ENZYMATIC HYDROLYSIS OF SOYMILK BYPRODUCT (OKARA) BY PROTEASES EXTRACTED FROM *CYNARA CARDUNCULUS*

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Soybeans are the main oilseed produced and consumed worldwide. Currently, Brazil, Argentina and China are the largest producers of soybeans. This oilseed has great commercial interest, especially, because of the oil extraction, soy proteins and soymilk. Soymilk is used as a base in a wide variety of products, including tofu, soy yogurt and cheese. This soybean product is achieved by aqueous extraction of whole soybeans. During this process, a by-product known as okara rich in fiber, protein and fat, is obtained [3,4 5]. Okara is produced in high amounts, since for each 1 kg of processed soybeans about 1.1 kg of okara is produced [1]. The okara by-product has a high nutritive value, as previously mentioned. However, during soymilk manufacturing, this by-product is submitted to a severe heat treatment, which causes a large protein denaturation and the resulting okara protein isolate has poor solubility, which restricts its direct use in food. Studies reported that protein can be produced from the okara and that the protein isolates are characterized as having good amino acid profile and showing good digestibility [2]. Beyond other functional properties emulsification, foaming and binding properties were comparable to those of commercial soy isolate. Therefore, the main objective of this work was to study the enzymatic hydrolysis of two okara substrates (dry okara, previously autoclaved (OA) and not autoclaved (ONA)), achieved by an enzymatic aqueous extract of *Cynara cardunculus*. The okara used in this study was provided by a local soybean producer in Portugal (NUTRE). After collection the okara samples were divided in two batches and one was submitted to heat treatment (1 atm, 121 ° C for 20 minutes) and the other was not heat treated. Finally, both were dried at 65 °C until constant weight and then milled with a 1 mm mesh. Two hydrolysis factors (reaction time and ratio of enzyme/substrate, E/S) were selected. The following parameters were analyzed: degree of hydrolysis (DH), antioxidant activity (ABTS) and the profile of hydrolysis (RH) determined by FPLC (fast protein liquid chromatography gel filtration). The hydrolysis was performed using commercial crude extract of *Cynara cardunculus* at 55 ° C and pH 5.2 for 2.5 to 5 h. No significant differences on the DH were observed between OA and ONA or between hydrolysis factors tested using the method of o-phthaldialdehyde (OPA). However, chromatographic analysis by FPLC showed strong protein hydrolysis after 2.5 h with formation of peptides fractions, with no significant difference between OA and ONA, but showing increasing concentration according increasing E/S rations. The antioxidant activity results showed a higher antioxidant potential for OA than for ONA and directly proportional to the time of hydrolysis, which proves that previous protein denaturation may promote the release of more antioxidant peptides. Therefore, it can be concluded that the hydrolysis of okara protein with *C. cardunculus* generated peptide extracts with relevant antioxidant activity, which is affected by the denaturation state of the proteins.

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