Food Hydrocolloids 69 (2017) 19–27

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Plant proteins mitigate *in vitro* wheat starch digestibility

Nataly López-Barón [a], Yuchen Gu [a], Thava Vasanthan [a, *], Ratnajothi Hoover [b]

[a] University of Alberta, Edmonton, Alberta, Canada
[b] Memorial University, St.Johns, Newfoundland, Canada

ARTICLE INFO

Article history:
Received 8 September 2016
Received in revised form
15 December 2016
Accepted 10 January 2017
Available online 12 January 2017

Keywords:
Cereal and pulse proteins
Denaturation
Protein hydrolysis
Starch-protein interaction
Amylolysis RDS content

ABSTRACT

Wheat is a major staple food in North America. The rapidly digestible nature of cooked wheat starch is linked to a greater incidence of health issues regarding insulin resistance. Plant proteins and their hydrolysates have shown insulinotropic activity and inhibitory activity against targeted metabolic enzymes, however, their direct effects on the susceptibility of wheat starch to amylolytic hydrolysis have not been systematically investigated. The objective of our study was to determine the *in vitro* amylolysis of wheat starch in the presence of wheat, corn, soybean, pea and rice proteins in their native, denatured and/or enzymatically hydrolyzed forms by pressure cooking or boiling. Native proteins (except rice) showed no significant effect on the RDS content of protein-starch mixtures. Denatured and/or hydrolyzed plant proteins significantly reduced the RDS content, while this effect could be influenced by the cooking method and protein origin. Confocal laser scanning microscopy and differential scanning calorimetry studies on selected starch-protein mixtures suggest that protein denaturation or protease hydrolysis promotes starch-protein interaction, and thus restricts starch hydration and enzymatic cleavage. Our study suggests the possibility of using this protein-based strategy to formulate low glycemic food products.

1. Introduction

Refined wheat flour, or “white” flour, is a staple food ingredient across the world. The rapidly digestible nature of cooked wheat starch negatively impacts human health, because it can decrease glucose tolerance leading to obesity and other complications. Consumption of foods low in rapidly digestible starch (RDS) and high in slowly digestible starch (SDS) and resistant starch (RS) has many human health benefits (Barros, Awika, & Lloyd, 2012; Dartois, Singh, Kaur, & Singh, 2010; Shi et al., 2014). SDS and RS are characterized by lowering the glycemic index (GI) after food intake. GI is a clinical measurement of the change in blood glucose concentration in response to consuming digestible carbohydrates (Jenkins et al., 2002). Foods high in rapidly digestible starch (RDS) content show a higher glycemic index and increase the glucose and insulin levels after consumption. Foods with a high content of SDS are digested gradually but completely in the small intestine between 20 and 120 min or longer, and thus stabilize blood glucose level (Englyst & Hudson, 1996). RS is not hydrolyzed by α-amylase and amyloglucosidase enzymes in the small intestine, and enters the large intestine where it is fermented by colonic microflora producing short chain fatty acids (acetate, propionate and butyrate), reducing colonic pH, glucose, and cholesterol blood level (Sajilata, Singhal, & Kulkarni, 2006), and improving hindgut immunity.

Starch digestibility is affected by extrinsic factors and mechanisms of resistance to amylolysis, based on which starch has been classified into five categories (Sajilata et al., 2006): a) RS1, physically entrapped by tissue structures and cell components such as protein; b) RS2, highly associated crystalline structures of native starch, especially those formed between the shorter branches of amylopectin; c) RS3, highly associated crystalline structures of native starch, especially those formed between amylose molecules; d) RS4, transglycosidated and chemically substituted or cross-linked starch; and e) RS5, V-amylose crystalline units formed by the tight association of amylose-lipid complexes.

Besides the above mentioned factors, the presence of other components such as protein has been found to play an important role in the mitigation of starch digestibility and the subsequent glycemic response. Clinical studies on healthy and diabetic subjects have revealed that proteins from pulse, cereal grains and their hydrolysates may be able to reduce blood glucose concentration and enhance insulin response. Studies on healthy patients

* Corresponding author. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.
E-mail address: tv3@ualberta.ca (T. Vasanthan).

http://dx.doi.org/10.1016/j.foodhyd.2017.01.015
0268-005X/

concluded that consumption of products without gluten may result in elevated blood glucose level (Jenkins et al., 1987). Likewise, one study demonstrated that the ingestion of a tomato soup containing isolated yellow pea protein (20 g) before a pizza meal can decrease the blood glucose level to 5.95 mM compared with 6.23 mM for the control group (Smith, Mollard, Luhovyy, & Anderson, 2012). In another study, the consumption of carbohydrates and wheat protein hydrolysates combined with a mixture of amino acids (leucine and phenylalanine) can stimulate the production and activity of insulin to a greater extent than carbohydrates alone (Loon, Saris, Verhagen, & Wagenmakers, 2000). Protein hydrolysates may be more potent than intact protein in inhibiting starch digestion, as protein hydrolysates from pea, wheat, rice and soybean generate a faster hormonal responses of insulin and glucagon in healthy participants than with the intact protein alone (Claessens, Calame, Siemensma, van Baak, & Saris, 2009). The insulin response in plasma appears to be related to the amino acid content from protein hydrolysates, especially leucine, isoleucine, phenylalanine, valine, and arginine (Calbet & MacLean, 2002).

In accordance with the findings on the beneficial effects of plant proteins *in vivo*, *in vitro* studies on cereal grains (wheat, corn, kodo millett, sorghum) and oil seeds (soy) have also shown that starch digestibility may be affected by the presence of protein (Berti, Riso, Monti, & Porrini, 2004; Colonna et al., 1990; Jenkins et al., 1987; Rooney & Pflugfelder, 1986; Ryan & Brewer, 2007; Singh, Dartois, & Kaur, 2010). Proteins from wheat are likely to form a resilient gluten network or sheet-like structures that entrap starch (RS1), reducing its access to digestive enzymes (Fleming, 1978; Venugopal, 2011). Furthermore, protein hydrolysates obtained from enzymatic hydrolysis may interact with starch (Lian, Zhu, Wen, Li, & Zhao, 2013).

Celiac disease due to intolerance to gluten affects millions of people worldwide, with the food industry responding by the rapid development of gluten-free food products. However, gluten-free products enhance starch digestion (Berti et al., 2004) due to the absence of proteins. This triggers our research interest in studying the potential application of employing exogenous proteins from plants to slow the rate starch digestion in gluten-free foods. Since current *in vitro* studies have been largely focused on starch-protein interactions that occur naturally in plants (Hesso et al., 2015; Jamilah et al., 2009), the effects of exogenous proteins and their hydrolysates on starch digestion via different methods of cooking remain unclear. Therefore, this study investigates the effect of isolated plant proteins from wheat, corn, soybean, pea and rice in their native, heat denatured & enzymatic hydrolyzed states on the susceptibility of wheat starch to *in vitro* hydrolysis by porcine pancreatic α-amylase.

2. Materials and methods

2.1. Materials

Protein isolates (corn and soy) and concentrates (wheat, pea and rice) as well as purified wheat starch were obtained from Agridient Inc (Farmington Hills, USA). Analytical kits for the determination of total starch, beta-glucan, phytates and starch digestibility were purchased from Megazyme (Megazyme International Ireland, Wicklow, Ireland). Protease from *Aspergillus Oryzae* ($p = 1.27$ g/ml), 2,4,6-trinitrobenzenesulfonic acid (TNBS) solution were obtained from Sigma-Aldrich. Sodium dodecyl sulfate (SDS) was purchased from Bio-Rad. All other chemicals & solvents were of ACS certified grade. Protein content was determined by combustion with a nitrogen analyzer (Model FP-428, Leco Corp., St. Joseph, MI). Samples were cooked in a water bath (Model BS-11, Jeio Tech Inc., Korea) and in a pressure cooker (Fresco, Model PC55A/PC90A). Samples were dried in a freeze-drier (VirTis model 50-SRC, Gardiner, NY, USA). Centrifugation was carried out using an Accuspin 400 (Fisher Scientific, USA) and Beckman J2-21 (Beckman Instruments Inc., Palo Alto, CA, USA) centrifuge.

2.2. Compositional analysis

Protein content was estimated by multiplying the determined nitrogen content by a nitrogen-to-protein conversion factor (6.25). Total starch and β-glucan were determined with kits from Megazyme according to AOAC Methods 996.11 and 995.16, respectively. Phytates were determined according to K-PHYT method using a kit from Megazyme. Lipids were extracted overnight in hexane, followed by gravimetric analysis. Phosphorus was determined using molybdenum blue method (Whistler, Smith, BeMiller, & Wolfrom, 1964). Total phenolics were evaluated according to the Folin-Ciocalteu spectrophotometric method and moisture content was determined by approved method 44–15.02.

2.3. Preparation of wheat starch and protein mixtures

The process is illustrated in Fig. 1. Wheat starch and protein blends consisted of starch (70%) and protein (12%) at a fixed proportion. These percentages were set in order to resemble the typical composition of bread-making flour (Goesaert et al., 2005; Shewry, 2009). Since the purity of the protein isolates/concentrates used in this study was not 100%, an inert filler (microcrystalline cellulose) was used to precisely adjust the starch and protein concentrations to 70% and 12%, respectively, in all the blends. Microcrystalline cellulose was selected because it is very unlikely to interact/bind with any of the components present in the mixtures and also not hydrolyzed by the digestive enzymes. In addition, its melting temperature (260–270 °C) is well above the cooking temperatures used in this study. A mixture of native wheat starch and cellulose was used as control (starch concentration 70%, dry basis). Purified plant proteins in four different forms: 1) native, 2) denatured, 3) hydrolyzed, and 4) denatured hydrolyzed were mixed and cooked with purified wheat starch containing cellulose (WSC). Specifically, each native protein was mixed with WSC in 15 ml sodium maleate buffer (pH 6). Protein denaturation was performed by dispersing each purified plant protein in sodium maleate buffer, followed by boiling (95 °C, 30min) or pressure cooking (100 °C, 30 min, 15 psi). Protein hydrolysis was carried out using a fungal protease from *Aspergillus Oryzae* (4% net protein basis) for 120 min at 50 °C. Denatured hydrolyzed proteins were obtained by following the same denaturation and hydrolysis conditions as mentioned above. Plant proteins in their four different forms were then mixed with WSC. Each “starch-protein-cellulose” mixture was vortexed and cooked by boiling or pressure cooking using the same conditions as mentioned above. After cooking, the mixture was cooled, freeze-dried and packaged air-tight for further analysis.

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

Pea and soybean proteins were dispersed in SDS-PAGE running buffer (250 mM Tris base, 1920 mM Glycine and 0.1% SDS) at 2 mg/mL, then diluted with a mixture of 10% β-mercaptoethonal (prepared in 2×Laemmli sample buffer) at a ratio of 1: 1 (v/v). The samples were then heated at 95 °C for 5 min in an Eppendorf thermomixer dry block heating and cooling shaker (Eppendorf Canada, Mississauga, ON), and cooled down to room temperature prior to SDS-PAGE analysis.

Rice protein was dispersed in SDS-PAGE running buffer