brauzyn®, respectively. The pH was 6.5 and temperatures were maintained for 60 minutes. All processing conditions were established based on previous studies and tests.

At the end of hydrolysis, the enzymes were inactivated thermally at 85°C for 15minutes,[6] followed by the addition of phosphoric acid to adjust the products pH to 3.0, for the preservation. TR hydrolysates underwent filtration process in stainless steel sieve (1.0mm) to remove the bones and spines.

Proximate composition

The analyses of moisture, crude protein (Nx6.25), ether extract and ash content were performed according to AOAC.[9]

Total and free amino acids profiles

Total and free amino acids were determined by reverse phase liquid chromatography (RP - HPLC) using a HPLC system (Shimadzu Corporation, Tokyo, Japan) equipped with UV detector (254nm) and a Luna - Phenomene Y C18 column (250mm x 4.6mm, 5µ - Phenomenex Inc. Torrance, CA, USA).The amino acids were quantified.[10] Tryptophan was determined after enzymatic hydrolysis with pronase at 40°C, followed by colorimetric reaction with 4-dimethylaminobenzaldehyde (DAB), in 21.1 N sulfuric acid, and reading using a spectrophotometer at 590nm.[11]

Chemical score

To evaluate the order of the limiting amino acids in the different hydrolyzed products, a chemical score (ChS) was calculated as follows:

$$ChS = \frac{\text{mg amino acid/g test protein}}{\text{mg amino acid/g reference protein}}$$

The essential amino acids of the standard protein were considered as reference: histidine 1.50; isoleucine 3.0; leucine 5.90; methionine + cysteine 2.20; phenylalanine + tyrosine 3.80; threonine 2.30; tryptophan 0.60; lysine 4:50; and, valine 3.90 (g 100g of protein^{-1}). The standard protein for adults according to FAO/WHO[4] was chosen due to the high requirement compared to other animal species.

Fatty acids profile

After lipid extraction with petroleum ether in the extractor Butt,9 the samples were esterified.[9,12] The methyl esters of fatty acids were quantified by gas chromatography (VARIAN 3900 Mod) equipped with CP-SIL 88 capillary column, flame ionization detector (FID) and workstation with STAR software. The peaks were identified by comparison to SUPELCO IM 37 Component FAME MIX (Sigma Aldrich) standards.

Statistical analysis

The 2x2 factorial ANOVA was used to compare the means and when statistical difference was observed, Turkey test ($P<0.05$) was applied. The software utilized was the Statistic 7.1.[13]

Results and discussion

After 40minutes into the hydrolysis process, the raw material was liquefied and filtration was performed to remove bone residues from the TR hydrolysate. The hydrolysate appearance reflects the characteristics of the different raw materials used (Table 1). These characteristics were also maintained when the chemical composition was evaluated, the hydrolysates differed significantly regarding the crude protein and ether extract contents ($P<0.05$). The crude protein content of PL hydrolysate was approximately 1.5times higher than of TR hydrolysate (Table 1).

Table 1 Chemical composition of hydrolysates obtained from pig liver and tilapia residue byproducts with different enzymes (Alcalase® and Brauzin®)

	Parameters (g/100g protein)			
	DM	**CP(%)**	**F(%)**	**Ash(%)**
Raw materials	**Averages**			
PL	28.98	69.36[a]	12.06[b]	14.31
TR	27.92	42.35[b]	40.04[a]	15.93
Enzymes				
A	27.98	56.54	25,25	15.22
B	28.93	55.16	26.85	15.03
Hydrolysates				
PLA	28.42	71.64	13.73	14.95
PLB	29.55	67.11	10.39	13.67
TRA	27.53	41.45	36.8	15.47
TRB	28.31	43.2	43.27	16.39
Statistics	F values for the variables shown above			
Raw material RM)	0.66[NS]	77.28**	9.63**	0.65[NS]
Enzyme(E)	1.47[NS]	1.66[NS]	00:04[NS]	12:15[NS]
Interaction(RM x E)	2.82[NS]	0.99[NS]	3.04[NS]	2.67[NS]
VC (%)	1.71	8.87	7.16	10.04

PL-pig liver; TR-tilapia processing residue; A-alcalase®; B brauzin®, PLA-pig liver hydrolyzed with alcalase®; PLB-pig liver hydrolyzed with brauzyn®; TRA-tilapia processing residue hydrolyzed with alcalase®; TRPB-tilapia processing residue hydrolyzed with brauzyn®; (N=3)

[a,b] Means followed by different lowercase letters in the column differ by Turkey test ($P<0.05$)

** ($P<0.01$)* ($P<0.05$)[NS] non significant

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Table 1 shows that crude protein and ether extract is inversely correlated for hydrolysates of different raw materials. RT hydrolysates displayed higher ether extract content.

The enzymes did not affect the chemical composition of the hydrolysates while there was no interaction of raw materials and enzyme factors (RM x E) (P>0.05) (Table 1). Although TR constitutes a source rich in mineral matter (MM), the similar mineral content observed in the different hydrolysates can be attributed to the removal of the bones from the TR hydrolysate by filtration and the addition of phosphoric acid.

The proximate composition of the hydrolysates depends and reflects the substrate used as raw material. The results corroborate those obtained by other authors,[14] who also found that the raw material directly influenced the chemical composition of hydrolysates produced with different residues of Alaska Pollock. The same correlation was reported for yellow stripe trevally hydrolysates.[15]

In general, essential and nonessential amino acids (Table 2) profiles of the different hydrolysates also maintained the characteristics of the raw materials (g 100g protein^{-1}).However, significant differences (P<0.05) were observed for the essential and nonessential amino acids profiles among the raw materials used. Pig liver hydrolysates (PLA and PLB) showed higher amounts (g/100g protein) of histidine, phenylalanine, isoleucine, leucine and valine compared to tilapia residue hydrolysates (TRA and TRB), which have higher arginine content (P<0.05). Furthermore, cystine, proline and glutamic acid were higher in TR products (Table 2).

Table 2 Profiles of essential and non-essential amino acids of the hydrolysates obtained from pig liver and tilapia processing residue with different enzymes (Alcalase® and Brauzin®)

Essential and nonessential amino acids (g / 100g protein)																	
	His	**Ile**	**Leu**	**Met**	**Phen**	**Thr**	**Trp**	**Lis**	**Val**	**Arg**	**Cis**	**Tir**	**Pro**	**Ser**	**Asp**	**Glu**	**Gli**	**Ala**
RM	**Averages**																	
PL	2.97^{a}	4.76^{a}	9.52^{a}	1.7	5.11^{a}	4.44	1.16^{a}	7.53	6.24^{a}	4.77^{b}	1.37^{b}	5.24^{a}	4.73^{b}	4.10^{a}	9.18^{a}	12.58 ^{b}	6.03 ^{a}	1.12
TR	2.32^{b}	3.82^{b}	6.85^{b}	1.62	3.73^{b}	4.29	0.87^{b}	7.66	4.44^{b}	7.03^{a}	2.18^{a}	3.03^{b}	5.96^{a}	3.71^{b}	8.95^{b}	13.76 ^{to}	1.0 ^{b}	0.76 ^{b}
E																		
A	2.65	4.38	8.34	1.62	4.39	4.33	0.99	7.55	5.55	6.02	1.87	4.07	5.34	3.82	9.1	13.23	3.59	0.95
B	2.63	4.2	8.03	1.71	4.46	4.41	1.04	7.63	5.09	5.79	1.68	4.2	5.35	3.99	9.03	13.11	3.5	0.93
Hydrolysates																		
PLA	2.99	4.98	9.86	1.64	5.09	4.37	1.13	7.38	6.45	4.65	1.36	5.13	4.71	3.94	9.23	12.71	6.1	1.13
PLB	2.94	4.54	9.18	1.76	5.13	4.52	1.18	7.67	6.02	4.89	1.37	5.35	4.74	4.26	9.12	12.44	5.98	1.11
TRA	2.31	3.77	6.82	1.59	3.69	4.28	0.85	7.72	4.64	7.38	2.37	3.01	5.97	3.69	8.96	13.74	1.08	0.77
TRB	2.32	3.86	6.88	1.66	3.78	4.3	0.89	7.6	4.16	6.69	1.99	3.05	5.96	3.72	8.93	13.78	1.03	0.75
	F values for the variables shown above																	
RM	208.20**	31.15**	65.09**	0.89^{NS}	47.01**	1.11^{NS}	20.72**	1.09^{NS}	95.62**	51.03**	52.71**	247.12**	98.28**	29.07**	55.98**	27.05**	163.06**	49.61**
E	1.47^{NS}	1.64^{NS}	00.13^{NS}	2.06^{NS}	00.02^{NS}	12.49^{NS}	1.32^{NS}	3.67^{NS}	2.47^{NS}	3.32^{NS}	1.74^{NS}	12.05^{NS}	0.008^{NS}	00.41^{NS}	3.03^{NS}	2.26^{NS}	0.005^{NS}	0.71^{NS}
RMxE	2.69^{NS}	2.88^{NS}	0.99^{NS}	1.01^{NS}	0.87^{NS}	12.15^{NS}	2.79^{NS}	1.99^{NS}	12.57^{NS}	2.02^{NS}	0.49^{NS}	12.35^{NS}	1.04^{NS}	1.57^{NS}	1.42^{NS}	1.48^{NS}	1.77^{NS}	1.39^{NS}
CV (%)	7.6	7.87	5.64	3.98	6.11	8.35	7.52	7.4	5.36	6	3.81	3.51	8.16	10.16	14.74	13.49	4.01	10.58

RM -raw materials; PL- pig liver; TR-tilapia processing residue; A-alcalase®; B-brauzin®; PLA-pig liver hydrolyzed with alcalase®; PLB-pig liver hydrolyzed with Brauzyn®; TRA-tilapia processing residue hydrolyzed with alcalase®; TRPB- tilapia processing residue hydrolyzed with Brauzyn®; E-enzyme. (N=3)

$^{a, b}$ Means followed by different lowercase letters in the column differ by Turkey test (P<0.05)

** (P<0.01). ^{NS} non-significant

Table 2 shows that methionine, threonine, tryptophan and lysine were not significantly different (P>0.05), probably due to the fact that they are animal proteins. The enzymes used did not influence the essential and nonessential amino acids profiles of the different hydrolysates, and no interaction was observed between factors (P>0.05) (Table 2). Probably, protein fragments (peptides) may have formed with different amino acid sequence and composition depending on the enzyme used. However, no difference was observed between the hydrolysates and the raw materials since the profiles were determined in the total hydrolysate.

The order of limiting amino acids in different hydrolysates was evaluated by the chemical score (Table 3), using as reference the essential amino acids requirements of the standard protein according to FAO/WHO[4] for adults. The results indicated that all hydrolysates are above the requirements regarding essential amino acids. These values (Table 3) are in agreement with previous publications[1,16] and reflect the high biological quality of the protein present in the hydrolysate.

In general, the hydrolysis process improves protein digestibility, increasing amino acid bioavailability and resulting in nutritional gain. The PL and TR hydrolysates displayed adequate essential amino acids profile and, therefore, can be targeted as alternative products or ingredients of high nutritional quality with potential application in the food industry for pets and aquaculture.[14,17]

The amounts of essential and nonessential free amino acids (Table 4) in PL (7.62g/100g of protein) and TR (2.52g/100g of protein) before the hydrolysis process show that the enzymatic process released free amino acids. Enzymatic catalysis performed with Alcalase® provided

3.40times more free amino acids in the PLAhydrolysate compared to PLB. Similar behavior was also observed for TR, where TRA showed 1.90 times more free amino acids than TRB. This feature can be attributed to the hydrolytic action of Alcalase, which can cleave the ends of the protein, thereby releasing larger amounts of free amino acids.

Table 3 Chemical score of protein hydrolysates derived from pig liver and tilapia processing residue with different enzymes (Alcalase® and Brauzin®) and compared with the minimum requirements for adults, FAO/WHO (2007)

Amino acids	Hydrolysates				FAO/ WHO
	PLA	PLB	TRA	TRB	
Histidine	1.99	1.96	1.54	1.55	1.5
Isoleucine	1.66	1.51	1.26	1.29	3
Leucine	1.67	1.56	1.16	1.17	5.9
Methionine+cystine	1.36	1.42	1.8	1.66	2.2
Phenylalanine+tyrosine	2.24	2.76	1.76	1.8	3.8
Threonine	1.9	1.97	1.86	1.87	2.3
Tryptophan	1.88	1.97	1.42	1.48	0.6
Lysine	1.64	1.7	1.72	1.69	4.5
Valine	1.65	1.54	1.19	1.07	3.9

PLA-pig liver hydrolyzed with Alcalase®; FB-pig liver hydrolyzed with Brauzyn®; TRA-tilapia processing residue hydrolyzed with Alcalase®; TRPB-tilapia processing residue hydrolyzed with Brauzyn®

Table 4 The composition of total and free amino acids (g/100g protein) of pig liver and tilapia processing residue

	Total amino acids*		Free amino acids*	
	PL	TR	PL	TR
Histidine	2.93	2.35	0.86	12.01
Isoleucine	4.94	4.69	12.3	12.16
Leucine	10.07	7.96	0.61	0.26
Methionine	1.91	2.01	0.13	0.13
Phenylalanine	5.23	4.69	0.56	0.14
Threonine	4.25	3.94	0.45	12.01
Tryptophan	1.22	0.92	0	12.23
Lysine	7.78	10.39	0.69	0.11
Valine	6.41	5.03	0.2	0.2
Arginine	4.74	6.71	0.21	0.04
Cystine	1.32	2.51	0	12.01
Tyrosine	5.13	3.43	0.2	0
Proline	4.69	4.61	12.16	0.11
Serine	3.77	3.52	0.31	12.33
Aspartic acid	10.66	9.05	12.17	0.02
Glutamic acid	12.86	14.02	1.68	0.31
Glycine	5.57	7.37	12.32	12.01
Alanine	1.33	0.82	0.76	12.42

* Average of results in triplicate; PL-pig liver; TR-tilapia processing residue

The composition of essential and non-essential free amino acids was influenced by the raw materials and the enzymes used ($P<0.05$), but no interaction was observed between factors ($P>0.05$) (Table 5).

The results of the free essential amino acids profile of the PL protein hydrolyzed with Alcalase® (100g CP^{-1}) show the following descending order: leucine, histidine, valine, phenylalanine, lysine, threonine, isoleucine and methionine. On the other hand, the lower means for the same essential free amino acids were found in TRB, with a higher content of arginine (Table 5).

Nitrogenous compounds such as amino acids are considered highly palatable to pets like dogs and cats. The chemoreceptor units of the sensory ganglia of cats are stimulated by amino acids described as "sweet" by humans. This group includes proline, cysteine, lysine, histidine and alanine. On the other hand, they are inhibited by "bitter" amino acids, including tryptophan, isoleucine, phenylalanine and arginine, which have hydrophobic side chains and aromatic rings.[18]

The products formed may exhibit distinct sensory characteristics, and in general, the sensory quality varies according to the amino acid concentrations.[19] Among the hydrolysates, PLA and PLB had twice as much sweet amino acids whereas TRA was more balanced and TRB had a higher proportion of "bitter" amino acids. This result may indicate that PLA and PLB might be more palatable in a diet for cats than TRA and TRB (Table 5).

The hydrolysis process involves a number of variables; enzyme specificity and activity, enzyme/substrate ratio, pH, temperature, time and interaction between the nutrients present in the raw material during hydrolysis. Specific characteristics of the different hydrolysates may be associated with the bond type cleaved by the different proteases used. The Alcalase® enzyme preferentially acts on peptide bonds with hydrophobic side chain at the C-terminal portion[7] while Brauzin® acts on ester and amide bonds, preferably on the bonds adjacent to the amino acids phenylalanine, valine and leucine.[8]

The fatty acids content in the hydrolysates (g/100g lipid) was not affected by the different enzymes tested, reflecting, as expected, the composition of raw materials used (Table 6).

TR hydrolysates had higher average monounsaturated fatty acids, whereas saturated fatty acids predominated in PLA and PLB, a characteristic of swine fat. Among the polyunsaturated fatty acids, the hydrolysates showed higher levels of n-6 fatty acids, especially PL hydrolysates, a result that is attributed to the composition of the raw material.

The fatty acids may originate from dietary lipids or biosynthesis of the non-lipid sources. The monounsaturated fatty acids can be synthesized from a saturated fatty acid, via enzymatic activity.[20] Deemed essential, then-3 and n-6 polyunsaturated fatty acids cannot be synthesized by vertebrates, and should, therefore, be supplemented in the diet of these animals, in order to allow normal development, growth and reproduction.[21]

Fish from colder regions are good sources of n-3 polyunsaturated fatty acids while fish from tropical regions have higher levels of n-6 fatty acids. This fact has been reported for tilapia fillets,[22] whose oil samples contained 20.44g of n-6 and only 3.99g of n-3 per 100g.

The n-6 requirements in tilapia, carp, trout and salmon vary between 0.5 to 1.0%,[20] thus fitting in the values presented in different hydrolysates developed (Table 6). Therefore, the potential use of PL and TR hydrolysates as sources of n-6 in diets for fish should be highlighted.

Table 5 Profiles of free essential amino acids in hydrolysates obtained from pig liver and tilapia processing residue with different enzymes (Alcalase ® and Brauzin®)

Essential and non-essential free amino acids (g/100g protein)																	
	His	**Ile**	**Read**	**Met**	**Phen**	**Thr**	**Trp**	**Lis**	**Val**	**Arg**	**Cis**	**Tir**	**Pro**	**Be**	**Asp**	**Glu**	**Gli**	**Ala**
RM	Averages																	
PL	1.94^{a}	0.84^{a}	2.79^{a}	0.54^{a}	1.53^{a}	0.96^{a}	0.17	1.60^{a}	1.65^{a}	0.27^{b}	0.55	1.23^{a}	0.75^{a}	0.70^{a}	0.75^{a}	3.7	0.71^{to}	1.90 ^{to}
TR	0.48^{b}	0.19^{b}	0.87^{b}	0.22^{b}	0.45^{b}	0.13^{b}	0.1	0.51^{b}	0.19^{b}	2.10^{a}	0.4	0.36^{b}	0.36^{b}	0.09^{b}	0.10^{b}	3.78	0.28 ^{b}	0.45 ^{b}
E																		
A	1.65^{a}	0.83^{a}	2.94^{a}	0.56^{a}	1.42^{a}	0.83^{a}	0.15	1.40^{a}	1.35^{a}	1.18	0.51	1.09^{a}	0.81^{a}	0.53^{a}	0.69^{a}	4.15^{a}	0.55	1.74^{to}
B	0.77^{b}	0.20^{b}	0.72^{b}	0.20^{b}	1.07^{b}	0.25^{b}	0.13	0.71^{b}	0.49^{b}	1.19	0.39	0.49^{b}	0.30^{b}	0.27^{b}	0.15^{b}	3.33^{b}	12.44	0.61^{b}
Hydrolysates																		
PLA	2.51	1.31	4.34	0.73	2.04	1.49	0.19	1.99	2.36	0.30 b	0.34	1.58	1.04	0.94	1.26	4.43	0.9	2.89
PLB	1.37	0.36	1.24	0.35	1.02	0.42	0.15	1.2	0.94	0.24 b	0.66	0.87	0.46	0.46	0.23	2.97	12.51	0.91
TRA	0.79	0.34	1.53	0.38	0.79	0.17	0.1	0.8	0.33	2.06a	0.68	0.6	0.57	0.11	0.12	3.87	0.19	0.59
TRB	0.17	0.03	0.2	0.05	0.1	0.08	0.1	0.21	0.04	2.14a	0.11	0.11	0.14	0.07	0.07	3.68	0.36	0.31
F values for the variables shown above																		
RM	33.60**	49.21**	99.32**	52.19**	82.48**	79.77*	0.91^{NS}	33.57**	77,25**	63.48**	3.09^{NS}	66.17**	43.61**	99.16**	103.14**	0.84 ^{NS}	78.26 **	9307 **
E	88.30**	71.45**	69.07**	80.66**	57.77**	62,69*	3.02 ^{NS}	20.14**	47.88*	2.34 ^{NS}	1.14 ^{NS}	28.63**	82.17**	50.02**	65.78**	81.36 **	1.96 ^{NS}	49.44 **
RMxE	0.99 ^{NS}	2.04 ^{NS}	1.76 ^{NS}	3.40 ^{NS}	0.19 ^{NS}	2.53 ^{NS}	2.01^{NS}	1.29 ^{NS}	0.71 ^{NS}	3.81 ^{NS}	1.87 ^{NS}	1.11^{NS}	2.99 ^{NS}	2.41 ^{NS}	3.07 ^{NS}	2.27 ^{NS}	1.48 ^{NS}	2.21 ^{NS}
CV(%)	0.47	0.61	0.5	0.43	0.48	0.65	0.3	0.39	0.72	0.98	0.64	0.45	0.61	0.6	0.77	0.43	0.49	0.41

RM-raw materials; PL-pig liver; TR-tilapia processing residue; A-alcalase®; B-brauzin®; PLA-pig liver hydrolyzed with Alcalase®; PLB-pig liver hydrolyzed with Brauzyn®; TRA-tilapia processing residue hydrolyzed with Alcalase®; TRPB - tilapia processing residue hydrolyzed with Brauzyn®; E-enzyme (N = 3)

$^{a, b}$ Means followed by different lowercase letters in the column differ by Turkey test (P<0.05)

** (P<0.01), ^{NS} non-significant

Table 6 Fatty acid composition of the hydrolysates obtained from pig liver and tilapia processing residue with different enzymes (Alcalase® and Brauzin®)

	Parameters (g/100g lipid)					
	Saturated	**Monounsaturated**	**Polyunsaturated**	**Omega 6**	**Omega 3**	**Trans**
Raw Material	**Averages**					
PL	42.41^{a}	18.72^{b}	31.48^{a}	29.45^{a}	0.81^{b}	0.33^{b}
TR	32.40^{b}	41.57^{a}	16.53^{b}	14.70^{b}	1.83^{a}	1.06a
Enzymes						
A	36.63	30.43	24.41	22.41	1.32	0.69
B	38.17	29.86	23.59	21.74	1.32	0.69
Hydrolysates						
PLA	40.88	19.34	32.29	30.07	0.8	0.34
PLB	43.93	18.11	30.67	28.82	0.82	0.33
TRA	32.39	41.52	16.54	14.74	1.84	1.05
TRB	32.4	41.61	16.51	14.66	1.82	1.06
Statistics	F values for the variables shown above					
Raw material(RM)	33.29**	163.81**	255.14**	338.25*	146.71*	367.94*
Enzyme(E)	2.47^{NS}	0.03^{NS}	0.24^{NS}	0.09^{NS}	0.0001^{NS}	0.0004^{NS}
RM x E interaction	0.87^{NS}	1.77^{NS}	1.21^{NS}	1.02^{NS}	0.27^{NS}	0.41^{NS}
CV(%)	0.15	0.4	0.33	0.1	0.34	0.56

PL-pig liver; TR-tilapia processing residue; A-alcalase®; B-brauzin®; PLA-pig liver hydrolyzed with alcalase®; PLB-pig liver hydrolyzed with Brauzyn®; TRA-tilapia processing residue hydrolyzed with alcalase®; TRPB-tilapia processing residue hydrolyzed with brauzyn®; E-enzyme (N=3)

$^{a, b}$ Means followed by different lowercase letters in the column differ by Turkey test (P<0.05)

** (P<0.01), ^{NS} non-significant

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Due to the intrinsic characteristics of enzymes and different compositions of raw materials, the products obtained had different free amino acids profiles. The hydrolysates maintain the features of raw material; however, the characteristics of the free amino acids were different due to the specificity of the enzymes used. The raw materials are converted to liquid form by the action of peptidases.

The processing of the agroindustrial residues, PL and TR, by enzymatic hydrolysis using Alcalase® and Brauzyn® resulted in products with distinct characteristics and composition, depending on the feedstock. PL hydrolysates had higher protein content and lower fat content, but regardless, all hydrolysates showed adequate essential amino acid levels. However, the action of different biocatalysts reflected in different composition of free amino acids in the products. The hydrolysis in the presence of Alcalase® yielded more free amino acids, probably due to exopeptidases present in the enzyme system.

Final comments

Hydrolysis is a process highly suitable for using the agroindustrial waste with high protein content, causing the release of free amino acids and protein fragments. The characteristics of the resulting products allow using them as potential new ingredients in animal feed as a source of essential nutrients due to the desirable amino acid and essential fatty acid profiles, as well as a flavoring agent due to the presence of free amino acids.

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Conflict of interest

Author declares that there is no conflict of interest.

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