Food Hydrocolloids 61 (2016) 843–850

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Functionality and digestibility of albumins and globulins from lentil and horse gram and their effect on starch rheology

Atinder Ghumman, Amritpal Kaur, Narpinder Singh*

[a] Department of Food Science and Technology, Guru Nanak Dev University, Amritsar 143005, Punjab, India

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form
5 July 2016
Accepted 8 July 2016
Available online 11 July 2016

Keywords:
Pulses
Functional properties
Digestibility
Starch rheology
Albumins
Globulins

ABSTRACT

Functional properties of albumins, globulins and starches from lentil and horse gram were determined. The affect of incorporation of proteins in starch suspensions on *in vitro* digestibility of proteins (IVPD) and rheology of starches were also evaluated. Albumins showed better foaming capacity and foam stability as compared to globulins. Globulins from both the pulses showed higher emulsifying activity index and water absorption capacity than albumins. Globulins from both the pulses showed higher proportion of serine, aspartic acid, alanine, methionine, valine, lysine and proline and lower glutamine, glycine and cystine content as compared to albumins. Globulins showed higher IVPD as compared to albumins for both the pulses. Both albumins and globulins in the presence of starch showed improvement in IVPD. Storage modulus (G'_{Peak}) and loss modulus (G''_{Peak}) of starches from both the pulses decreased on addition of albumins and globulins; however, greater decrease was observed upon incorporation of globulins. The retrogradation tendency of starch gels from both the pulses decreased on incorporation of both the proteins.

1. Introduction

Globulins are the major storage proteins in pulse seeds constituting 35–72% of total protein and the remaining protein fraction mainly consists of albumins. Albumins usually have physiological role (Machuca, 2000). Globulins have highly packed rigid structure due to disulfide bonds and hydrophobic interactions (Utsumi, 1992). Among the four major classes of protein in pulses, albumins are unique due to their soluble nature in water (Bean & Lookhart, 2001). Owing to this solubility, albumins are capable of interacting and competing with starch for water more efficiently. However, poor digestibility of these pulse proteins is the major nutritional constraint in their use. Digestibility can be used as measure to determine susceptibility of a protein to proteolysis and an indicator of protein availability. Highly digestible proteins are more desirable as they would provide more amino acids for absorption on proteolysis and are therefore of better nutritional value than proteins of low digestibility. Functional properties, such as foaming properties, water and oil absorption capacities, emulsification and solubility determine the suitability of proteins to be used as hydrocolloids in food formulations (Kinsella & Phillips, 1989). Determination of processing and storage conditions of proteins is possible only with the knowledge of their functional properties. Functionality and interaction of different food constituents influence each other and are interdependent. Functional properties of food constituents determine type of interactions and these interactions influence functional properties of food products (Tang, Sun, & Foegeding, 2011). Functional properties of proteins are directly related to its physiochemical and structural properties. Albumins are very diverse structurally and functionally. Polypeptide chains constitute protein subunits, two or more subunits join to form oligomers which are typically seed storage proteins (Ward, Uknes, & Ryals, 1994). The knowledge on interaction of food ingredients is important for understanding their functionalities in real food systems as these interactions influences properties of food products (Zhang & Hamaker, 2003). Interaction of protein with starch significantly influences the pasting and textural properties of starch and determines properties of food products such as flow, stability functionality, structure, texture and mouth feel of food products (Chinma, Ariahu, & Abu, 2013; Li, Yeh, & Fan, 2007). Proteins and polysaccharides are being used widely to improve food texture or functional properties in various food formulations. Recent studies suggest that interaction of maize starch with soybean protein influence various starch properties such as pasting,

* Corresponding author.
E-mail address: narpinders@yahoo.com (N. Singh).

http://dx.doi.org/10.1016/j.foodhyd.2016.07.013
0268-005X/

thermal, retrogradation, rheological and microstructure properties of starch gel (Li & Yeh, 2003; Zhang & Hamaker, 2003). The stability of food dispersions are also significantly influenced by protein-starch interactions in bulk solutions and at interfaces. Electrostatic interaction between the positively charged groups of the protein and the anionic groups of the starch can exhibit one of the three different equilibrium situations: (a) miscibility, (b) thermodynamic incompatibility and (c) complex coacervation (or complexation) (de Kruif, & Tuinier, 2001; Martinez, Baeza, Millan, & Pilosof, 2005). Many studies consisting starch and wheat gluten proteins as model systems have been conducted, while similar systems on pulse proteins have not been evaluated. Associations between gluten proteins have been reported to be electrostatic in nature and pH and concentration dependent (Eliasson & Tjerneld, 1990; Eliasson, 1993). The interactions occurring at neutral pH have been attributed to the hydrophobic nature of gluten proteins. The surface proteins were reported to be basically involved in gluten-starch interaction (Lindahl & Eliasson, 1986). Proteins from horse gram and lentil have been studied for their composition and effect of germination on their functional properties (Ghumman, Kaur, & Singh, 2016). Although starches and proteins form horse gram and lentils have been extensively studied separately, literature on the interaction between their protein and starch is scanty. Some studies suggest that proteins in the starch granule obstruct the access of amylolytic enzymes. The present study examined how the presence of albumin and globulins affect the starch properties. The information obtained was developed to clarify the interactions between the starch and the protein and their possible technological application. The aim of the study was to determine functional and digestibility properties of albumin and globulin from lentil and horse gram and to study their effect on starch rheology.

2. Materials and methods

2.1. Protein fractionation

The protein fractions were extracted according to solubility in different solvents as described by Osborne and Mendel (1914) with a minor modification. The grains were ground to pass all through a 60 mesh sieve. The flours were defatted using hexane (1:4) for 24 h. The solvent was separated and residue was dried at 30 °C. Defatted flour (40 g) was extracted twice with 400 mL 1 M NaCl (globulin) for 1 h at room temperature, then centrifuged at 8000 rpm for 30 min. The supernatant was dialyzed against distilled water for 24 h. Supernatant obtained was centrifuged at 8000 rpm for 20 min. Pellet and supernatant obtained were collected as globulins and albumins, respectively. Both the proteins were then freeze dried and collected in plastic pouches. Nitrogen content for each fraction was measured using Kjeldahl method and protein content was calculated using conversion factor 6.25 (AOAC, 1990).

2.2. Functional properties of protein fractions

2.2.1. Foaming and emulsifying properties

Foaming capacity (FC), foam stability (FS) were determined by following the method described by Shevkani, Singh, Rana, and Kaur (2014) and emulsifying activity index (EAI) was determined by the method of Pearce and Kinsella (1978). Protein suspensions (1% w/v, 30 ml) at pH 7.0 were homogenized with a digital homogenizer (ULTRA-TURRAX T 25, IKA Co., Germany) at 15,000 rpm for 2 min. FC was calculated as the percent increase in volume of the suspensions upon homogenizing, while FS was estimated as the percentage of foam remaining after 30 min.

2.2.2. Water and oil absorption capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) were determined following the methods described by Ogunwolu, Henshaw, Mock, Santros, and Awonorin (2009) and expressed as weight of oil/water absorbed per gram of the sample.

2.3. Zeta potential determination

The zeta potential (ζ) of protein fractions was measured using Zetasizer Nano ZS (Malvern Instrument Ltd., UK). Freshly prepared protein suspensions in de-ionized water (1 mg/mL) were filtered through a 0.45 μm membrane prior to analysis.

2.4. Amino acid analysis

The amino acids (AAs) were analyzed using method described by Dhillon, Kumar, and Gujar (2014).

2.5. Starch isolation

Starch was isolated from lentil and horse gram seeds using method of Singh, Kaur, Singh, and Guraya (2004). Seeds (100 g) were soaked in distilled water and left overnight at 40 °C. The excess water was drained off and seeds were ground in minimum amount of water using laboratory scale grinder to get fine slurry. The slurry obtained was filtered through 4 layers of muslin cloth with continuous washings with distilled water. The filtrate was given washings with distilled water for 2 days for removing protein and fiber portion followed by centrifuge at 3000 g for 10 min. Starch obtained was dried overnight at 40 °C.

2.6. Scanning electron microscopy (SEM)

Scanning electron micrographs of starches were obtained with a Scanning Electron Microscope (S_3400 N, Hitachi, Japan) using an accelerating potential of 20 kV as described earlier by Kaur and Singh (2007). Starch samples were suspended in ethanol to obtain 1% suspension. A drop of the starch-ethanol suspension was placed on an aluminum stub using double-sided adhesive tape to obtain uniform smear and the starch was coated with gold.

2.7. Particle size analysis (PSA)

Granule size of starches was measured using Mictrotrac S3500 Particle Size Analyzer, (Microtrac Ins. Ltd., USA). The sample (dried starch) was directly added to the sample port to reach an obscuration to ~40%. The size distribution of starch granules was expressed in terms of the volumes of equivalent spheres.

2.8. In vitro protein digestibility (IVPD)

The *in vitro* protein digestibility of extrudates was determined using the method of Akeson and Stahmanna (1964). Sample (250 mg) in 0.1 mol/L HCl-pepsin solution was incubated at 37 °C for 3 h followed by neutralization and treatment with pancreatin in 0.2 mol/L phosphate buffer (pH 8.0) for 24 h. After incubation, the sample was treated with 10 g/100 g TCA and centrifuged at 10,000 × g for 20 min. Protein in the supernatant was estimated using the Kjeldahl method (AOAC, 1990). The percentage of protein digestibility was calculated by the ratio of protein in supernatant to protein in sample as equation. Protein digestibility % = Nitrogen (in supernatant) -Nitrogen (in blank)\ Nitrogen (in sample) * 100.