Available online at www.sciencedirect.com

ScienceDirect

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

Effects of thermal, microwave, and ultrasound pretreatments on antioxidative capacity of enzymatic milk protein concentrate hydrolysates

Hankie Uluko [a,b], *Shuwen Zhang* [a], *Lu Liu* [a], *Madalitso Tsakama* [a,c], *Jing Lu* [a], *Jiaping Lv* [a,*]

[a] *Institute of Agro-Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, China*

[b] *Faculty of Agriculture, Agricultural Engineering Department, Bunda College of Agriculture, Lilongwe, Malawi*

[c] *Department of Physics and Biochemical Sciences, The Polytechnic, University of Malawi, Blantyre, Malawi*

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form 21 November 2014
Accepted 26 November 2014
Available online 22 December 2014

Keywords:
Antioxidative
Milk protein concentrate
Pretreatment
Hydrolysis
Ultrafiltration

ABSTRACT

The antioxidant capacity of milk protein concentrate (MPC) subjected to hydrolysis with digestive enzymes (pepsin and trypsin) after heat (HT), microwave (MW) and ultrasound (US) pretreatments were investigated using DPPH radical scavenging and ferric reducing antioxidative power (FRAP) assays. Pretreatments were carried out for 10 min, MW and HT pretreatments at 90 °C, US at 800 W and 20 kHz. Samples were jacketed with ice during sonication while a control received no pretreatment. Compared with the control (EC_{50} = 0.316 mg mL^{-1}), most pretreatments (US, US + MW, US + HT, US + MW + HT) significantly improved ($P = 0.05$) the radical scavenging activity and US pretreated samples showed the highest activity (EC_{50} = 0.283 mg mL^{-1}) but the pretreated samples showed similar antioxidant capacity when the FRAP assay was used which could be due to the large number of peptides in the pretreated samples. US pretreatment has the potential to improve antioxidant capacity of aggregated proteins such as MPC during enzymatic hydrolysis with digestive enzymes.

1. Introduction

Antioxidative peptides have been isolated successfully from different animal and plant protein sources such as milk, egg, fish, cowpea, soy and wheat (Power, Jakeman, & FitzGerald, 2013; Quansah, Udenigwe, Saalia, & Yada, 2013; Samaranayaka & Li-Chan, 2011). Many researchers have explained the mechanisms by which antioxidative peptides play an important role in food systems and in the human body particularly the reduction of the oxidative processes (Dudonne, Vitrac, Coutière, Woillez, & Mérillon, 2009; Gomez-Ruiz, Lopez-Exposito, Pihlanto, Ramos, & Recio, 2008; López-Alarcón & Denicola, 2013; Pihlanto, 2006; Segura-Campos, Salazar-Vega, Chel-Guerrero, & Betancur-Ancona, 2013). In general, the functions attributed to antioxidative peptides in food matrices include the reduction in lipid peroxidation and its associated products (Peng, Kong, Xia, & Liu, 2010) that help in the maintenance of flavor, texture and the color of food during storage while in the human body, the antioxidative peptides reduce the effects of oxidative stress which is responsible for aging and various diseases (Finkel & Holbrook, 2000; Halliwell, 2007). Due to the

* *Corresponding author.* Institute of Agro-Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), No.2 Yuan Ming Yuan West Road, Haidian District, P.O.Box 5109, Beijing 100193, China. Tel.: +86 10 62819421; fax: +86 10 62819421.
E-mail address: lvjp586@vip.sina.com (J. Lv).
http://dx.doi.org/10.1016/j.jff.2014.11.024
1756-4646/

difference in mechanisms, several assays have to be used to determine the antioxidant capacity of peptides (López-Alarcón & Denicola, 2013).

Several studies on enzymatic hydrolysis have reported the successful production of potent antioxidative hydrolysates and peptides from individual milk proteins (whey and casein) with different enzymes. For example, Zhang, Wu, Ling, and Lu (2013) and Peng et al. (2010) demonstrated the potential of whey proteins as antioxidants while Gomez-Ruiz et al. (2008), Correa et al. (2011) and Ao and Li (2013) indicated the antioxidative capacity of casein fractions. However, studies on antioxidative capacities of aggregated milk proteins such as milk protein concentrate (MPC) are few. Recently, Uluko et al. (2014a) reported that Neutrase-derived hydrolysates of MPC exhibited antioxidative capacity after ultrasound (US) pretreatment. The pretreatment of proteins before enzymatic hydrolysis has been shown to improve the release of bioactive peptides from various proteins. Such methods include heat (HT), hydrostatic pressure (HP), ultrasound (US), microwave (MW) and pulsed electric field (PEF) treatments (Knezevic-Jugovic et al., 2012; Quiros, Chichón, Recio, & López-Fandiño, 2007a; Uluko et al., 2014b). It is thought that the pretreatments help in protein unfolding and increased accessibility of enzymes to peptide bonds.

This study was conducted to determine the effects of US, HT and MW pretreatment on the antioxidative capacity of MPC hydrolysates after hydrolysis with digestive enzymes (pepsin + trypsin). A previous study (Uluko et al., 2014a) used US pretreatment prior to Neutrase (microbial protease) hydrolysis and membrane filtration to improve antioxidative capacity of MPC hydrolysates. The current research compared the antioxidative capacities of hydrolysates from untreated samples with samples pretreated with US, HT, MW or their combinations (US + MW; US + HT; MW + HT and US + MW + HT) using enzymes present in the digestive system. In other studies, the bioactivity of hydrolysates of some legume species was improved with the use of HT (Akillioglu & Karakaya, 2009; Quansah et al., 2013), while US has been used to improve the bioactivity of egg white proteins (Knezevic-Jugovic et al., 2012) and MW has been used for the same purpose in whey proteins (Izquierdo, Penas, Baeza, & Gomez, 2008). The determination of antioxidative capacities was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidative power (FRAP) assays. The effect of ultrafiltration of MPC hydrolysates on the antioxidative capacities was also studied.

2. Materials and methods

2.1. Substrate and chemicals

The protein substrate used was a commercial MPC with protein, lactose, water, fat and ash contents of 74.56, 11.94, 4.40, 1.52 and 7.12%, respectively, bought from Ningxia Cezanne Dairy Industry Co. Ltd (Ningxia, China). Trypsin (EC 3.4.21.4, from bovine pancreas) and pepsin (EC 3.4.23.1, from porcine stomach mucosa) were purchased from Pangbo Enzyme (Guangxi, China). Trifluoroacetic acid (TFA), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) and high performance liquid chromatography (HPLC) peptide standard mixture were sourced from Sigma-Aldrich China (Beijing, China). Hydrochloric acid (HCl), sodium hydroxide (NaOH), glacial acetic acid, sodium acetate, sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, ethanol, potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and chemicals required for o-phthaldialdehyde (OPA) assay were procured from Beijing Biodee Biotechnology Co. Ltd (Beijing, China). During chromatographic analyses, MilliQ water was used.

2.2. Thermal, microwave and ultrasound pretreatments

The samples for different experimental treatments were made from 5% (w/w) protein solutions. Each sample was prepared by mixing 6.70 g of MPC in 100 mL double deionized water in a cylindrical beaker. During HT pretreatment, the samples were immersed in a water bath set at 90 °C for 10 min with manual stirring. For MW pretreatment, the samples were left in the microwave (Sineo, MDS-6, Shanghai, China) for 10 min with the temperature programmed not to exceed 90 °C by inserting a temperature probe in the sample well. Ultrasound pretreatment, using a cell disruptor (Ningbo Scientz, JY92-IIN, Zhejiang, China), followed the procedure of Uluko et al. (2013) with minor changes. The samples were sonicated for 10 min while jacketed with ice.

2.3. Hydrolysis with digestive enzymes and ultrafiltration

During enzymatic hydrolysis, a protocol described by Parrot, Degraeve, Curia, and Martial-Gros (2003) was followed with modifications. In brief, digestion was conducted in an incubation shaker set at 37 °C and 180 × *g* in 250 mL conical flasks. Before digestion, the substrates were immersed in a water bath at 37 °C for 30 min. Pepsin was added to the MPC solution (pH 2.0) at an E/S ratio of 1:100 (w/w) to initiate digestion. The peptic digestion was conducted for 2 h and was stopped by increasing the pH to 7.5 with 2 M NaOH. Then trypsin was added to the pepsin predigested mixture at an E/S ratio of 1:200 and carried out for 2 h. The enzyme deactivation was achieved by heating in a water bath at 95 ± 3.0 °C for 20 min. After cooling to room temperature, the samples were lyophilized then stored at –20 °C until the chromatographic and amino acid analyses. The OPA assay was used for the determination of the degree of hydrolysis (DH) and peptide content of samples (Nielsen, Petersen, & Dambmann, 2001; Uluko et al., 2014a).

Before storage, some MPC hydrolysates were fractionated using stirred ultrafiltration cell on a Minimate™ TFF system (Pall Life Sciences, New York, NY, USA) first employing a 10 kDa (Omega, made with polyethersulphone) to remove enzymes and unhydrolyzed proteins from the hydrolysates, then a 5 kDa membrane (Pall Life Sciences, New York, NY, USA) was used to separate the hydrolysates into 2 fractions (filtrate, < 5 kDa and retentate, > 5 kDa). The fractions were also freeze dried before storage at –20 °C for further analysis.