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Screening of *in vitro* bioactivities of a soy protein hydrolysate separated by hollow fiber and spiral-wound ultrafiltration membranes

Cyril Roblet [a,b], Jean Amiot [a,b], Charles Lavigne [b,c], André Marette [b,c], Martin Lessard [b,d], Julie Jean [a,b], Charles Ramassamy [b,e], Christine Moresoli [f], Laurent Bazinet [a,b,*]

[a] Department of Food Sciences and Nutrition, Université Laval, 2425 rue de l'Agriculture, Quebec (QC), Canada, G1V 0A6
[b] Institute of Nutraceuticals and Functional Foods (INAF), Université Laval, 2440 boulevard Hochelaga, Quebec (QC), Canada, G1V 0A6
[c] Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, 2725 chemin Ste-Foy, Quebec (QC), Canada, G1V 4S3
[d] Dairy and Swine R & D Centre, Agriculture and Agri-Food Canada, Lennoxville, (QC), 2000 rue College, Canada, J1M 0C8
[e] INRS-Institut Armand-Frappier, 531 boul des Prairies, Laval (QC), Canada, H7V 1B7
[f] Biotechnology and Health Engineering Centre, University of Waterloo, 200 University Avenue West, Waterloo (ON), Canada, N2L 3G1

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ABSTRACT

The aims of this work were 1) to determine the bioactivities resulting from the hydrolysis of soy (*Glycine max*) proteins isolate (SPI) by a double enzymatic treatment of pepsin and pancreatin and 2) to investigate whether fractionation of the protein hydrolysates by two ultrafiltration techniques using either hollow fiber (HF) or spiral-wounded (SW) membrane (10 kDa cut-off), increases biological activity of the recovered peptides. The focus was on the screening of bioactivity of the peptide fractions using multiple *in vitro* bioassays such as the proliferation of cancer cells, destruction of murine norovirus-1 (MNV-1), anti-oxidative and immunomodulating properties, and finally glucose metabolism in a muscle cell line. Significant biological effects of the peptides for both antioxidant capacity (ORAC assay) and metabolism (muscle glucose uptake assay) were found. These results show that ultrafiltration of SPI hydrolysates is a convenient process for the recovery of large amount of bioactive peptides.

1. Introduction

First cultured in south-east Asia, soybean (*Glycine max*) is nowadays present all around the world. It is one of the most important crop plants for seed protein and oil content. Since a long time, consumption of soy food, and especially soy proteins, was associated with nutritional and health benefits for human, including hypocholesterolemic effect (Sugano et al., 1988), prevention of heart (Castiglioni et al., 2003) and breast (Barnes, Grubbs, Setchell, & Carlson, 1990) diseases. Many studies have also been conducted on soybean protein hydrolyzates produced with various enzymes such as microbial, gastric and pancreatic enzymes during the last four decades. *In vitro* studies have demonstrated that peptide fractions have stronger biological effect than whole protein, and many soy peptide sequences having bioactivity have been reported (Wu & Ding, 2001; Shin et al., 2001; Yoshikawa et al., 2000).

Most studies on protein hydrolyzates have used long and expensive technologies for the separation of bioactives peptides, such as High Performance Liquid Chromatography (HPLC) or ion exchange column (Schlimme & Meisel, 1995; Recio & Visser, 1999; Bouhallab, Henry, & Boschetti, 1996). Nevertheless, UF seems to be a trivial technology for the separation of proteins or peptides according to their molecular weights, and allows the production of large amount of purified peptides fraction (Drioli, 1986). Previous studies have shown the feasibility of using UF to enhance bioactivity, such as ACE inhibitor effects (Fujita, Yamagami, & Ohshima, 2001), antioxidative properties (Je, Kim, & Kim, 2005), in various complex matrices (milk, plant or fish proteins). However, no study has yet been conducted on a pepsin-pancreatin hydrolyzate of soy protein isolate fractionated by

Abbreviations: 2-DG, 2-Deoxyglucose; α-MEM, α-Minimum essential medium; AA, amino acids; BBI, Bowman-Birk inhibitor; BCA, bicinchoninic acid; BSA, bovine serum albumin; DH, demineralised hydrolysate; DMEM, Dulbecco's minimal essential medium; ED, Electrodialysis; FBS, foetal bovine serum; HF, Hollow fiber; HFP, Hollow fiber permeate; HFR, hollow fiber retentate; ICP, inductively coupled plasma; IEM, Ion exchange membrane; IL-6, interleukine-6; KTI, Kunitz trypsine inhibitor; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MNV-1, Murine norovirus strain 1; MW, molecular weight; ORAC, Oxygen Radical Absorbance Capacity; PBMC, Peripheral Blood Mononuclear Cell; PES, polyether sulfone; PFU, plaque-forming unit; RH, raw hydrolysate; ROS, reactive oxygen species; RPMI, Roswell Park Memorial Institute medium; SPI, Soy proteins isolate; SW, Spiral-wound; SWP, Spiral-Wound permeate; SWR, spiral-wound retentate; TNF-α, tumor necrosis factor-α; UF, ultrafiltration; w/v, weight/volume.

* Corresponding author at: Department of Food Sciences and Nutrition, Université Laval, 2425 rue de l'Agriculture, Quebec (QC), Canada, G1V 0A6. Tel.: +1 418 656 2131x7445; fax: +1 418 656 3353.
E-mail addresses: cyril.roblet.1@ulaval.ca (C. Roblet), jean.amiot@fsaa.ulaval.ca (J. Amiot), charles.lavigne@criucpq.ulaval.ca (C. Lavigne), andre.marette@criucpq.ulaval.ca (A. Marette), martin.lessard@agr.gc.ca (M. Lessard), julie.jean@fsaa.ulaval.ca (J. Jean), charles.Ramassamy@iaf.inrs.ca (C. Ramassamy), cmoresoli@uwaterloo.ca (C. Moresoli), laurent.bazinet@fsaa.ulaval.ca (L. Bazinet).

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ultrafiltration (UF), with focus on biological properties such as anti-cancer, anti-viral, immunologic or other metabolic properties.

The aim of the present study was therefore to establish 1) if a pepsin-pancreatin hydrolyzate presents any bioactivity by a screening of different techniques, 2) if UF fractionation is a potential mean to enhance the bioactivity of this raw hydrolyzate, and finally (3) if UF membrane configuration has an influence on the biological activities of the recovered peptides. In this study, the focus was on the screening of bioactivity of the peptide fractions using multiple *in vitro* bioassays.

2. Materials and methods

2.1. Raw material

SPI, Profam® 974, was obtained from NEWLY WEDS (lot 8502, Boucherville, Qc, Canada). The analysis of the soy isolate, as provided by the company, was: moisture max, 6%; protein min, 90%; lipid max, 4.0%; and ash max 5.0%. The pH of the soy isolate was 7.0–7.4

The two proteases used for the hydrolysis of the SPI were obtained from SIGMA-ALDRICH (Oakville, Canada): Pepsin from porcine gastric mucosa and pancreatin from porcine pancreas with respective optimal pH of 1–3 and 7–9.

2.2. Production of raw hydrolyzate

Hydrolysis protocol of the SPI aqueous dispersion was adapted from the digestion conditions described by Vilela, Lands, Chan, Azadi, & Kubow, (2006). The SPI was hydrolyzed in a 25 liters thermoregulated batch laboratory reactor (Scanima a/s Mixer SRB 25, Aalborg, Denmark). First, a 3.12% (w/v) soy protein solution was hydrated and preheated during 30 min at 37 °C. Then, pH was adjusted at 1.5 with HCl 10 M (SIGMA-ALDRICH, Oakville, Canada). After another 30 min of hydration at pH 1.5, the SPI was hydrolyzed during 45 min by 750 ml of a pepsin solution diluted in HCl 0.01 M, with a final enzyme-substrate ratio (E/S) of 1/100. The residual pepsin activity was stopped by increasing pH to 7.8, with 10 M NaOH (SIGMA-ALDRICH, Oakville, Canada).

Thereafter, the temperature of the SPI peptic hydrolyzate solution was adjusted to 40 °C, and pancreatic hydrolysis was carried-out during 120 min using 3 L of a pancreatin solution diluted in a 0.1 M sodium phosphate buffer (Na_2HPO_4) (SIGMA-ALDRICH, Oakville, Canada), with a final 1/10 E/S ratio. The hydrolysis was stopped by heating the solution at 85 °C during 15 min. The soy hydrolyzate was cooled as quickly as possible and frozen at −30 °C: the final concentration was 2.4% (w/v). It was then lyophilized (lyophylisator model FFD-42-WS, VirTis RePP, Gardiner, NY). The powder obtained (named raw hydrolyzate (RH)) was stored at −20 °C in hermetic food grade plastic bag. Nine batches of hydrolyzates were produced, checked for comparable molecular weight distribution and pooled to obtain a sufficient quantity of SPI hydrolyzate.

2.3. Demineralization

Before being separated by UF, raw hydrolysate (RH) was demineralized by electrodialysis (ED) (Fig. 1), using the EUR-2C type cell (Eurodia, Rungis, France) delivered with 200 cm^2 graphite electrodes. Seven pairs of ions exchange membranes (IEM) were stacked in the ED cell, with one Neosepta CMX-SB cationic membrane and one Neosepta AMX-SB anionic membrane (both from Tokoyuma Soda Ltd, Tokyo, Japan) per pair. The objective of the demineralization was to reduce mineral salt content of RH by 70–75% for the needs of the further *in vitro* tests. 10 L of a 2.4% (w/v) RH aqueous solution was hydrated during 1 h at room temperature. Twenty liters of KCl solution (2 g/L) and ten liters of Na_2SO_4 solution (20 g/L) were also prepared and placed into appropriate ED compartments (KCl solution into mineralisation compartment, Na_2SO_4 into rinsing electrode compartment). After hydration, RH solution was placed into the demineralisation compartment, then solutions were homogenized by recirculation and pH was adjusted to 7–7.2 with NaOH 1 M (SIGMA-ALDRICH, Oakville, Canada). After pH stabilization, demineralization was performed at room temperature: the flow rates were adjusted at 4 L/min for Na_2SO_4 solution and 2 L/min for hydrolyzate and KCl solutions. The voltage was adjusted at 14 V (less than 80% of the limiting current). At the beginning of experiments, current density was 90 A/m², and decreased to 15 A/m² at the end of demineralization. After demineralization, solutions were frozen at −30 °C, and then lyophilized. Powder obtained was stored at −20 °C in a hermetic food grade plastic bag. Five repetitions of demineralization were performed, to obtain a sufficient quantity of demineralized hydrolyzate (DH) for the study.

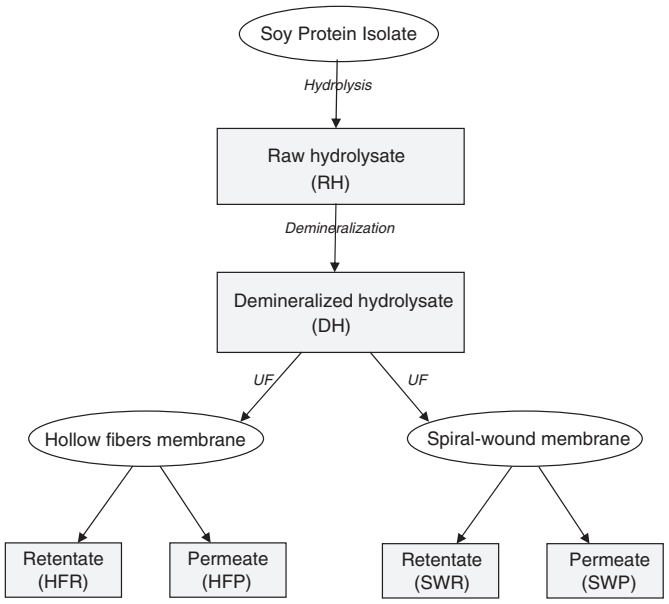

Fig. 1. SPI processing for the production of different peptide fractions by UF using hollow-fiber and spiral-wound membranes. Overview of the hydrolysis, demineralization and UF fractionation process: raw hydrolyzate (RH), demineralized hydrolyzate (DH), hollow fiber membrane retentate (HFR), hollow fiber membrane permeate (HFP), spiral-wound membrane retentate (SWR), and spiral-wound membrane permeate (SWP).

2.4. UF fractionation

UF fractionation was performed on a Lab Unit 1812 (Filtration Engineering Co., Inc., Champlin, MN) using two different membranes configuration, presenting both the same 10 kDa cut-off (Table 1):

1) A polyethersulfone (PES) spiral-wound (SW) membrane, with 4200 cm² (4.5 square feet) (1812-T UF spiral-wound membrane, Filtration Engineering Co., Inc. Champlin, MN)

Table 1
Comparison of the UF process and membrane parameters of the two membrane configurations used.

Variable	Spiral wound	Hollow fiber
TMP (kPa)	173 ± 21	
Membrane surface area (cm^2)	4200	420
Mean permeate flux (L/m^2/hour)	12.86	7.14
Initial volume (ml)	1800	
Final volume (ml)	900	
Filtration time (min)	10	180