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Enzymatic hydrolysis of chicken viscera proteins in the presence of an ionic liquid as a strategy to improve the antioxidant properties of the hydrolysates

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Abstract

Aiming to explore the use of ionic liquids (ILs) not yet described in the literature, this work evaluated the hydrolysis of proteins from chicken viscera using the protease Alcalase modified and unmodified by the IL tetramethylammonium bromide. The protein hydrolysates produced in the presence of the IL presented values of antioxidant activities 40% higher than the hydrolysates obtained without IL. In addition, with the presence of the IL, it was possible to obtain protein hydrolysates from chicken viscera with similar antioxidant activities, compared to the protein hydrolysates produced without IL, using 1/3 of the amount of enzyme.

Key words: *ionic liquids, enzymatic hydrolysis, antioxidant activity*

Introduction

The use of ionic liquids (ILs) in the modification of enzymes have been highlighted due to their advantages such as high conversion rates, high enantioselectivity, better recovery and possibility of recycling. In addition, ILs act to potentiate the enzymatic action by substantially increasing enzyme activity and stability. On the other hand, enzymatic hydrolysis has industrial application as a possibility of using by-products, transforming them into products of interest and avoiding environmental problems.

Thus, the objective of this study was to evaluate the modification of the commercial protease Alcalase by the addition of an ionic liquid, aiming the production of protein hydrolysates from chicken viscera with antioxidant properties using a central composite rotatable design.

Results and Discussion

The presence of the ionic liquid was responsible for higher enzymatic activities during the hydrolysis, may resulting in a greater efficiency in the hydrolysis of the proteins (Table 1).

In addition, the antioxidant activities (Table 3) were up 40% higher in the presence of the IL for the assays 4 (measured by the DPPH method) and 1, 4, 8, 9 and 12 (measured by the FRAP method).

Table 1. Relative protease activity as a function of time during the enzymatic hydrolysis of protein from chicken viscera by Alcalase.

Time (min)	Relative protease activity (%)[1]	
	Alcalase	Alcalase + IL
0	100.00[a]	100.00[a]
5	118.18[b]	121.21[a]
10	120.32[b]	163.01[a]
15	145.34[a]	159.49[a]
20	138.48[b]	184.53[a]
30	102.14[b]	147.95[a]
45	99.60[b]	174.06[a]
60	95.52[b]	188.62[a]
75	102.08[b]	165.83[a]
90	101.61[b]	143.06[a]
105	102.64[b]	166.13[a]
120	96.02[b]	171.33[a]

[1]The relative protease activity was calculated considering the initial value (time 0) as 100% for each enzymatic treatment (Alcalase modified and unmodified by the IL tetramethylammonium bromide). Results are presented as the mean (n = 3), and those with different letters are significantly different ($p < 0.05$) on the same line.

Table 2. Matrix of the central composite rotatable design used to obtain the protein hydrolysates from chicken viscera.

Assay	Hydrolysis parameters		
	Enzyme (U/mL)	pH	Temperature (°C)
1	60	7	40
2	60	7	60
3	60	9	40
4	60	9	60
5	120	7	40
6	120	7	60
7	120	9	40
8	120	9	60
9	39.6	8	50
10	140.4	8	50
11	90	6.32	50
12	90	9.68	50
13	90	8	33.2
14	90	8	66.8
15, 16 e 17	90	8	50

Table 3. Antioxidant activities of the protein hydrolysates from chicken viscera obtained by enzymatic hydrolysis.

Assay	DPPH (µmol TE/g)		FRAP (µmol TE/g)	
	Alcalase	Alcalase + IL	Alcalase	Alcalase + IL
1	119.60[a]	115.19[a]	570.31[b]	811.41[a]
2	110.29[a]	94.25[b]	421.12[b]	460.07[a]
3	14.70[a]	11.45[b]	848.64[b]	1126.33[a]
4	19.62[b]	28.11[a]	689.60[b]	978.78[a]
5	159.65[b]	188.11[a]	693.15[b]	885.32[a]
6	93.26[b]	101.11[a]	396.69[b]	489.70[a]
7	19.23[a]	14.36[b]	862.42[b]	1057.59[a]
8	35.19[b]	43.23[a]	620.64[b]	893.02[a]
9	60.96[a]	60.74[a]	576.63[b]	794.44[a]
10	93.61[a]	81.27[b]	718.76[a]	713.05[a]
11	136.74[b]	156.98[a]	615.60[b]	719.66[a]
12	31.57[a]	3.23[b]	693.18[b]	941.52[a]
13	68.06[a]	66.82[a]	676.07[b]	763.75[a]
14	68.40[b]	77.48[a]	525.54[b]	659.66[a]
15	75.22[a]	77.14[a]	630.45[b]	669.41[a]
16	73.27[a]	76.05[a]	619.50[b]	673.52[a]
17	70.55[a]	75.50[a]	629.41[b]	672.90[a]

Results are presented as the mean (n = 3), and those with different letters are significantly different ($p < 0.05$) for the same method on the same line.

Conclusions

The remarkable results obtained in this study were: 1) protein hydrolysates produced in the presence of the IL showed antioxidant activities 40% higher than those obtained without IL and 2) with the IL, it was possible to obtain protein hydrolysates from chicken viscera with similar antioxidant activities, compared to the protein hydrolysates produced without IL, using 1/3 of the amount of enzyme.