STUDY OF TECHNO-FUNCTIONAL CHARACTERISTICS OF OKARA FLOUR HYDROLYZED WITH ALCALASE

Gabriella N. Ricarte[1*], Flávia Mylenna S. Martins[2], Amanda Hamad[2], Cristiane N. da Silva[1], Ailton C. Lemes[2]
Eveline L. Almeida[2], Bernardo D. Ribeiro[2] & Maria Alice Z. Coelho[2]

[1] Instituto de Química, Universidade Federal do Rio de Janeiro, 21941-909, Rio de Janeiro, RJ, Brazi
[2] Escola de Química, Universidade Federal do Rio de Janeiro, 21941-909, Rio de Janeiro, RJ, Brazil
** gabriellaricarte123@gmail.com*

ABSTRACT

Okara flour, a by-product of the manufacture of soy milk, is nutritionally rich, has a high protein and fibre content, and contains minerals and bioactive compounds, such as phenolic compounds. Okara can be used to enrich various food products. Still, some studies have shown reduced digestibility of proteins in foods to which okara flour was added, which can be attributed to antinutritional compounds, such as trypsin inhibitors. Enzymatic hydrolysis with protease can be an effective strategy to overcome this issue. However, a hydrolysis process can change the techno-functional properties of okara, which can affect the sensory aspects of final products, being necessary for investigation. Hydrolysis with Alcalase ® enhanced water holding capacity and the minor gelation concentration but reduced the emulsifying capacity and stability of the emulsion. This change shows the importance of a prior investigation of the modified material before its application in food matrices.

Keywords: Protease, degree of hydrolysis, water holding capacity, emulsifying capacity, least gelation concentration.

1 INTRODUCTION

The consumption of water-soluble soy extract (EHS), a vegetable drink analogous to milk, has been growing worldwide. In 2017, the global EHS market moved 14.4 billion dollars, and it is expected that in 2026, it will move 28.4 billion dollars. The production of EHS generates a by-product called "okara", whose main dry composition is approximately 50-65% fibres, 20-30% proteins, and 10-20% lipids, in addition to containing vitamins, minerals and phenolic compounds. Because it is nutritionally rich, it can be used to nutritionally enrich foods, which would be beneficial from an environmental and nutritional point of view. However, some studies have shown reduced digestibility in foods to which okara flour was added. Enzymatic hydrolysis with proteases can be an alternative to overcome this problem, as it could increase protein solubility, reduce the size of proteins, and reduce the trypsin inhibitor content, which are factors that impact digestibility. However, hydrolysis can alter the techno-functional characteristics of okara, directly influencing the final product's quality. It is possible to find data in the literature on how hydrolysis with proteases affects such characteristics in okara protein isolates. Still, there needs to be more knowledge related to the impacts on whole okara flour. This study aims to evaluate the effect of enzymatic hydrolysis with Alcalase®, a commercial endoprotease, on the techno-functional characteristics of whole okara flour.

2 MATERIAL & METHODS

2.1 Hydrolysis of okara flour and determination of degree of hydrolysis

Hydrolysis was carried out by mixing 10% okara flour in 25 mM phosphate, pH 7, at 60°C, adding 40 U/g of enzyme. The hydrolysis time was chosen based on the maximum degree of hydrolysis (DH)[1]. After hydrolysis, the liquid phase was separated and dried in a spray dryer at 70°C, while the solid phase was dried in a convective oven at 70°C.

2.2 Determination of water holding capacity (WHC) and oil holding capacity (OHC)

Approximately 0.1 g of okara flour or okara flour hydrolyzed with Alcalase was dispersed in 1 mL of distilled water or commercial sunflower oil in a microtube (previously weighed), and the dispersions were mixed in a vortex for 1 min. Then, the dispersions were kept for 30 min at room temperature, and the tubes were centrifuged at 15,000 g for 20 min. The supernatant was discarded, and the edge of the microtube was placed against absorbent paper to drain the residual supernatant. Then, the microtube with the sediment was weighed, and the WHC was calculated through the mass relation between the wet flour and the initial quantity of material[2].

2.3 Emulsifying capacity (EC) and emulsion stability (ES)

Solutions 7% (w/v) okara were prepared in a 50 mL centrifuge tube, and the pH was adjusted to 7 with 1 N NaOH. Soybean oil was added at a 1:1 ratio, and the mixture was homogenized in an Ultra-Turrax® (IKA, T 25, Brazil) at a speed of 9,500 rpm for 1 min and, subsequently, the mixture was centrifuged at 3000 g for 5 min. The volume of the emulsified layer was measured, and the emulsifying capacity was calculated as the percentage of the emulsified layer in relation to the total volume. To determine emulsion stability, the tubes previously used for EC calculation were heated for 15 min at 85 °C, cooled to room temperature, and centrifuged at 3000 g for 5 min. Then, the emulsion stability (ES) is determined as the percentage of remaining emulsified layer[3].

2.4 Least gelation concentration

Different concentrations of okara (0.02–0.2 g/mL in water) were prepared in glass test tubes, which were kept in a water bath at 100 °C for 1 h. Then, the tubes were immediately cooled in an ice bath and kept at 4 °C for 2 h. The tubes were inverted, and it was verified if the solutions flowed, in increasing order of concentration. The least gelation concentration is defined as the lowest concentration of material required to form a gel network, exhibiting hard gel without gravity drop when the tubes are inverted[4].

2.5. Statistical analysis

The analyses were conducted in triplicate and data were expressed as mean ± standard deviation. One-way ANOVA and *t*-test were carried out with a level of significance of ($p < 0.05$). The statistical tests were done by using Microsoft Excel 365 (Microsoft Corporation, USA).

3 RESULTS & DISCUSSION

The degree of hydrolysis (GH) was performed until achieve a plateau, obtaining 16.3 % after 1:10h, calculated as showed below:

$$GH\ (\%) = \frac{h}{h_{total}} \times 100 = \frac{V_{naOH} \times N_b}{MP \times \alpha \times h_{total}} \times 100$$

Where: Nb is the normality of the base; MP is the protein mass (g, determined in N × Kjeldahl factor), α is the degree of dissociation, h total is the total number of peptide bonds before the reaction

Regarding techno-functional properties, shown in Table 1, the hydrolysis with Alcalase improved the water holding capacity but decreased the emulsion capacity and emulsion stability. Due to enzymatic hydrolysis, it was expected to obtain a material with greater protein solubility and, consequently, greater water-holding capacity and emulsifying capacity since protein solubility facilitates protein interaction at the water/oil and air/water interface. However, some protein functionality can be lost due to a high extent of hydrolysis or denaturation of protein, which could occur due to the drying process.

Table 1: Techno-functional characteristics of okara flour (OF) and okara flour hydrolyzed with Alcalase (OFA)

	WHC (w/w)	OHC	EC (%)	ES (%)
OF	4.4 ± 0.1^a	1.4 ± 0.0^a	42.9 ± 0.1^b	56.1 ± 1.2^b
OFA	7.8 ± 0.1^b	1.4 ± 0.2^a	41.6 ± 0^a	39.9 ± 0^a

Different letters in the same column indicate statistical differences
WHC- Water holding capacity; OHC- oil holding capacity; EC- emulsifying capacity; EE- emulsion stability

Least gelation concentration (Table 2) is a techno-functional property that can contribute to some foods, confer texture and juiciness, influence products such as jellies and mousses, and increase the viscosity of bread and cakes[5]. A three-dimensional network of carbohydrates, proteins, and lipids forms the gel[6]. According to the data in Table 3, the capacity of gel formation was improved due to the hydrolysis.

Table 2: Analysis of least gelation concentration of okara flour (OF) and okara flour hydrolyzed with Alcalase (OFA) at different concentrations (g/mL)

	0.02	0.04	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20
OF	-	-	-	-	-	+-	+	+	+	+
OFA	-	-	-	-	+	+	+	+	+	+

4 CONCLUSION

Hydrolysis with Alcalase improves water absorption, an essential property for bread, cakes, and sausages. The capacity of forming gel was also enhanced, requiring less material to form gel. This property can help increase the viscosity and confer structure to foods, such as puddings, cakes, sauces, and sausages. However, hydrolysis decreases the emulsifying capacity and emulsion stability by approximately 7% and 29%, respectively. More studies on techno-functional characterizations are necessary for more precise identification of the application potential of okara flour hydrolyzed with Alcalase.

REFERENCES

[1] Adler-Nissen, J. (1986). Enzymic hydrolysis of food proteins. London: Elsevier Applied Science.
[2] Okezie, B.O., & Bello, A.B. (1988). Physicochemical and functional properties of winged bean flour and isolate compared with soy isolate. Journal of Food Science, 53(2), 450–454.
[3] Du, S., Jiang, H., Yu, X., & Jane, J. (2014). *Physicochemical and functional properties of whole legume flour*. *LWT - Food Science and Technology*, *55*(1), 308‑313.
[4] *Coffmann, C.W., & Garcia J, V.V. (2007). Functional properties and amino acid content of a protein isolate from mung bean flour. International Journal of Food Science and Technology, 12(5), 473‑484.*
[5] *Zayas, J.F. (1997). Functionality of proteins in food. Springer Berlin Heidelberg.*
[6] *Kaur, M., & Singh, N. (2005). Studies on functional, thermal and pasting properties of flours from different chickpea (Cicer arietinum L.) cultivars. Food Chemistry, 91(3), 403‑411.*

ACKNOWLEDGEMENTS

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ).