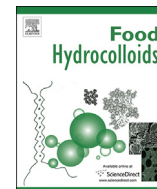


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## Rheological and microstructural characteristics of lentil starch–lentil protein composite pastes and gels

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## ABSTRACT

The rheological and structural changes in heat induced pastes and gels of lentil starch and lentil protein composites were investigated at various starch to protein ratios. The starch fraction ( $\phi_s$ ) in the mixtures was varied from 0 to 1 and the total solid content was maintained at 25% (w/w). Results showed that the gel strength of the composite gels increased exponentially with the increase in lentil starch fraction. The pasting temperature increased and the paste viscosity decreased with the increase in the lentil protein fraction. The high starch composite gels ( $\phi_s > 0.5$ ) showed higher elastic ( $G'$ ) and loss ( $G''$ ) moduli at higher temperature (60 °C) than at lower temperature (10 °C). The high protein composite gels ( $\phi_s \leq 0.5$ ) showed higher  $G'$  and  $G''$  values at lower temperature (10 °C) than at higher temperature (60 °C). Segregation of protein-rich domains was observed in the high starch gels ( $\phi_s > 0.7$ ) whereas low starch composite gels ( $\phi_s \leq 0.5$ ) appeared more homogeneous. The microstructure of composite gels appeared to be more fragile with larger pore size and thinner wall compared to the microstructure of starch gel. Both non-covalent interactions (hydrophobic and hydrogen bonding) and covalent bonding were found to contribute to the gel structure and firmness of these composite gels. The NaCl concentration increased the paste viscosity and gel firmness of the composite gels up to 0.25 mM above which the magnitudes of these parameters were decreased. Both the paste viscosity and the gel firmness of the composite gels were found to be higher above the isoelectric point of lentil protein and vice versa. From this study, textural properties of the composite gel/paste were found to be strongly affected by the proportion of the starch and protein as well as the extrinsic factors (pH, ionic strength, presence of reducing agents). Therefore, understanding of gelling behaviour of lentil starch and protein in composite gel would be helpful for structure formation of these two biopolymers in mixtures and would help their application in new product development.

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### 1. Introduction

The ability to design and control desired gel structure is very important aspect in ensuring consumer satisfaction in many food products such as yoghurt, cheese, pudding, sausage, and tofu. Gel formation is basically a transformation of hydrocolloid dispersion from sol to the gel state during which a three-dimensional gel network is formed. Gel matrix exhibits a solid-like behaviour and immobilizes large quantity of water as well as other important food constituents (Aguilera & Lillford, 2008). In order to achieve gels of desired characteristics, it is essential to understand the mechanism

of gelling process which depends primarily on the nature and molecular interactions among the constituents.

Starch and protein are very important bio-macromolecules ubiquitous in foods. Both starch and protein are responsible for the formation of characteristic texture in many food products due to their ability to form gel (Doublier, Launay, & Cuvelier, 1992). When both components are applied to form gel, the resulting systems are usually described as composites. The composite gels are biphasic in nature and each component gels within its own phase. The gel structure of a protein–starch composite gel depends on the interactions between protein and starch. Processing environment (pH, ionic strength) also significantly affect the interaction between protein and starch and hence determine the nature of the gel formed (Aguilera & Rojas, 1996; Shim & Mulvaney, 2001). Understanding of the gelling behaviour of lentil starch and lentil

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protein in composite gel would be helpful for the better design of the structure and function of the gels in which these two biopolymers are major constituents.

There is increased interest in plant proteins such as legume proteins (including lentil proteins), as they can be used as good substitute for conventional proteins such as soy and milk proteins (Alsohaimy, Sitohy, & El-Masry, 2007; Nunes, Batista, Raymundo, Alves, & Sousa, 2003). Previously, we characterized lentil protein for its physicochemical as well as surface properties and found that this protein is capable of forming a good gel (Joshi, Adhikari, Aldred, Panozzo, & Kasapis, 2011; Joshi et al., 2012). In general, protein gelation consists of two steps: conformational change or partial denaturation of protein molecules followed by gradual association or aggregation of the denatured protein molecules. The gelation of protein involves the formation of non-covalent bonds (hydrophobic interactions, hydrogen bonds and electrostatic interactions) and also the covalent disulphide bonds (Matsumura & Mori, 1996).

Starch is widely used in food industry both as a major ingredient and as a minor additive to impart some functional properties. When starch is heated in the excess water, it swells irreversibly and undergoes order-to-disorder phase transition at a characteristic temperature range depending on its source (Cook & Gidley, 1992). Important functional properties such as gelling and pasting of starch are derived once the starch is gelatinized. The critical concentration for the formation of a gel network is dependent on the source and type of starch. In our previous study, we found that lentil starch possesses strong gel forming propensity at relatively low concentration compared to the corn and potato starch (Joshi et al., 2013).

Protein–starch mixed systems are widely used in food and have been extensively studied as the interactions between these two biopolymers can be harnessed to impart some specific and desired texture. Various aspects of starch–protein interactions have been studied in the past (Aguilera & Rojas, 1996; Li, Yeh, & Fan, 2007; Shim & Mulvaney, 2001). These studies have used better characterized proteins (milk proteins, soy proteins and glutens) and starches (corn starch, potato starch cassava starch) to investigate the protein–starch interactions in starch–protein composite gels.

In this study, we investigated the effect of variation of (lentil) starch to (lentil) protein ratios on pasting and gelation behaviour of composite gels and pastes. We also investigated the effects of extrinsic factors such as pH, reducing agent (dithiothreitol) and ionic strength (NaCl) to the pasting and gelation behaviour of composite gels and pastes. It is expected that this study will provide better understanding of the structure–function relationships of lentil protein–lentil starch composite gels/pastes and also help better formulation of textured lentil products.

## 2. Materials and methods

### 2.1. Raw materials

The lentil seeds of Aldinga cultivar were provided by Department of Primary Industries, Horsham, Australia. Seeds were dehulled and ground to pass through 0.5 mm sieve to obtain lentil flour and packed into airtight containers before further analysis.

#### 2.1.1. Protein/starch isolation and purification

The lentil protein isolate and lentil starch were obtained following the method previously reported by Joshi et al. (2011) and Joshi et al. (2013), respectively. Briefly, dehulled lentil flour was extracted in alkaline condition (1:10 w/v, pH 8.0) for one hour using a magnetic stirrer to solubilize the protein and to facilitate the separation of starch. The mixture was centrifuged at 17,500 g for 15 min (Sorvall Instruments, Wilmington, DE, USA). The clear

protein extract obtained was acidified (pH 4.5) and the protein was allowed to precipitate. The protein precipitate was recovered by centrifugation, washed with distilled water and subsequently neutralized. The neutralized sample was frozen at  $-80\text{ }^{\circ}\text{C}$  and subsequently freeze dried. The solid residue obtained from centrifugation after protein extraction was washed several times by resuspending in distilled water and the supernatant was decanted off. Finally, starch dispersion was recovered by sieving through sieve no 325 (US standard sieve size). Purified starch was then air dried at  $25\text{ }^{\circ}\text{C}$  for 24 h.

### 2.2. Pasting properties

The pasting properties of starch, protein, and starch–protein mixed samples were measured by using Rapid Visco Analyzer (Newport, Scientific Pty Ltd., NSW, Australia). Total solid content of 15% (w/v) was used in all these tests. The starch–protein suspensions maintained at different starch fractions ( $\phi_s$ ) were heated from  $50\text{ }^{\circ}\text{C}$  to  $95\text{ }^{\circ}\text{C}$  at the heating rate of  $12.5\text{ }^{\circ}\text{C}/\text{min}$ , held at  $95\text{ }^{\circ}\text{C}$  for 5 min and finally cooled down to  $50\text{ }^{\circ}\text{C}$  at the same cooling rate. During heating, the samples were stirred at 960 rpm for the first 1 min and 160 rpm for the remaining testing time. Viscosity (mPa s)–time and viscosity–temperature profiles of starch–protein suspensions/pastes were recorded during heating and subsequent cooling.

### 2.3. Starch–protein composite gels

#### 2.3.1. Gel preparation and experimental design

The lentil starch and lentil protein isolate were dry mixed in a plastic tube with a magnetic stirrer. Water was added subsequently and the slurry was further stirred for a minimum period of 1 h at 300 rpm to properly disperse and hydrate both the protein and starch. This starch–protein mixture was then preheated at  $60\text{ }^{\circ}\text{C}$  with continuous stirring for 20 min. Then these starch–protein mixed samples were heated in a water bath (maintained at  $90\text{ }^{\circ}\text{C}$ ) with continuous stirring at 300 rpm for 20 min. These fully dissolved starch–protein mixed samples (pastes) were immediately cooled under tap water. The gels formed in this way were stored overnight at  $4\text{ }^{\circ}\text{C}$  and equilibrated to room temperature ( $25\text{ }^{\circ}\text{C}$ ) for at least 30 min before further tests. Cylindrical gel samples (6.7 mm diameter) were prepared by extruding the gel by using a stainless steel cylindrical plunger. Gel cylinders were then cut into 6 mm length with a cutting guide and a razor for texture profile analysis and stress relaxation tests.

#### 2.3.2. Texture profile analysis (TPA) of gels

Texture profile analyses (TPA) of starch–protein composite gels were carried out using a texture analyser (TA.XT plus, Stable Micro Systems Ltd., UK). The gel pieces were compressed twice to 30% of their original height at a constant speed of 0.3 mm/s. A cylindrical probe with a diameter of 10 mm was used for this purpose. The force–deformation data were used to calculate the TPA characteristics: hardness, cohesiveness, adhesiveness, springiness, and gumminess (Bourne, 2002). Each gel sample was compressed with 10 mm derlin cylindrical probe. At least five replicate tests were made for each test condition. To avoid the effect of variation of gel properties among the different batches, the gels from the same batch were tested. No apparent rupture of the gels was observed during these tests.

#### 2.3.3. Stress relaxation properties of gels

Stress relaxation tests of the starch–protein composite gels were conducted using the texture analyzer (TA.XT plus, Stable Micro Systems Ltd., UK). The cylindrical gel samples were compressed to a fixed strain and the decay of stress (required to

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