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Antioxidant activity of casein hydrolysates produced using *Bromelia serra* leaf extract

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INTRODUCTION & AIM

Obtaining casein hydrolysates from the partial or total degradation of proteins into lower molecular weight peptide products or even amino acids is carried out by enzymes. This hydrolysis can be catalyzed by acids, bases, or proteolytic enzymes. Enzymatic hydrolysis is superior because it can be carried out under physiological-like conditions, produces hydrolysates containing well-defined peptide mixtures, and avoids the destruction of L-amino acids and the formation of toxic substances such as lysine-alanine. The biologically active peptides released during hydrolysis may be hidden or inactive in the amino acid sequence of dairy proteins and be released or activated in vivo during gastrointestinal digestion, or even more so, during food processing via specific enzymatic proteolysis.

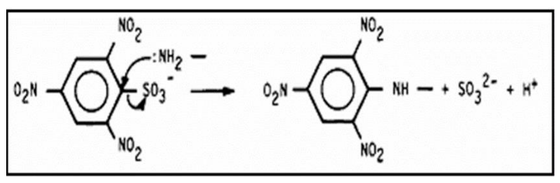
The aim of this work was to study the hydrolysis of casein using enzyme isolates from *Bromelia serra* leaves.

METHOD

Plant material: Adult leaves of *Bromelia serra* (BS) were collected in the town of Santa Ana, Corrientes

Obtaining enzyme isolate: A crude extract of BS leaves was obtained and then treated with four volumes of ethyl alcohol (-20°C) to minimize protein denaturation. The suspension was gently stirred on a magnetic stirrer and left to stand for 20 min at 0°C, then centrifuged at 4000 xg for 20 min. The pellets were dried in a vacuum desiccator to remove organic solvent residues and suspended with 4 mL of sodium phosphate buffer at 0°C. This resuspended precipitate was called enzyme isolate (EIB).

Preparation of hydrolysates: The trinitrobenzene sulfonic acid (TNBS) method was used, based on the reaction of this compound with the primary amino groups that are exposed when hydrolyzing the peptide bonds (Adler-Nissen, 1979) on bovine casein.



Denaturing electrophoresis (SDS-PAGE) of the hydrolysates according to Laemmli (1970).

Optimal time for casein hydrolysis: The hydrolysis reaction was carried out at times 90, 120 and 240 minutes at 55°C in an oven with constant stirring at 300 rpm.

Determination of degree of hydrolysis (DH): Using the TNBS method, a leucine calibration curve was used since it contains a free amino group. For this purpose, a 45 mM leucine stock solution was used, from which diluted solutions (0.225–2.25 mM) corresponding to the calibration curve points were prepared, which were read at 340 nm.

Antioxidant activity of hydrolysates: To quantify the antioxidant capacity of a sample based on the ability to neutralize the cationic radical ABTS [2,2'-azinobis (3-ethylbenzothiazolin-6-sulfonic acid)], the method of Re et al. (1999) was used.

RESULTS & DISCUSSION

Profile of casein hydrolysates: control or blank casein (lane 2), presenting high molecular weight polypeptides that did not enter the gel and mainly the protein fractions of casein with a range of 23 to 25 kDa which may belong to α, β and another band of 19 kDa to κ casein. Also, a high intensity polypeptide of ≥ 31 kDa was observed. The progression of the hydrolysis process at 55 ° C can be observed at 90 minutes (lane 4), 180 minutes (lane 5) and 240 minutes (lane 6). At 90 minutes, the same polypeptides present in the control casein are still observed, but with the appearance of new bands with molecular weight ranges from 15 to 10 kDa. After 180 minutes of hydrolysis (lane 5), not much difference was observed compared to the previous treatment, with the exception of a decrease in the intensity of high molecular weight bands in the 50 to 150 kDa range. In the last treatment, 240 minutes (lane 6), the majority of the 23 and 31 kDa bands were no longer observed, with a 36 kDa polypeptide and lower molecular weight proteins of 19, 15, and 10 kDa predominating.

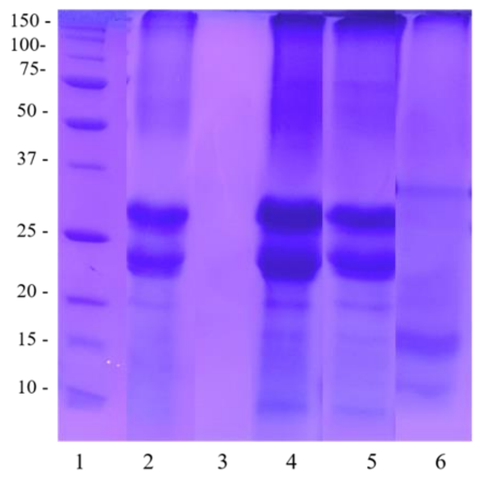


Figure 1: SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Lane 1: molecular weight standards; lane 2: unhydrolyzed casein; lane 3: EIB enzyme blank; lane 4: 90 minutes; lane 5: 180 minutes; lane 6: 240 minutes.

Table 1: Degree of casein hydrolysis expressed as a percentage for each hydrolysis time (0, 90, 180, and 240 minutes) and their corresponding IC50 and TEAC values. Different letters indicate significant differences between treatments ($p<0.05$).

Time hydrolysis (minutes)	Degree of hydrolyis (%)	Antioxidant activity	
		IC_{50} (mg/mL)	TEAC (µg de Trolox equivalent/mg)
0	0	1,98 c	231,23 b
90	5,68 b	2,21 b	206,97 c
180	6,91 b	2,91 a	150,54 d
240	12,01 a	1,68 d	272,89 a

CONCLUSION

Analyzing multiple time for hydrolysis, at 240 minutes of incubation, the highest DH value was obtained at 12%. . The lowest IC50 value was obtained for the 240-minute hydrolysate, followed by unhydrolyzed casein. This indicates that 1.68 mg/mL of hydrolyzed casein (55°C for 240 minutes) is required to achieve 50% inhibition of the ABTS cation radical, compared to 1.98 mg/mL for unhydrolyzed casein.

REFERENCES

Adler-Nissen, J. (1979). Journal of Agricultural and Food chemistry, 27(6), 1256-1262;

Laemmli, U. K. (1970). Nature, 227, 680-68; Re et a.. (1999). Biology and Medicine, 26(9–10).