Food Chemistry 221 (2017) 590–598

Contents lists available at ScienceDirect

Food Chemistry

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

Electro-activation of sweet defatted whey: Impact on the induced Maillard reaction products and bioactive peptides

Ourdia Kareb [a,b], Ahmed Gomaa [a,b,e], Claude P. Champagne [a,c], Julie Jean [a,b], Mohammed Aïder [a,d,*]

[a] Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec, Qc G1V 0A6, Canada
[b] Department of Food Sciences, Université Laval, Quebec, Qc G1V 0A6, Canada
[c] Food R&D Centre, Agriculture and Agri-food Canada, 3600 Casavant, St. Hyacinthe, Qc J2S 8E3, Canada
[d] Department of Soil Sciences and Agri-Food Engineering, Université Laval, Quebec, Qc G1V 0A6, Canada
[e] Food Science and Nutrition Department, National Research Center, Egypt

ARTICLE INFO

Article history:
Received 30 August 2016
Received in revised form 4 November 2016
Accepted 24 November 2016
Available online 25 November 2016

Keywords:
Whey
Electro-activation
Bioactive peptides
Maillard reaction
Glycation

ABSTRACT

Electro-activation was used to add value to sweet defatted whey. This study aimed to investigate and to characterize the bioactive compounds formed under different electro-activation conditions by molecular and proteomic approaches. The effects of electric current intensity (400, 500 or 600 mA) and whey concentration (7, 14 or 21% (w/v)) as a function of the electro-activation time (0, 15, 30 or 45 min) were evaluated. The targeted dependent variables were the formation of Maillard reaction products (MRPs), protein hydrolysates and glycated compounds. It was shown that the MRPs derived from electro-activated whey at a concentration of 14% had the highest potential of biological activity. SDS–PAGE analyses indicated the formation of hydrolysates and glycated compounds with different molecular weight distributions. FTIR indicated the predominance of intermediate MRPs, such as the Schiff base compounds. LC–MS/MS and proteomics analysis showed the production of multi-functional bioactive peptides due to the hydrolysis of whey proteins.

1. Introduction

Whey is a dairy industry co-product that contains approximately 55% of milk components (Smithers, 2008). It is rich in proteins such as β-lactoglobulin (β-Lg) and α-lactalbumin (α-La) with high nutritional and physiological health-promoting effects (Korhonen & Pihlanto, 2006; Mohanty, Mohapatra, Misra, & Sahu, 2016; Möller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008; Patel, 2015). Moreover, whey-derived ingredients are increasingly used in different food formulations due to their technological, functional and organoleptic characteristics (Chatterton, Smithers, Roupas, & Brodkorb, 2006). Furthermore, the growth of foods with nutraceutical properties involves intensive use of whey-derived ingredients for their possible bioactivity and health promoting benefits. To achieve this objective, different approaches were proposed to improve the functionality of whey by producing whey-derived products containing bioactive compounds with special focus on antioxidant activity (Brandelli, Daroit, & Corrêa, 2015; de Castro & Sato, 2014; Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2014; Peng, Kong, Xia, & Liu, 2010). Enzymatic treatments are generally used to improve the bioactive potential of whey through the formation of active peptides (Hernández-Ledesma, Dávalos, Bartolomé, & Amigo, 2005; Peng et al., 2010; Pihlanto, 2006; Pihlanto & Korhonen, 2015). Indeed, whey protein hydrolysates demonstrated high efficacy as natural antioxidants by preventing lipid oxidation in different foods. Several antioxidant hydrolysates from β-Lg and α-La with high radical-scavenging activity were isolated (Hernández-Ledesma et al., 2005). Unfortunately, this strategy suffered from serious drawbacks, such as lack of specificity, high process cost, low catalysis efficiency and the need for using huge volumes of chemicals and thermal treatments to stop the hydrolysis process (Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, & Recio, 2011; de Castro & Sato, 2014; Kosseva, Panesar, Kaur, & Kennedy, 2009; Power, Jakeman, & FitzGerald, 2013).

Recently, the Maillard reaction (MR), also known as non-enzymatic browning, was pursued as a promising mean of enhancing the antioxidant activity of whey (Oliveira et al., 2014). Maillard reaction products (MRPs) occur between available ε-amino groups

* Corresponding author at: Université Laval, Pavilion P. Comtois, Department of Soil Sciences and Agri-Food Engineering, 2255 Rue de l'Agriculture, Quebec, Qc G1V 0A6, Canada.
E-mail address: mohammed.aider@fsaa.ulaval.ca (M. Aïder).

http://dx.doi.org/10.1016/j.foodchem.2016.11.134
0308-8146/

in the proteins, peptides, free amino acids and the reducing-end carbonyl group of sugars. This reaction is highly enhanced by heating (Arena, Renzone, D'Ambrosio, Salzano, & Scaloni, 2017). Different studies have shown that MRPs from whey exhibit high antioxidant activities in different model and real food systems (Dong et al., 2012; Jiang & Brodkorb, 2012; Wang, Bao, & Chen, 2013). Various mechanistic approaches have been suggested to explain the observed antioxidant activities of MRPs-whey. Among these mechanisms, scavenging of reactive oxygen species, inactivation of free radical chain, inhibition of lipid peroxidation and chelation of transition metals were suggested (de Castro & Sato, 2014; Liu, Kong, Han, Sun, & Li, 2014; Oh et al., 2013; Stanic-Vucinic, Prodic, Apostolovic, Nikolic, & Velickovic, 2013; Wang et al., 2013). Moreover, MRPs-whey could present other interesting properties such as a prebiotic effect, antimicrobial activity and antihypertensive and anti-allergenic activities (Chevalier, Chobert, Genot, & Haertlé, 2001; Corzo-Martínez, Hernandez-Hernandez, Villamiel, Rastall, & Moreno, 2013; Hwang, Kim, Woo, Lee, & Jeong, 2011). Nevertheless, the major challenge in this area remains the need to develop efficient technologies that can control the extent of the MRPs and confer the desired end-product characteristics without the need of energy-intensive high thermal and lengthy processing conditions (Chawla, Chander, & Sharma, 2009; Jaeger, Janositz, & Knorr, 2010; Jiang & Brodkorb, 2012).

In this context, we have previously reported the electro-activation (EA) as an emerging technology to promote the antioxidant activity of water-soluble whey under wet conditions (Kareb, Champagne, & Aïder, 2016). The enhancement of the antioxidant properties of electro-activated whey (EA-whey) resulted from the combined effect of hydrolysed proteins/peptides and glycation of amino group containing molecules of whey with reducing sugars under alkaline conditions which are favorable for the formation of Maillard reactions products (MRPs). Interestingly, a conversion of lactose to lactulose (a prebiotic) was also achieved concurrently under the same conditions. Therefore, EA-whey could be considered as a complex mixture of bioactive compounds in which several conjugates and distinct antioxidant mechanisms are potentially acting. Furthermore, the antioxidant activity of EA-whey can be influenced by many factors, such as time, pH, concentration and type of reactants, in addition to the intrinsic reaction conditions related to the EA processing.

In this study, we investigated the potential of electro-activation (EA) as a promising efficient technology to enhance the bioactive properties of whey under controlled conditions. The main objective of this work was to investigate the effect of electro-activation under different experimental conditions (electric current intensity, whey total solids concentration, and reaction time) on the induced Maillard reaction products (MRPs) and bioactive peptides formation.

2. Materials and methods

2.1. Chemicals and reagents

Food-grade whey powder (lactose 75%, proteins 12% and moisture content less than 5%) was purchased from Agropur Cooperative (St-Hubert, Quebec, Canada). Sodium sulfate (Na_2SO_4) was purchased from Anachemia (Montreal, Quebec, Canada). All reagents and chemicals used for this study were of analytical grade and were purchased from Sigma Chemical Co. (Oakville, Ontario, Canada). Reagents were freshly prepared and stored under adequate conditions to prevent deterioration. All the solutions were prepared in deionized water and were filtered through 0.45 μm Millipore filters before use.

2.2. Maillard reaction products (MRPs) generation from EA-whey

MRPs derived from EA-whey were prepared under alkaline conditions following a previously described procedure (Kareb et al., 2016) (Fig. 1). Briefly, whey powder was freshly reconstituted to the required concentrations (7, 14 or 21% (w/v)) in deionized water and stored overnight at 4 °C. An equal volume (125 mL) of each preparation was kept in the cathodic compartment, while the central and anodic compartments were filled with Na_2SO_4 solution at 0.5 M concentration to ensure the passage of current, as shown in Fig. 1. Solutions were submitted to different current intensities (400, 500 or 600 mA) and were gently stirred at 200 rpm min^{-1}, corresponding to 0.54 RCF, using an agitator Model: RW20DS1 (Coleparmer Canada, Montreal, Canada). Samples from the cathodic compartment were collected at 15 min intervals during the 45 min EA process. The samples were then stored at 4 °C. All experiments were performed in triplicate. The pH was measured by a digital multimeter (Keithley, Inerga Series, Cleveland, OH) equipped with a pH-probe (model Oakton pH 700, Eutech Instruments, Cole-Parmer, Montreal, Canada).

2.3. Determination of free and sugar-bound amino acids

Free amino acid groups and glycation degree of the conjugates derived from EA-whey after 45 min of treatment were analyzed using an EZ:faast kit according to the manufacturer's instructions (Torrance, CA, USA). The derivatized free amino acids were separated, identified and quantified by gas chromatography (GC) equipped with an AOC-20i auto-injector and a FID 2010 Plus (Fisons Instruments Plus) that is connected to GC solution software (Mandel Scientific Inc., Guelph, ON, Canada). The data corresponded to the concentration of free amino acid (μmol). The degree of glycation of EA-whey was expressed as the glycation ratio of EA samples to the native sample (C_0).

2.4. Determination of reducing sugars in MRPs-whey

Reducing sugars such as lactose, lactulose, glucose, galactose and fructose in whey subjected to EA for 45 min were quantified using an HPLC Agilent Technology (Millipore Corp., Milford, MA, USA). A column 300 × 6.5 mm Carbohydrate Analysis (Waters Co., Milford, MA USA) and a refractive index detector (Waters, Model 410) were used. The supernatant was diluted 100 times, and then, a 25 μL aliquot was injected. The running time was set at 30 min per sample. Once the separation of sugars was achieved, peaks were identified by comparing their retention time to that of the standard sugars.

2.5. Structure characterization of MRPs-whey induced by electro-activation

2.5.1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with or without β-mercaptoethanol (β-ME) of EA-whey proteins was performed according to a previously described method (Laemmli, 1970). Briefly, the 1% (w/v) protein solutions were diluted in a 1:1 ratio with an SDS-PAGE Laemmli sample buffer and then heated at 95 °C for 5 min. A 10 μL aliquot of each sample was loaded into a 4–20% gradient of polyacrylamide gel. The separation was performed at a constant voltage of 30 mA. After the run, the protein bands were stained by Coomassie Brilliant Blue R-250 (0.2%) in 40% methanol and 10% acetic acid for 2 h and destained overnight with 10% methanol and 10% glacial acetic acid.