Food Chemistry 217 (2017) 517–523

Contents lists available at ScienceDirect

Food Chemistry

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

Digestibility and antigenicity of β-lactoglobulin as affected by heat, pH and applied shear

Toheder Rahaman, Todor Vasiljevic, Lata Ramchandran *

Advanced Food Systems Research Unit, College of Health and Biomedicine, Victoria University, Melbourne, VIC, Australia

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form 30 August 2016
Accepted 31 August 2016
Available online 3 September 2016

Keywords:
Beta-lactoglobulin
Antigenicity
Digestibility
Heat
pH
Shear

ABSTRACT

Processing induced conformational changes can modulate digestibility of food allergens and thereby their antigenicity. Effect of different pH (3, 5, 7), temperature (room temperature, 120 °C) and shear (0 s^{-1}, 1000 s^{-1}) on simulated gastrointestinal digestibility of β-lg and post digestion antigenic characteristics have been studied. At all pH levels unheated β-lg showed resistance to peptic digestion with high antigenic value while it was fairly susceptible to pancreatin with moderate reduction in antigenicity. Heating at 120 °C significantly improved both peptic and pancreatic digestion attributed to structural alterations that resulted in much lower antigenicity; the level of reduction being pH dependant. The lowest antigenicity was recorded at pH 5. Shearing (1000 s^{-1}) had a minor impact reducing digestibility and thereby enhancing antigenicity of unheated β-lg at pH 5 and 7 slightly; however in conjunction with heating (120 °C) it reduced antigenicity further irrespective of the pH. Overall, treatment at pH 5, 120 °C and 1000 s^{-1} could potentially reduce post digestion antigenicity of β-lg.

1. Introduction

Beta-lactoglobulin (β-lg) is the major whey protein of bovine milk comprising about 50% of total whey proteins and 10% of total milk proteins (Lozano, Giraldo, & Romero, 2008). It is a globular protein composed of 120 amino acids with two disulfide bridges and one free cysteine residue with a molecular weight of 18 kDa (Brownlow et al., 1997). It has nine antiparallel β-sheets (β barrel) and one α-helix on outer surface of barrel which folds into a calyx and acts as a carrier for some hydrophobic ligands such as retinoids, fatty acids, vitamin D, and lipids (Ron, Zimet, Bargarum, & Livney, 2010). β-Lg is a good source of many essential amino acids (Smithers, 2008) and also has some highly regarded physical functional properties such as gelling, emulsifying and foaming (Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001). In spite of its diversified functional properties and nutritional benefits, its application in food manufacturing is limited due to its allergenic potential (Hattori et al., 2004). It is one of the major bovine milk allergens, responsible for almost 80% of cow milk allergies (Bu, Luo, Zheng, & Zheng, 2009). The relative resistance of β-lg to peptic digestion appears to be due its native conformation and its absence from human milk could be the underlying reasons for observed allergenicity in individuals allergic to cow's milk (Villas-Boas, Vieira, Trevizan, de Lima Zollner, & Netto, 2010).

Digestive stability is one of the common features for most food allergens, which allows them to pass intact through the gastrointestinal mucosa that consequently lead to retention of a sufficient number of epitopes to bind with present antibodies thereby triggering allergenic reactions (Astwood, Leach, & Fuchs, 1996; Moreno, 2007). For this reason, it is necessary to gain a thorough understanding how known allergens present in food behave during gastrointestinal passage and what impact this passage may have on their allergenic potential in order to properly manage food allergenicity. Most food products are subjected to various processing operations in order to produce diversified end products. Processing conditions affect conformation of food proteins, recognized as the most important allergen property, which in turn impacts on accessibility of antigenic epitopes to digestive enzymes (Rahaman, Vasiljevic, & Ramchandran, 2016a, 2016b) and thereby increase (Maleki et al., 2003), decrease (López-Expósito, Chicón, Belloque, López-Fandiño, & Berin, 2012) or maintain (Bu, Luo, Chen, Liu, & Zhu, 2013) their allergenic potential. Milk, in particular, undergoes various processes such as heating (pasteurization, sterilization), high pressure treatment, fermentation, homogenization, centrifugation and filtration prior to distribution and consumption. Among these, effect of heat treatment on the conformation of milk allergens and subsequent impact on digestibility and allergenicity has been well documented (Morisawa et al., 2009; O'Loughlin, Murray, Kelly, FitzGerald, & Brodkorb, 2012; Rudloff & Lönnerdal, 1992). Heating above 90 °C induced conformational changes in β-lg resulting in exposure of susceptible peptic bonds and disruption

* Corresponding author.
E-mail address: lata.ramchandran@vu.edu.au (L. Ramchandran).

http://dx.doi.org/10.1016/j.foodchem.2016.08.129
0308-8146/

of conformational and sequential epitopes that consequently reduced the extent of antigenic reaction (Rahaman et al., 2016b). Another important environmental factor is pH, which modulates heat induced changes in the conformation of β-lg and its digestive behaviour (Zhang & Vardhanabhuti, 2014). Since diverse milk products are prepared at different pH levels and treated at various temperatures accompanied with flow induced shear, it is necessary to establish the impact of pH in combination with temperature on digestibility of β-lg and its post digestion antigenic potential. Shear is an another important processing factor that milk proteins are subjected to during various processing operations such as mixing, centrifugation, homogenization, general flow and membrane processing (ultrafiltration, microfiltration) (Yim & Shamlou, 2000). Shear stress can affect the native physico-chemical and functional properties of protein molecules (Bekard, Asimakis, Bertolini, & Dunstan, 2011). Our previous work (Rahaman, Vasiljevic, & Ramchandran, 2015) has shown that shear in combination with pH and temperature exerts substantial conformational changes on β-lg and its antigenicity. However, stability of such changes during digestion and post digestion antigenic potential has not been well studied and requires to be investigated in order to establish if any carryover effect exists from processing to a physiological level. Therefore, the present work was aimed at establishing effects of selected pH, temperatures and applied shear on the digestion of β-lg and antigenicity of the resulting digests.

2. Materials and methods

2.1. Treatment of sample

Based on the result of our earlier work (Rahaman et al., 2015), treatments that significantly influenced the antigenicity of β-lg were selected for further investigation. These included prepared samples of β-lg with high antigenicity observed at pH 3, room temperature, 0 s^{-1}; and pH 3, 120 °C, 1000 s^{-1} and samples with low antigenicity at pH 5, room temperature, 0 s^{-1}; pH 5, 120 °C, 1000 s^{-1}; pH 7, room temperature, 0 s^{-1}; and pH 7, 120 °C, 1000 s^{-1}. As reported previously (Rahaman et al., 2015), a dispersion (0.3%, w/v) of β-lg powder (Davisco Co. Ltd., Le Sueur, MN, USA) was prepared in citric acid-phosphate buffer at pH 3, 5 or 7 with 0.02% sodium azide to prevent microbial growth. The concentration was selected on the basis of reported concentration of β-lg in bovine milk (Dupont, Croguennec, Brodkorb, & Kouaouci, 2013). Samples at each pH were treated to different temperatures (room temperature or 120 °C) and shear (0 or 1000 s^{-1}) using a pressure cell (CC25/PR-150) of an Anton Paar rheometer (Physica MCR 301 series, Graz, Austria) as described previously (Rahaman et al., 2015). Thereafter the samples were subjected to digestion and then assessed for post digestion antigenicity. The control (0.3% β-lg solution in MilliQ water, pH 7.6, without any shear and heating) was also included in the assessment. It was assessed intact (undigested) and subjected to digestion protocol and antigenicity determination to establish effects of different treatments on digestibility and antigenicity.

2.2. Sample digestion

All the samples and controls were digested following the method described by Corzo-Martínez, Soria, Belloque, Villamiel, and Moreno (2010) with some modifications. The samples were subjected to the conditions of the stomach by lowering the pH to 2.0 with 2 M HCl, followed by addition of 0.3% (w/v) solution of porcine pepsin (EC 3.4.23.1, Sigma-Aldrich, 2500 units mg^{-1} protein activity) in 2 M HCl to each sample to obtain enzyme-substrate ratio 1:20. The gastric digestion was performed at 37 °C for two hours in a shaking water bath to ensure continuous mixing. The pH of the mixture was then increased to 7.5 by addition of 1 M NaOH to inactivate the pepsin. A 0.1% solution of pancreatic protease (United States Pharmacopeia (USP) in Milli Q water) was then added to obtain enzyme-substrate ratio of 1:100. The simulated intestinal digestion was performed for 2 h at 37 °C. At the end of digestion, the pancreatin was inactivated by placing the samples in an oil bath at 100 °C for 10 min. The resulting digests were frozen and stored at −80 °C until required.

2.3. Determination of degree of hydrolysis (DH)

The DH of different treated samples was assessed by measuring the ratio of nitrogen soluble in 10% trichloroacetic acid (TCA-SN) to the total nitrogen following a method described previously (Drago & González, 2000). Briefly, at the end of digestion, 2 ml of the digested samples was mixed with the equal volume of 20% TCA. The mixture was allowed to stand for 1 h and then centrifuged at 3000×*g* (RT7, Sorvall, Newtown, CT) at room temperature (20 °C). The nitrogen content of the supernatant was determined by Kjeldahl method (Helrich, 1990). The DH% was calculated as follows:

$$\mathrm{DH}(\%) = \frac{(\text{Soluble nitrogen in } 10\%\ \text{TCA in hydrolysate} - \text{soluble nitrogen in } 10\%\ \text{TCA in sample without hydrolysate}) \times 100\%}{\text{Total nitrogen in dispersion}}$$

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

Non-reducing SDS-PAGE of the digests was used to establish the fate of proteins in all β-lg samples, including the control before and after digestion and treated samples after digestion. The protocol has been described previously (Rahaman et al., 2015). Briefly, aliquots of exactly 20 µl of each sample were mixed with equal volume of Tris-Tricine sample buffer and loaded onto a 16.5% Mini-PROTEAN® Tris-Tricine Gel (Bio-Rad Laboratories, NSW, and Australia). Electrophoresis was carried out at a constant voltage of 100 V for 100 min. The gels were then stained with a staining solution followed by destaining for 2 h and immersion in distilled water until the background became clear (Rahaman et al., 2015). Gels were visualized using an imager (Chemidoc MP, Bio-Rad Laboratories, NSW, Australia).

2.5. Antigenicity of digests

The digested samples (Section 2.2) were assessed for antigenicity following a protocol reported previously (Rahaman et al., 2015) using sandwich enzyme-linked immunosorbent assay (ELISA) with