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THE IMPACT OF ENZYMATIC HYDROLYSIS ON THE ANTIOXIDANT CAPACITY OF SOY AND POTATO VEGETABLE PROTEINS

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1. INTRODUCTION

Enzymatic hydrolysis is a mechanism that allows the breakdown of large protein molecules, reducing their complex structure to specific peptides and amino acids. As a result of hydrolysis, the physicochemical and functional properties of proteins also change, making them more interesting for the pharmaceutical and food industry (GOMES; KUROZAWA, 2020). Therefore, the main objective of this study was to investigate the effect of enzymatic hydrolysis, using the commercial Flavourzyme protease, on the antioxidant capacity of two isolated vegetable proteins: soy (*Glycine max*) and potato (*Solanum tuberosum*).

2. MATERIAL AND METHODS

The evaluation of Flavourzyme's ability to perform hydrolysis on soy protein isolate (SPI) and potato protein isolate (PPI) was performed according to the pH-stat method, described by Adler-Nissen (1986). For that, two different solutions containing 4 % (dry mass) of SPI and PPI were prepared with deionized water and stirred for 1 h at room temperature (≅ 25 °C), in order to ensure complete rehydration. Protease was added at an enzyme:substrate ratio of 2 g/100 g protein under optimal protease conditions (50°C and pH 6.0).

The hydrolysis process was repeated under the same conditions, but this time, two degrees of hydrolysis (*DH*, %) were selected for each protein, in which, when the target *DH* was reached, Flavourzyme was inactivated by heating to 85 °C for 10 min and cooled in an ice bath until reaching room temperature. The hydrolyzed mixture was centrifuged at 10,000 rpm for 20 min and the supernatant was frozen for analysis of antioxidant capacity.

The antioxidant capacity of protein isolates and hydrolysates were evaluated by different methods: ability to capture the free radical ABTS+ (RE et al., 1999), ferric reducing antioxidant power (FRAP) (BENZIE; STRAIN, 1999), and ability to donate hydrogen to stable free radical DPPH (BRAND-WILLIANS et al., 1995). All assays were performed in triplicate and data were subjected to analysis of variance (ANOVA), followed by Tukey's post-hoc test ($p<0.05$). Antioxidant capacity results were expressed as µmols of Trolox equivalent (TE)/g protein, using Trolox calibration curves (50–2000 µmol/g) as a standard.

3. RESULTS AND DISCUSSION

The maximum values of *DH* reached were 5.10±0.41% and 27.53±0.30% for PPI and SPI, respectively, indicating that Flavourzyme was able to hydrolyze SPI 5.4 times more than PPI. It should be noted that the soy protein used was 80% isolated, against 90% of the potato protein, suggesting that even with a greater amount of solids in relation to protein, SPI was more susceptible to the action of Flavourzyme. This protease effectiveness in hydrolyzing SPI may be related to the fact that Flavourzyme contains a specific active site capable of more easily cleaving this protein.

Protein modification by the action of Flavourzyme protease increased the antioxidant capacity of SPI and PPI, showing a positive relationship with *DH* (Table 1).

Table 1. Antioxidant capacity (FRAP, ABTS, and DPPH assays) of soy protein isolate (SPI) and potato (PPI) and of hydrolysates obtained by Flavourzyme, with different degrees of hydrolysis (*DH*).

	FRAP (uMol/g)		ABTS (uMol/g)		DPPH (uMol/g)	
Proteins	**Soy**	**Potato**	**Soy**	**Potato**	**Soy**	**Potato**
Isolated	457,8±12,0^{B}	155,8±2,0^{A}	1210,0±34,6^{C}	497,8±150,6^{A}	983,3±11,3^{C}	822,5±13,2^{A}
***DH* 2 %**	699,8±8,7^{C}	812,5±7,6^{E}	1990,0±63,3^{D}	1008,9±111,0^{B}	1010±8,7^{D}	947,5±9,0^{B}
***DH* 5 %**	735,1±4,1^{D}	877,8±12,2^{F}	2208,9±19,2^{E}	1366,6±166,5^{C}	1114,2±12,3^{F}	1037,5±30,7^{E}

Mean values followed by standard deviation. The letters indicate statistical convergence or divergence ($p<0.05$) between samples. Comparisons took place for each method.

The ability of hydrolysates to reduce iron through FRAP analysis increased up to 1.6-fold for SPI and up to 5.6-fold for PPI. Regarding the antioxidant power by capturing the ABTS+ radical, enzymatic hydrolysis increased up to 2 times for SPI and 2.7 times for PPI, while for the DPPH method they showed an increase in antioxidant capacity 1.1x and 1.3x, respectively, compared to intact proteins. When performing enzymatic hydrolysis of isolated rice protein using Flavourzyme, Gomes and Kurozawa (2020) also observed a positive correlation between *DH* and antioxidant capacity, noting an increase of 3.3x and 1.4x in antioxidant capacity by FRAP and DPPH, respectively.

Soy protein showed higher antioxidant capacity by ABTS and DPPH methods, while potato protein achieved superior results with its hydrolysates, by FRAP method. SPI is considered a good natural antioxidant (SINGH; VIJ; HATI, 2014). However, this study demonstrated that it is possible to increase the antioxidant capacity of SPI by increasing enzymatic hydrolysis. As for PPI, despite having a lower antioxidant capacity than SPI, hydrolysis contributed to the reduction of this difference.

4. CONCLUSIONS

This study showed that Flavourzyme has a greater ability to hydrolyze SPI compared to PPI. Enzymatic hydrolysis has proven to be a good alternative to increase the antioxidant capacity of plant proteins, enabling its use in food supplements and in the fight against degenerative diseases, contributing to the current green trend in the market.

5. REFERENCES

ADLER-NISSEN, J. 1986. Enzymic Hydrolysis of Food Proteins. London, Elsevier Applied Science Publishers. 427 p.

BENZIE, I.F.F., STRAIN, J.J. 1999. Ferric reducing (antioxidant) power as a measure of antioxidant capacity: the FRAP assay. Methods Enzymol., 299: 15-36.

BRAND-WILLIAMS, W., CUVELIER, M.E., BERSET, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol., 28: 25-30.

GOMES, M.H.G., KUROZAWA, L.E. 2020. Improvement of the functional and antioxidant properties of rice protein by enzymatic hydrolysis for the microencapsulation of linseed oil. J. Food Eng., 267: 109761.

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 Radic. Biol. Med., 26: 1231-1237.

SINGH, B.P., VIJ, S., HATI, S. 2014. Functional significance of bioactive peptides derived from soybean. Peptides, 54: 171–179.