LWT - Food Science and Technology 68 (2016) 523–531

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Hydrolysis of rapeseed meal protein under simulated duodenum digestion: Kinetic modeling and antioxidant activity

Cunshan Zhou [a, b, c], Xiaojie Yu [a], Xiaopei Qin [a], Haile Ma [a, b, c, *], Abu ElGasim A. Yagoub [d], Jiali Hu [a]

[a] School of Food and Biological Engineering, Jiangsu University, No.301 Xuefu Road, Zhenjiang 212013, China
[b] Jiangsu Provincial Key Laboratory for Physical Processing of Agricultural Products, Jiangsu University, No.301 Xuefu Road, Zhenjiang 212013, China
[c] Jiangsu Provincial Research Center of Bio-process and Separation Engineering of Agri-products, Jiangsu University, No.301 Xuefu Road, Zhenjiang 212013, China
[d] Faculty of Agriculture, University of Zalingie, PO Box 6, Zalingie, Sudan

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form
25 November 2015
Accepted 27 November 2015
Available online 9 December 2015

Keywords:
Rapeseed meal protein
Simulated duodenum digestion
Kinetics model
Peptides
Antioxidant activity

Chemical compounds studied in this article:
Cytochrome C (PubChem CID: 439171)
Aprotinin (PubChem CID: 16197280)
L-leucine (PubChem CID: 6106)
L-tryptophan (PubChem CID: 6305)
Ferric chloride (PubChem CID: 24380)
Trichloroacetic acid (PubChem CID: 6421)
Potassium ferricyanide (PubChem CID: 26250)
Sulfosalicylic acid (PubChem CID: 7322)
1,1-Diphenyl-2-picrylhydrazyl (PubChem CID: 2735032)
2,4,6-Trinitrobenzenesulfonic acid (PubChem CID: 498085)

ABSTRACT

Kinetics modelling, DPPH radical scavenging activity and reducing power of rapeseed meal protein were investigated under simulated duodenum digestion. Kinetics of the reaction was considered in relation to initial substrate concentration, initial enzyme concentration, and hydrolysis time. Optimum hydrolysis conditions were trypsin concentration of 10 g/L, substrate concentration of 4 g/L, time of 40 min and temperature of 37 °C. From the results a general kinetic enzymolysis equation was suggested, providing a rational theoretical basis for determining the parameters of the reaction. The highest antioxidant activity was in accordance with the optimum reaction conditions. Antioxidant free amino acids increased after hydrolysis. Antioxidant oligopeptides of molecular weights of 2608, 1695 and 211 Da were identified. At a concentration of 2 mg/mL, the peptide fraction of CS-F_2-CC_2; with molecular weight of 1000–1500 Da; showed the highest DPPH radical scavenging activity and reducing power, accounting for 70.32% and 0.538, respectively. The trypsin-hydrolysate displayed a potential capacity to scavenge free radicals and binding irons after duodenal digestion, appearing as promising ingredient to formulate functional foods with antioxidant activity.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Rapeseed is one of the most important oilseed crops worldwide and also it is the world's second leading source of protein meal (World Health Organization, 2002). The protein content of defatted rapeseed meal, a by-product of oil industry, is high (35–45%). The nutritive and functional properties of the rapeseed are characterized by two main protein families; cruciferin (12S globulin) and napin (2S albumin), with molecular weights around 300 kDa and 14 kDa, respectively (Bos et al., 2007). It is, thus, a kind of ideal and high quality protein resource, with good utilization value. Therefore, efforts have been made to develop efficient methods to prepare acceptable products from rapeseed meal for human benefit (Pan, Jiang, & Pan, 2011). Food proteins may act as sources of bioactive peptides. These peptides can be released from proteins *in vitro* during food processing or *in vivo* by the gastrointestinal

* Corresponding author. School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China.
E-mail address: mhl@ujs.edu.cn (H. Ma).

http://dx.doi.org/10.1016/j.lwt.2015.11.057
0023-6438/© 2015 Elsevier Ltd. All rights reserved.

digestion. Peptides with low molecular weights, which easily absorbed in the gastrointestinal tract, have been reported displaying potential antioxidative, antihypertensive and immune effects (Byun, Lee, & Park, 2009; Monchi & Rat, 1993; Grimble, Rees, & Keohane, 1987). Antioxidative peptides derived from soy proteins (Chen, Muramoto, Yamauchi, & Nokihara, 1996), soy milk whey (Peña-Ramos & Xiong, 2003), egg-yolk proteins (Sakanaba & Tachibana, 2006), amaranth protein or their hydrolysates (Orsini Delgado, Tironi, and Añón 2011), and buck wheat proteins (Ma, Xiong, Zhai, Zhu, & Dziubla, 2010) were monitored. Yet, there are only few studies examining the rapeseed meal as a source of bioactive peptides to enhance the value of this industry by-product. These studies were focused on production of antihypertensive peptides (Marczak, Ohinata, Lipkowski, & Yoshikawa, 2006; Marczak et al., 2003), HIV protease inhibitory hydrolysate (Yust et al., 2004), and ACE-inhibitory peptides (Mäkinen, Johannson, Gerd, Pihlava, & Pihlanto, 2012).

Recently, it is found that digestion of food proteins by human gastrointestinal tract can approximately be simulated in an *in vitro* environment. However, different food proteins under simulated gastrointestinal digestion showed different digestive characteristics. To render the enzymatic process much more close to the human digestion, maximum utilization of trypsin is indispensable. In respect to the release of potential bioactive peptides of low molecular weights, trypsin is reported to be more efficient than pepsin (Wang, Ma, Ma, & Fan, 2014). Moreover, trypsin-catalysed canola protein digestion produce high yields of bioactive hydrolysates, and less amounts of free amino acids compared to pepsin/trypsin digestion. (Alshai et al., 2014). Toward this aim, many kinetic models including empirical and mechanistic models have been proposed (Bansal, Hall, & Realff, 2009; Xu & Ding, 2007). It has been generally accepted that enzyme deactivation during the enzymatic hydrolysis process results in some hydrolytic rate slowdown (Gan, Allen, & Taylor, 2003; Ghadge, Patwardhan, & Sawant, 2005). However, many models have been proposed without considering enzyme deactivation (Shen & Agblevor, 2008).

Peptides presenting radical scavenging capacity have been released by simulated gastrointestinal digestion from diverse food sources (Ma et al., 2010). Despite that, gastrointestinal digestion of proteins may increase probability of release of more amounts of free amino acids; and simultaneously a decrease in amounts of the bioactive peptides, produced by pancreatic enzymes, due to low acidity. Díez Marqués, Ramírez-Moreno, Sánchez-Mata, and Goñi (2011) reported that gastrointestinal digestion starts at acidic medium (pH 1.3 to 3) and then followed by intestinal digestion, starting at duodenum (fasting pH 6.5) where gastric acid lowers the pH to 5.4 before increasing to 5.8 at the end of duodenal digestion In this respect, simulated duodenal digestion may be a perspective method of preparation of bioactive peptides. Accordingly, no research has been reported on the simulated duodenum digestive conditions and kinetics for producing antioxidant peptides from rapeseed protein. So that, we deduced a simple mathematical equation that directly described the relationship between the degree of hydrolysis and two reaction conditions (initial enzyme concentration and initial substrate concentration). Antioxidant activity of rapeseed protein under the simulated duodenum digestion was also investigated. This study may help providing antioxidant peptides that conform to the human physiological environment, hence increasing their efficacy when incorporated into food and pharmaceutical systems.

2. Material and methods

2.1. Material and reagents

Rapeseed meal with 42.03% protein (Kjeldahl, AOAC, 2000), was purchased from Hubei Weipu Biologic Technology Company (Hubei, China). The meal was ground to pass 80 mesh screen and kept until use. Trypsin (EC 3.4.21.4) with an activity of 50,000 U/g and L-leucine were purchased from Sinopharm Chemical Reagent Company (Zhenjiang, China). 2,4,6-trinitrobenzenesulfonic acid (TNBS), cytochrome C (12,500 Da), aprotonin (6500 Da), L-tryptophan (204 Da) were from Sigma Chemical Co. (St. Louis, MO, USA). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Aladdin Reagent Co. Ltd (Shanghai, China). Sephadex G-25 (Shanghai Yuanye Biotechnology Co. Ltd). All the other reagents were of analytical grade.

2.2. Simulated duodenum digestion of the rapeseed meal protein

The method used in simulated duodenum digestion was an adaptation of published methods (Fallingborg, 1999; Emma, Hala, & Abdul, 2008). The digestion experiments were done at different trypsin/protein ratios and hydrolysis times. After pre-incubation of the simulated duodenum digestive fluid (0.04 M KH_2PO_4 buffer, pH 6.4) for 5 min at 37 °C, solutions with different concentrations of rapeseed meal (1, 2, 4, 8 and 16 g/L) and trypsin (2, 4, 6, 10 and 12 g/L) in the digestive fluid were prepared according to the method in our previous study (Zhou, Qin, Yu, Yang, Hu, & Ma, 2015). Digestion experiments were done at different times (0–120 min) and at 37 °C with continuous agitation. In the end, reactions were terminated by boiling the mixtures for 10 min. Finally, the digests were cooled and centrifuged at 4360 × g for 20 min. The centrifuge supernatant (CS) was collected, lyophilized and kept at −20 °c until analysis. Experiments were replicated three times.

2.3. Determination of degree of hydrolysis of the rapeseed meal protein

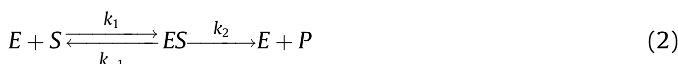
The degree of hydrolysis (DH) was measured by the reaction of free amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS) as described by Orsini Delgado et al. (2011).The DH was calculated using Eq. (1):

$$DH(\%) = \frac{[-NH_2]_h - [-NH_2]_0}{[-NH_2]_\infty - [-NH_2]_0} \times 100 \quad (1)$$

Where, $[-NH_2]$ indicates the concentration of free amino groups in the non-hydrolysed samples (0), or in the hydrolysed samples (h), $[-NH_2]_\infty$ indicates the concentration of total free amino groups.

2.4. Enzymolysis kinetics of the rapeseed meal protein

Kinetics of enzyme-catalysed reactions is the science of studying enzymatic reaction velocity and its influence factors. The kinetic equation, however, is explained by modeling the hydrolysis as a zero order reaction and inactivation of the enzyme as a second order reaction (Camacho, González, Páez, Márquez, & Fernández, 1993; Zhou et al., 2013; Zhou, Yu, Zhang, He, & Ma, 2012). According to the enzyme reaction intermediate complex theory, the process of enzymatic hydrolysis of a protein is expressed as follows:



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \quad (2)$$

where, S is the concentration of substrate (g/L), E is the

Download English Version:

https://daneshyari.com/en/article/4563677

Download Persian Version:

https://daneshyari.com/article/4563677

Daneshyari.com