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Proceeding Paper

Hydrolysates from a Whey Protein Concentrate Are a Promising Functional Ingredient for Diabetes Control via DPP-IV Inhibition †

Selene Isabel Alvarez-Chávez [1], Itzury Reyes-Ramírez [1], Luis Guillermo González-Olivares [1], Jesús Guadalupe Pérez-Flores [1], Elizabeth Contreras-López [1], Laura Berenice Olvera-Rosales [1], Laura García-Curiel [2] and Emmanuel Pérez-Escalante [1,*]

[1] Área Académica de Química, Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca-Tulancingo km. 4.5, Ciudad del Conocimiento, Mineral de la Reforma 42184, Mexico; al414334@uaeh.edu.mx (S.I.A.-C.); re299248@uaeh.edu.mx (I.R.-R.); lgonzales@uaeh.edu.mx (L.G.G.-O.); jesus_perez@uaeh.edu.mx (J.G.P.-F.); elizac@uaeh.edu.mx (E.C.-L.); lauolver@gmail.com (L.B.O.-R.)

[2] Área Académica de Enfermería, Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca-Actopan Camino a Tilcuautla s/n, San Agustín Tlaxiaca 42060, Mexico; laura.garcia@uaeh.edu.mx

* Correspondence: emmanuel_perez@uaeh.edu.mx

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Abstract: The circular economy has been a strategy for diminishing waste and improving the use of natural resources and energy in different industrial sectors. The food industry is a manufacturing and service sector with few incorporations into sustainable development from the circular economy philosophy due to the absence of concrete or real scenarios to be carried out. The dairy industry has incorporated some strategies to mitigate the contamination, producing whey concentrate powders and their hydrolysates as alternatives. Thus, the work aimed to produce hydrolysates with antidiabetic functions from the hydrolysis with alcalase and flavourzyme of whey protein concentrate with 80% protein. Dispersions of whey powder were prepared in phosphate buffer at pH = 7.5 and hydrolyzed for 6 h at 60 °C and 130 rpm. The hydrolysates produced maintained an antidiabetic activity between 43% and 52% from dipeptidyl peptidase IV (DPP-IV) inhibition, with the alcalase enzyme slightly better. Thus, the enzymatic process tested on whey protein concentrate generated a promising ingredient for glycemic control.

Keywords: bovine whey powder; whey contamination mitigation; enzymatic hydrolysis; antidiabetic peptides

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1. Introduction

The circular economy has emerged as a powerful strategy to reduce environmental problems caused by diverse industries worldwide [1]. This approach is focused mainly on reducing and recycling waste, enhancing the consumption of natural resources, and developing an efficient energy system [2]. Although the last conceptual methodology has gained certain acceptance by industries to align it into a sustainable future, the food and agricultural sector lacks concrete strategies for achieving real sustainability [1,3]. In this context, milk processing companies have little experience applying circular economy concepts, and the proposal solutions are usually limited [4]. The main dairy product where the circular business models have been incorporated is cheese-making, with strategies focused on cleaning processes, improving manufacturing energy efficiency, and whey recycling or biorefinery [5–8]. Bovine whey is the principal contaminant in cheese companies' producers, where nearly the middle of the total whey worldwide produced is destinated for non-human food uses, highlighting its use as animal feed, fertilizer, or discharged in water bodies as waste [5,7]. Whey contains lactose, soluble proteins, essential amino

acids, and minerals, being the organic compounds responsible for higher chemical and biochemical oxygen demands; however, most of those components could be leveraged for different purposes [5,7,8]. In this sense, some of the attributes shown by whey are its techno-functional properties, where emulsifying, water binding, fat replacement, protein enrichment, stabilizer, whipping, or viscosity controller are the highlighted functions [9]. Another proposed strategy for mitigating whey contamination is its inclusion in developing beverages for athletes, exploiting its nutritional value, or mixing it with other functional ingredients to produce functional formulations for human well-being [8,9]. On the other hand, from a classical circular economy methodology for whey valorization, producing whey powders from filtration, electrodialysis, and whey drying is common. The powders obtained have storage and transport facilities and a better shelf life [9]. It contains 35 to 80% protein, converting it into promising candidates for enzymatic or bacterial hydrolysis, enhancing protein digestibility and absorption, and producing smaller peptides with human health benefits [9,10]. In this context, hydrolysates from whey protein concentrate (WPC) have shown interesting human health and well-being properties, such as cytoprotective, antioxidant, antihypertensive, or antidiabetic activities [11–13]. Indeed, finding alternatives for diabetes control is imperative because type 2 diabetes is considered a pandemic public health problem [14]. Thus, the work aimed to release peptidic fractions with inhibitory DPP-IV activity from enzymatic hydrolysis of WPC with alcalase and flavourzyme, enzymes with a broad application in the bioactive peptide production from different sources but limited for whey powders [15,16].

2. Materials and Methods

2.1. Chemical Reagents

Whey protein concentrate with 80% protein (WPC-80) was acquired from Fudtech (Cuautitlán Izcalli, Edo. de Mexico, Mexico). Alcalase from *Bacillus licheniformis* (>2.4 U/g Anson Units), Flavourzyme from *Aspergillus oryzae* (>500 U/g), picryl sulfonic acid solution (5%, *w*/*v*), Dipeptidyl Peptidase IV (DPP-IV) recombinant enzyme from human (>1 unit/vial), and Tris (hydroxy) aminomethane were bought from Sigma-Aldrich (Saint Louis, MO, USA). Gly-Pro-p-nitroanilide was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). HCl, KH_2PO_4, K_2HPO_4, and K_2CO_3 were obtained from JT Baker Thermo Fisher Scientific (Benito Juárez, Mexico City, Mexico). All chemicals were ACS grade, and Milli-Q deionized water at 18.2 MΩ·cm (Merck Millipore; Darmstadt, Hesse, Germany) was used for dissolutions.

2.2. Instrumentation

Vertical autoclave (Evar, Guadalajara, Jalisco, Mexico). Convection incubator (Benchmark, Guadalajara, Jalisco, Mexico). Refrigerated microcentrifuge Sorvall Fresco and UV–vis spectrophotometer (Thermo Fisher Scientific, Benito Juárez, Mexico City, Mexico). Dry-Bath (Barnstead Thermolyne; Dubuque, IA, USA).

2.3. Enzymatic Hydrolysis

Dispersions of commercial WPC-80 at 10% (*w*/*v*) in sterile phosphate buffer (0.01 M, pH = 7.5) were made and thermally treated at 90 °C for 10 min. The hydrolysis was performed according to the proposed by Hussein et al. [17] with some modifications. The enzymatic reaction was started by alcalase or flavourzyme addition at a mass ratio of 100:2.5 (soluble protein: enzyme), maintaining it at 60 °C and 130 rpm for 6 h. Later, the reaction was stopped with thermal treatment using a boiling water bath for 10 min. Supernatants were obtained through centrifugation at 10,000 rpm and 4 °C for 10 min and used in posterior analysis.

2.4. Free Amino Groups Determination

The release of amine groups for enzymatic hydrolysis was measured by the Adler-Nilssen method [18]. Thus, 0.25 mL of supernatants obtained after enzyme inactivation and

centrifugation was blended with 2 mL of phosphate buffer (0.21 M, pH = 8.2). The reaction started with adding 2 mL of pycrylsulfonic acid solution (0.1% in buffer) and incubating for 1 h at 50 °C. To finish the reaction, 4 mL of 0.1 N HCl was added, and the absorbance was recorded at 340 nm. Free amino group concentration was determined using a glycine calibration curve ranging from 0 to 200 mg/L.

2.5. Antidiabetic Determination by DPP-IV Inhibition Test

The antidiabetic activity was evaluated through the spectrophotometric method proposed by Nongonierma et al. [19] with slight modifications described by Islas-Martínez et al. [20]. The process is based on the hydrolysis of Gly-Pro-p-nitroanilide by the DPP-IV enzyme, measuring the p-nitroanilide release in the electromagnetic spectra visible region. Thus, three systems were tested. The sample (A_s), positive control (A_{100}), and control sample (A_{sc}) systems were constituted. The first contains the hydrolysate, DPP-IV substrate (1.6 mM), and the enzyme (0.01 U/mL). The second system contained buffer Tris-HCl (0.1 M, pH = 8) instead of sample, substrate, and enzyme. Both last systems were incubated at 37 °C for 60 min and arrested with potassium carbonate addition. For the third system, a blending with the sample, potassium carbonate, and the amount of buffer instead of substrate and enzyme was made. Finally, absorbance for all systems was measured at 405 nm, and the following equation calculated the DPP-IV inhibitory activity:

$$\text{DPP} - \text{IV inhibition}(\%) = \frac{A_{100} - (A_s - A_{sc})}{A_{100}} \times 100$$

2.6. Statistical Analysis

Minitab 18 software, version 1 (State College, PA, USA) was employed to carry out a one-way ANOVA. Tukey's contrast was performed to determine differences at a confidence level $\alpha = 0.05$.

3. Results and Discussions

Table 1 shows the results obtained during the following of free amino groups released by enzymatic hydrolysis and the corresponding antidiabetic capacity.

Table 1. Following hydrolysis and antidiabetic activity by alcalase and flavourzyme enzymes.

Enzyme	Free Amino Groups Concentration (mg/L)		DPP-IV Inhibition (%)	
	0 h	6 h	0 h	6 h
Alcalase	1198.04 ± 24.21 [b]	2283.26 ± 96.07 [a]	22.03 ± 1.92 [b]	52.14 ± 0.78 [a]
Flavourzyme	735.00 ± 0.00 [b]	1585.44 ± 110.68 [a]	27.75 ± 1.85 [b]	43.26 ± 1.42 [a]

Different lowercase letters show significant differences between the evaluation times. $n = 3$ for free amino groups determination, while $n = 2$ for antidiabetic capacity.

Both enzymatic hydrolysis showed a significant increment in the free amino groups released according to the concentration determined at the initial time. That increase was similar for hydrolysis, 1.9 and 2.2-fold for alcalase and flavourzyme, respectively. Usually, alcalase is recognized as a more proteolytic enzyme than flavourzyme [21]; however, this also depends on the substrate. Specifically for whey protein concentrate or isolate, Kim et al. [22] found a hydrolysis degree of 12.4% for alcalase and 12% for flavourzyme after the hydrolysis of WPC-80 for 4 h, which demonstrated very slight differences. In more recent years, a hydrolysis degree of around 12% was also shown by Dermiki and FitzGerald [23] when WPC-80 was hydrolyzed by alcalase after 4 h. In comparison, 20% was reported by Di Filippo et al. [24] for alcalase hydrolysis of whey protein isolate (WPI) with ~95% protein after 6 h. Also, Sereda et al. [25] found an improvement in hydrolysis degree when alcalase and flavourzyme were combined to hydrolyze WPC-80, reaching a ~30% hydrolysis degree after 4.5 h. Thus, previous works demonstrate that alcalase and

flavourzyme maintain a very similar whey protein hydrolysis, at least in the context of amine release, agreeing with the present study.

For antidiabetic activity, WPC-80 at the hydrolysis beginning showed DPP-IV inhibition between 22% and 28%, which was not statistically different according to the enzyme used. Sereda et al. [25] have shown that the WPC can present low levels of hydrolysis degree due to heating treatment; thus, it is possible to release specific peptidic fractions with biological activity. Nonetheless, results evidenced a significant increment in antidiabetic activity at the final hydrolysis (43–52%) attributed to the enzymatic activity. A few differences in bioactivity among the enzymes used were found, with a slightly better antidiabetic activity for alcalase hydrolysis. The last could be induced by the higher number of cutting sites exhibited by alcalase than flavourzyme [21], which produces amino acid sequences with higher antidiabetic capacity. These sequences are characterized by hydrophobic amino acids and significantly lower molecular weights [26].

WPC has been shown to inhibit the DPP-IV enzyme significantly. Konrad et al. [27] demonstrated that WPC-80 hydrolyzed by a serin protease from Asian pumpkin generated better antidiabetic peptides than β-lactoglobulin hydrolysis, with an IC_{50} lower than 0.55 mg/mL. In the same context, Nongonierma et al. [28] and Haj Mustafa et al. [13] found antidiabetic capacities from DPP-IV inhibition in hydrolysates from camel and bovine WPC, having IC_{50} of 0.55–1.52 mg/L and 0.9–1.98 mg/L, respectively. In addition, all bioactive hydrolysates showed molecular weights in the range of <10, 3–5, and <3 kDa, which could be released from WPC-80 hydrolyzed by alcalase and flavourzyme because both enzymes maintain the capacity to generate this type of peptides.

On the other hand, when the WPC-80 hydrolysates are compared with the most consumed synthetic DPP-IV inhibitors, such as sitagliptin and vildagliptin, results show that the hydrolysates are very competitive with the effect provided by a sitagliptin dose of 25 mg, which has shown a DPP-IV inhibition of 46.6% after 24 h of administration [29]. In the same context, hydrolysates are shown to be competitive with a dose of 200 mg of vildagliptin, which provided an antidiabetic effect by DPP-IV inhibition of 60.7% at 24 h postdose [30]. However, other deep pharmacokinetics studies must be employed to evaluate the incorporation of WPC-80 hydrolysates as nutraceuticals.

Finally, whey hydrolysates produced by the enzymatic process are very promising for use as functional ingredients in the food industry due to their health-promotion properties. Nonetheless, future technological functions, solubility, absorption, and sensorial attributes must be assessed to develop foods with positive biological effects that are acceptable to consumers.

4. Conclusions

The hydrolysates generated by alcalase and flavourzyme enzymes showed a promising antidiabetic activity from the in vitro DPP-IV inhibition found, which was highly comparable with the DPP-IV inhibition in plasma for the most common drugs for glycemic control. The last is very encouraging because the obtention of nutraceuticals or functional foods from a cheese by-product opens novel options for implementing a circular economy in the milk sector, increasing the opportunities for whey treatment and its incorporation in other industries like pharmaceutical and food supplements. However, different analyses with higher profundity must be employed to ensure the biological effect on animals and humans. Also, sensorial and technological properties must be assessed to increase the knowledge about better food products where the hydrolysates could be incorporated as functional ingredients.

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