APPLICATION OF TRANSGLUTAMINASE IN HYDROLYZED WHEY PROTEIN: EFFECT ON COLOR AND VISCOSITY

Rejane O. Ramos[1*], Cristiano J. Andrade[2] & Débora Oliveira[1-2]

[1] *Graduate Program in Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil.*
[2] *Graduate Program in Chemical Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil.*
** Corresponding author's email address: oliverjane4324@gmail.com*

ABSTRACT

Concentrated whey protein (WPI) solutions typically exhibit the rheological behavior of Newtonian fluids. However, this behavior can be changed to pseudoplastic, with variable viscosity, after the application of the microbial transglutaminase enzyme (MTGase) in conjunction with the use of Unconventional Technologies (UNC). The aim of this study was to apply UNC in the preparation of WPI, favoring the MTGase reaction. Colorimetric tests and rotational rheometer were performed to investigate the viscosity of samples treated by heating (HT), ultrasound probe (US), microwave (MI) and pressurized liquid extraction (PLE). In the results, the pre-treatments were significantly different in the analysis of the ΔE $p<0.05$ in relation to the control, the treatment fur was the most distant from the control sample, possibly the occurrence of Maillard reaction and the smallest for the PLE. In the rheometer, the treatments evaluated were compared with the control (without enzyme and UNC), and the viscosity found was non-significant $p>0.05$. Therefore, it is necessary to perform new assays and explore different conditions, such as substrate concentration, pH, pressure and temperature, protein analysis such as Dumas method, Near-infrared spectroscopy, electrophoresis and MALDI, when polymerization of WPI in the action of MTGase by UNC.

Keywords: Microbial transglutaminase. Polymerization. Emerging technologies. Whey Protein Isolate.

1 INTRODUCTION

Whey protein isolate (WPI) is a dairy by-product obtained during the manufacture of cheese or casein, which contains water-soluble proteins such as β-lactoglobulin, β-lactalbumin, immunoglobulins, and serum albumin, contributing significantly to protein intake. [1]

Enzymatic crosslinking, mediated by the microbial enzyme transglutaminase (MTGase; protein-glutamine gamma-glutamyltransferase, EC 2.3.2.13), is an efficient and promising alternative. This enzyme catalyzes acyl transfer, deamidation, and cross-linking (polymerization) reactions between intra- or interchain protein peptide residues (acyl donor) and lysine (acyl acceptor). Such a process can occur in the residues of glutamine and lysine peptides available in the WPI, thus making MTGase a relevant tool for sustainability in the food industry. [2]

The polymerization of WPI, induced by MTGase, can be combined with unconventional or emerging treatments. This may favor the increase in the size of WPI aggregates, culminating in the formation of three-dimensional networks of natural polymers. These nets have the ability to absorb large amounts of water through cross-linking, allowing for gelling, making WPI suitable for use in dairy products. In ultrasound, the induced chemical and physical effects cause changes in the constituents of the milk that lead to significant effects on the properties of the milk and dairy products, which can also occur in WPI under favorable conditions, as well as microwave to accelerate enzyme-catalyzed reactions and PLE. This last extraction with pressurized liquid (PLE) is based on the use of solvents at high pressure and temperature, but without reaching its critical point, which in this study was designed to modify the structures of the WPI, in order to expose the amino acids improving polymerization. [3]

The search for mild alternatives in food processing with improved modifications has led to a focus on non-thermal technologies (UNC), as mentioned above, which aim to avoid changes in the flavor or nutritional content of food during production. [4] Based on the context presented, this study was designed mainly to compare the effects of non-thermal technologies applied to WPI, evaluating the enzymatic crosslinking action of MTGase in relation to viscosity. The inference was the occurrence of gelation caused by the cross-linking promoted by the enzyme when the WPI modified by non-thermal technologies is reactivated and thus contribute to the development and improvement of more innovative and sustainable dairy products.

2 MATERIAL & METHODS

The WPI was obtained from local trade, the product had 95% protein (dry basis) according to the manufacturer. The enzyme Transglutaminase ACTIVA WM with a chemical composition ≥ 1% manufactured by Ajinomoto Ltda, obtained in powder form, was kindly provided by the company. [5]

The sample preparation protocol was carried out with WPI solutions (5% by weight%), which were prepared by dispersing the protein powder in deionized water, after which the samples were mixed with a magnetic stirrer at room temperature for 12 hours, then applied the non-thermal technologies for each group of samples: Heating (HT), microwave (MI), probe ultrasound US) and

pressurized liquid extraction (PLE) followed the same procedure for all samples before the addition of the enzyme. Table 1 shows the conditions of the experiment and the technologies used for each treatment.

Table 1 Treatment condition data: Heating (HT), Microwave (MI), Ultrasound (US) and Pressurized liquid extraction (PLE).

Non-thermal technologies	Equipament	Treatment conditions
Heating	Thermostatic Bath TE-184	Temp of 85 °C for 10 minutes
Microwave	Monowave 300 da Anton Paar GmbH	Temp of 85 °C with power of 350W
Ultrasound	Ultrassonicador UP400ST	Temp re of 85°C for 15 minutes and amplitude of 30%
Pressurized liquid extraction	Self-assembled SWE [6]	Temp from 85 °C to pressure from 100 bar

The amount of enzyme used in this experiment was estimated at 10% of the WPI mass, resulting in an activity/substrate of 10 U/g-WPI. Enzyme activity was estimated at 100/g according to supplier, where a unit (U) of activity was defined as the grams of the powdered enzyme capable of producing 1 µmol of hydroxamate per minute at pH 7.0 and 50 °C when substrates of N-carbobenzoxy-glutaminyl-glycine and hydroxylamine were catalyzed by the enzyme. The samples were directly dissolved by the enzymatic powder followed by incubation at 55 °C in a Shaker Incubator with agitation at 200 rpm for 4 hours for the action of MTGase in the polymerization reaction of the WPI, after which the samples were stored at 4 °C, the enzymatic activity ceased, causing the enzyme to hibernate at this temperature and then performed the analyses.

Color Analysis: The color characteristics of the samples were determined by the Hunter Lab colorimeter (color flex EZ, Reston, VA) based on the CIE Lab or L*, a* and b* color scale recommended by the Commission Internationale d'Eclairage. [7]

Rheology: To measure the viscosity of the samples, an Anton Paar MCR 72 rheometer was used under the following conditions: Fixed shear rate of 50 s^{-1}, 20 points per analysis in an interval of 45 seconds between each point with analysis at 25 °C and geometry used of concentric cylinder-CC 27, in rotational test in triplicates. Data analysis: Each experiment was carried out in triplicate and statistical considering $p < 0.05$ as a significant difference between the data, the data analysis graphs were plloted using Origin pro 2018R1 (Northampton, MA).

3 RESULTS & DISCUSSION

The research was based on the general observation of the action of MTGase on WPI modified by non-thermal treatments in protein polymerization, resulting in the viscosity or strength of the gel of the treatments. It was expected that the technologies employed would act on the WPI structures, allowing the enzymatic action of MTGase and thus promoting a better integration of high in the three-dimensional gel network. Figure 1 shows the appearance of the treatments and Figure 2 shows the colorimetric analysis.

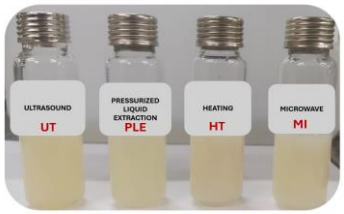

Figure 1 Appearance of the samples of WPI MTGase and non-thermal treatments.

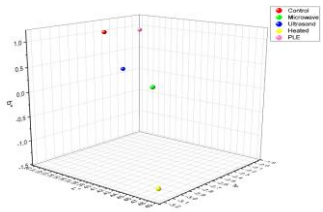

Figure 2 Color analysis for non-heat treatments.

Figure 1 shows the treatments that went through the four non-thermal technologies for four hours on the enzymatic action of MTGase in equal amounts that were compared to the control sample. In the statistical analysis, there was a difference at $p < 0.05$ (data not shown) and that there is a significant difference.

The technologies used influenced the structures and components of the samples, which was the expected result. In Figure 2, statistically, the values differed significantly at $p > 0.05$ (data not shown), in which the treatment furthest from the sample control was heating, which may have been the naturally occurring non-enzymatic Maillard reaction possibly due to the end products of the reaction in the changes in WPI that occurred at the molecular level when combined in solution and heating. not being noticeable to the naked eye. The one that was least distant from the control sample was the PLE.[8] In the analysis of ΔE, in comparison with heating, the treatments of UT and PLE were considered different, but without differentiation for MI.

In the rheological analysis, the values obtained in the polymerization of WPI by MTGase were measured and plotted in the graph in Figure 3. There was variation in the values of heating, microwave, ultrasound and PLE. However, in the statistical approach, no significant difference was observed between the treatments ($p < 0.05$).

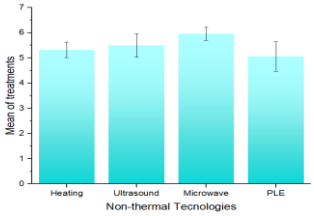

Figure 3 Mean treatment in non-thermal technologies and standard deviation.

In Figure 3 shows some of the unconventional technologies that have been employed in order to alter the properties of WPI by exposing its reactive groups, such as glutamine, lysine and amines, to gelation. This would cause covalent bonds of ε-(γ-glutamyl)lysine with intra- and intermolecular cross-links, nevertheless for this experiment there was no occurrence of gel strength. It is noteworthy that the structures of the WPI are compacted and considered to be poorly reactive to transglutaminase.

Apparently, the rheological behavior of whey protein dispersions (WPI), shown in Figure 3, may have been affected by WPI concentration or pH. According to existing reports in the literature, viscosity may increase with increasing pH or with increasing substrate concentration, culminating in the variation of the strength of the gel in which the suspension, which may have important implications for the processing of these dispersions.[9,10]

Due to the limited amount of information on WPI modified by non-thermal technologies, research is still ongoing. Recently, research has been done using whey protein soluble aggregates (WPISA) that have been pretreated with high-intensity ultrasound. The rheology of WPISA treatments cross-linked by TGase showed that the apparent viscosity and consistency index were significantly increased ($p < 0.05$), proposing that ultrasound facilitated the formation of higher molecular weight polymers. [11] The effects of ultrasound treatment on the physicochemical and emulsifying properties of whey proteins before and after thermal aggregation were explored, which caused statistically significant changes ($p < 0.05$) in the flow index (n), while ultrasound did not significantly influence the flow index ($p > 0.05$).

Combination treatments are also being researched to better obtain responses to MTGase use. For example, three physical pretreatments have been devised and combined with each other: ultrasound (400 W, U), microwave heating (75 °C for 15 min, M) and synergized ultrasound with microwave heating pretreatments (UM) to promote the formation of polymers in TGase cross-linked WPI, which between three physical methods, the pretreatment with M had the strongest effect on the structure and functional characteristics of the TGase-induced WPI and further by the results the catalysis of TGase brought out expansive polymers and viscosities of the WPI. [12]

4 CONCLUSION

The polymerization of MTGase in WPI was carried out using non-thermal treatments. However, it is necessary to investigate the redissolution conditions of the WPI powder modified in water to obtain a more pronounced apparent viscosity, since the tests related to color such as ΔE, according to the analysis made by the Kruskal-Wallis test, obtained a 0.01455 p-value, demonstrating that the UNC used here has an effect on the treatments, and with this inference possibly differentiating some parameters such as substrate concentration or pH, the promoted crosslinking may occur by MTGase. This study demonstrated that non-thermal technologies have been employed and can be employed with more improved parameters. It is recommended to test the modification of WPI for enzymatic action, since the substrate (WPI) is not very favorable to crosslinking. In addition, it is important to perform new tests in parallel with other approaches and conditions exploring new forms of analysis.

5 REFERENCES

[1] TAN, S. ZHONG, C. LANGRISH, T. 2020. Pre-gelation assisted spray drying of whey protein isolates (WPI) for microencapsulation and controlled release. LWT. 2020 (117) 108625.

[2] GASPAR, A. L. C. GÓES-FAVONI, S. P. 2015. Action of microbial transglutaminase (MTGase) in the modification of food proteins: A review. Food Chemistry. 171:315-22.

[3] OZEL, B. CIKRIKCI, S. AYDIN, O. OZTOP, M. H. 2017. Polysaccharide blended whey protein isolate-(WPI) hydrogels: A physicochemical and controlled release study. Food Hydrocoll. 71. 35-46.

[4] CARRILLO-LOPEZ, L .M. GARCIA-GALICIA, I. A. TIRADO-GALLEGOS, J. M. SANCHEZ-VEGA, HUERTA-JIMENEZ, M. ASHOKKUMAR, M. 2021. Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties. Ultrason Sonochem. 73.105467.

[5] HINZ, K. HUPPERTZ, T. KELLY, A. L. 2012. Susceptibility of the individual caseins in reconstituted skim milk to cross-linking by transglutaminase: influence of temperature, pH and mineral equilibria. JDR. 79 (4). 414-21.

[6] GONÇALVES, R. L. G. MAZZUTTI, S., VITALI L. MICKE, G. A. FERREIRA, S. R. S. 2019. Recovery of bioactive phenolic compounds from papaya seeds agroindustrial residue using subcritical water extraction. Biocatal. Agric. Biotechnol. 22.101367.

[7] AFSHARI-JOUYBARI, H. FARAHNAKY, A. 2011. Evaluation of Photoshop software potential for food colorimetry. J. Food Eng. (2).170–5.

[8] Qi, P. Xiao, Y. Wickham, E. D. 2018. Interactions, induced by heating, of whey protein isolate (WPI) with sugar beet pectin (SBP) in solution: Comparisons with a dry-state Maillard reaction. Food Hydrocoll. **83.** 61-71.

[9] ZHANG, L. WANG, X. QU, W. ZHANG, AO. WAHIA, H. GAO, X. 2022. Evaluation of dual-frequency multi-angle ultrasound on physicochemical properties of tofu gel and its finished product by TOPSIS-entropy weight method. Ultrason Sonochem. 90.106196.

[10] BAZINET, L. TRIGUI, M. IPPERSIEL, D. 2004. Rheological Behavior of WPI Dispersion as a Function of pH and Protein Concentration. J Agric Food Chem. 52 (17) . 5366–71.

[11] ZHANG, T. ZHAO, Y. TIAN, X. LIU, J, YE, H. SHEN, X. 2021. Effect of ultrasound pretreatment on structural, physicochemical, rheological and gelation properties of transglutaminase cross-linked whey protein soluble aggregates. Ultrason Sonochem. 74.105553.

[12] ZHANG, W. ZHAO, P. LI, J. WANG, X. HOU, J. JIANG, Z. 2022. Effects of ultrasound synergized with microwave on structure and functional properties of transglutaminase-crosslinked whey protein isolate. Ultrason Sonochem 83.105935.

ACKNOWLEDGEMENTS

We thank the Postgraduate Program in Food Engineering - PPGEAL of the Federal University of Santa Catarina - UFSC, the Coordination for the Improvement of Higher Education Personnel - CAPES for the support provided, as well as the company Ajinomoto.