Peptides 61 (2014) 48–55

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Hydrolysates of sheep cheese whey as a source of bioactive peptides with antioxidant and angiotensin-converting enzyme inhibitory activities

Ana Paula Folmer Corrêa[a], Daniel Joner Daroit[b], Roberta Fontoura[a], Stela Maris Meister Meira[a], Jeferson Segalin[a], Adriano Brandelli[a,*]

[a] Laboratório de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos (ICTA), Universidade Federal do Rio Grande do Sul (UFRGS), 91501-970 Porto Alegre, RS, Brazil
[b] Universidade Federal da Fronteira Sul (UFFS), Campus Cerro Largo, 97900-000 Cerro Largo, RS, Brazil

ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form 2 September 2014
Accepted 2 September 2014
Available online 10 September 2014

Keywords:
Cheese whey
Microbial protease
Protein hydrolysates
Bioactive peptides
Antioxidant activity
Antihypertensive activity

ABSTRACT

Enzymatic proteolysis may be employed to release bioactive peptides, which have been investigated for potential benefits from both technological and human health perspectives. In this study, sheep cheese whey (SCW) was hydrolyzed with a protease preparation from *Bacillus* sp. P7, and the hydrolysates were evaluated for antioxidant and angiotensin I-converting enzyme (ACE)-inhibitory activities. Soluble protein and free amino acids increased during hydrolysis of SCW for up to 4 h. Antioxidant activity of hydrolysates, evaluated by the 2,2′azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid radical scavenging method, increased 3.2-fold from 0 h (15.9%) to 6 h of hydrolysis (51.3%). Maximum Fe^{2+} chelation was reached in 3 h hydrolysates, and the reducing power peaked at 1 h of hydrolysis, representing 6.2 and 2.1-fold increase, respectively, when compared to that of non-hydrolyzed SCW. ACE inhibition by SCW (12%) was improved through hydrolysis, reaching maximal values (55% inhibition) in 4 h, although 42% inhibition was already observed after 1 h hydrolysis. The peptide LAFNPTQLEGQCHV, derived from β-lactoglobulin, was identified from 4-h hydrolysates. Such a biotechnological approach might be an interesting strategy for SCW processing, potentially contributing to the management and valorization of this abundant dairy byproduct.

Introduction

Whey is a major byproduct of cheese and casein industries, representing about 85–95% of the milk volume, and consisting basically of water (94–95%, v/v), lactose (3.8–4.0%, w/v), proteins (0.8–1.0%, w/v), and minerals (0.7–0.8%, w/v). Because of its low concentration of total solids (6–7% dry matter), whey has commonly been considered as a waste product. However, since millions of tons of whey are produced worldwide and it possess high chemical and biochemical oxygen demands, discard of this byproduct as an effluent represents an important source of environmental problems [16].

Due to its polluting potential, the disposal of untreated whey has been severely restricted, prompting the dairy industry to search for alternative management practices. Among the different strategies, whey can be used in supplemental feeding of livestock; irrigation of soil and pastures for agriculture; as a source of lactose and proteins for food and industrial applications; in fuel production (such as ethanol, hydrogen, methane) and obtainment of bioproducts through fermentation technologies; among others. Considering its volume and composition, cheese whey could be viewed as an important source of proteins with consistent functional and nutritious properties, for utilization in the food industry [29,33].

Technologies that allow the transformation of cheese whey into other products are increasingly focused, since such processes potentially act both as valorization strategies and effluent management practices. One of the processes that could promote value-aggregation to whey proteins is enzymatic hydrolysis. This treatment, which promotes the fractionation of parent proteins into smaller units, usually improves functional properties such as solubility, emulsifying power, texture, and therefore may increase the applicability of whey proteins into food products [37,38]. Also, amino acid sequences found within whey proteins are capable of modulating physiological responses after

* Corresponding author at: ICTA – UFRGS, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, RS, Brazil. Tel.: +55 51 3308 6249; fax: +55 51 3308 7048.
E-mail address: abrand@ufrgs.br (A. Brandelli).

http://dx.doi.org/10.1016/j.peptides.2014.09.001
0196-9781/

release by enzymatic hydrolysis, exerting antioxidant, antimicrobial, immunomodulatory, antiulcerogenic, antihypertensive, opioid, and hypocholesterolemic activities [22,24]. Considering their potential usefulness from both human health and food technology perspectives, increasing research efforts are focused on obtaining whey-derived bioactive peptides and hydrolysates. Particularly, Adjonu et al. [1] reported that hydrolysis of whey protein isolate (WPI) hydrolysed by chymotrypsin, trypsin or pepsin resulted in increased *in vitro* antioxidant and angiotesin-converting enzyme (ACE)-inhibitory activities. Similar results were observed after hydrolysis of WPI, bovine whey concentrate, and purified bovine whey proteins (β-lactoglobulin and α-lactalbumin) hydrolyzed by diverse commercial enzymes [3,12,17,28,40].

As presented above, the bulk literature reports the biological activities of bovine whey hydrolysates obtained with commercial proteases. However, whey proteins account for 17–22% of sheep milk proteins, mainly represented by β-lactoglobulin and α-lactalbumin which, in turn, make up to 70–80% of total whey proteins [29]. Also, the investigation of alternative proteolytic enzymes from different sources is an interesting approach to obtain protein hydrolysates and bioactive peptides with desired properties. Specifically, *Bacillus* sp. P7, a bacterium isolated from the intestine of an Amazon basin fish, produces high levels of extracellular proteases with biotechnological potential [5]. In this context, this study aimed to evaluate the antioxidant and the angiotensin I-converting enzyme (ACE)-inhibitory activities of sheep cheese whey hydrolysates obtained through treatment with a protease preparation from *Bacillus* sp. P7.

Materials and methods

Enzyme production and protease preparation

Bacillus sp. P7 was maintained in Brain-Heart Agar (BHA) plates. For protease production, the strain was cultivated in feather meal broth (10 g L^{-1} feather meal, 0.3 g L^{-1} Na_2HPO_4, 0.4 g L^{-1} NaH_2PO_4, 0.5 g L^{-1} NaCl) for 48 h at 30 °C in a rotary shaker (125 rpm). After this period, the culture was centrifuged (10,000 × *g* for 15 min at 4 °C) and the supernatant was submitted to a partial purification protocol involving ammonium sulphate concentration (60% saturation) followed by liquid chromatography on a Sephadex G-100 gel permeation column (25 × 0.5 cm), which was equilibrated and eluted with 20 mmol L^{-1} Tris-HCl buffer (pH 8.0). Fractions with proteolytic activity on azocasein [6] were pooled and used as the protease preparation for sheep whey hydrolysis.

Enzymatic hydrolysis of sheep cheese whey

Sheep cheese whey (SCW) (3.9 g L^{-1}), obtained from a local cheese manufacturer, was lyophilized and subsequently dissolved in Tris-HCl buffer (20 mmol L^{-1}, pH 8.0). This solution (10 g L^{-1}) was preheated at 45 °C for 15 min, and the hydrolysis was initiated by adding the protease preparation (2%, v/v; 1057 U mL^{-1}). Incubation was performed at 45 °C in a water bath with reciprocal shaking and, at specified intervals t_i (i = 0, 0.5, 1, 2, 3, 4 or 6 h), samples were removed and the hydrolysis reaction was terminated by heating at 100 °C for 15 min. After cooling, the hydrolysates were centrifuged (10,000 × *g* for 15 min) to remove insoluble materials, and the supernatants were lyophilized and stored at −18 °C until further analyses.

Determination of protein and free amino acids concentration

The concentration of protein on the supernatant of the hydrolysates was determined by the Folin phenol reagent method [23], using bovine serum albumin as standard. Concentration of amino acids was determined by the ninhydrin method [25], using glycine as standard. All measurements were performed using a Shimadzu UV mini-1240 spectrophotometer.

Determination of protein hydrophobicity

Hydrophobicity of the SCWH supernatants was determined using bromophenol blue (BPB) sodium salt for electrophoresis (Sigma), according to Chelh et al. [2], with modifications. To 1 mL of sample (50 mg mL^{-1}), 200 μL of 1 mg mL^{-1} BPB (in distilled water) was added and mixed well. A control, without sample, consisted of the addition of 200 μL of BPB solution to 1 mL of 20 mmol L^{-1} Tris-HCl buffer (pH 8.0). Samples and controls were kept under agitation, at room temperature, during 10 min, and then centrifuged for 15 min at 2000 × *g*. The absorbance of the supernatant (diluted 1:10) was measured at 595 nm against a blank of Tris-HCl buffer. In this assay, a higher amount of bound BPB indicates a higher hydrophobicity. The amount of BPB bound is given by the formula: Bound BPB (μg) = 200 μg × [(absorbance of control − absorbance of sample)/absorbance of control].

2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical scavenging assay

Scavenging activity of SCW hydrolysates (SCWH) supernatants against the ABTS radical was determined by the decolorization method described by Re et al. [34]. The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by reacting 5 mL of ABTS solution (7 mmol L^{-1}) with 88 μL of K_2SO_4 solution (140 mmol L^{-1}) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the assay, $ABTS^{\bullet+}$ solution was diluted with 5 mmol L^{-1} phosphate buffered saline (pH 7.4) to an absorbance of 0.7 (±0.02) at 734 nm. A 10 μL sample (50 mg mL^{-1}) was mixed with 1 mL of diluted $ABTS^{\bullet+}$ solution and absorbance (at 734 nm) was measured after 6 min. The percentage decrease of absorbance was calculated in comparison to that of controls.

Iron (II) chelating activity assay

The ferrous ion chelating ability of hydrolysate supernatants was determined according to Tang et al. [39], with the following modifications. A sample volume of 1 mL (50 mg mL^{-1} concentration) was mixed with 3.7 mL of distilled water, 0.1 mL of 2 mmol L^{-1} $FeSO_4$ (Fe^{2+}) and 0.2 mL of 5 mmol L^{-1} ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine). After 10 min, the absorbance of the reaction mixture was read at 562 nm. Likewise, 1 mL of distilled water, instead of sample, was used as a control. EDTA (20 mg mL^{-1}) was used as standard. Chelating activity was calculated as follows: Chelating activity (%) = [1 − (absorbance of sample/absorbance of control)] × 100.

Reducing power

The reducing power of SCWH supernatants was assessed according to the method of Duh et al. [13]. Samples (50 mg mL^{-1}) in phosphate buffer (2.5 mL, 0.2 mol L^{-1}, pH 6.6) were added to 2.5 mL of 10 g L^{-1} potassium ferricyanide solution, and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 mL of a 10% solution, w/v) was added to the mixture, which was then centrifuged at 3000 × *g* for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and, after addition of 0.5 mL of ferric chloride solution (1%, w/v), the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a greater reducing power. Butylated hydroxytoluene (BHT) at the same concentration of samples was used as a positive control.