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Determination of molecular driving forces involved in heat-induced corn germ proteins gelation

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ABSTRACT

Molecular forces involved in corn germ proteins (CGP) gelation were investigated using a rheometer. With the inclusion of urea and guanidine hydrochloride (GuHCl), the storage moduli (G′) of CGP gels tends to decrease. This is an indication that hydrophobic interactions and hydrogen bonds are present. The involvement of propylene glycol (PG) provides evidence that hydrogen bonds and electrostatic interactions are present. The impact of thiocyanate (NaSCN) and sodium sulfate (Na_2SO_4) further proves the involvement of hydrogen bonds. The effects of 2-mercaptoethanol (2-ME) and N-ethylmaleimide (NEM) reveal that involvement of disulfide bonds contribute to CGP gel stiffness. Reheating and recooling tests revealed that during the initial cooling phase the gel is thermally reversible and higher levels of G′ suggested more hydrogen bonds are formed when recooled. It can be deduced that hydrogen bonds play a greater role than hydrophobic interactions from the increasing G′ during cooling and recooling.

1. Introduction

Besides the predominant constituent oil, corn germ contains 18–22% proteins, and these proteins account for around 29% of the total proteins in corn seeds (Watson, 1987). Water-soluble albumins plus saline-soluble globulins account for more than 60% of corn germ proteins (CGP) (Wilson, 1987). These proteins are proven to possess a good balance of amino acids, which presents a superior nutritional value to endosperm protein (Wilson, 1987) and have better functional properties than corn endosperm proteins. Corn germ is generally used to extract oil through solvent extraction and high temperature processing, which results in serious denaturation of the residual protein; these denatured proteins almost completely lose their functional properties (Kinsella, 1979), and thus are mainly used as animal feed.

Due to increasing demand for high quality proteins with good functional characteristics in the food industry, researchers have been attempting to recover proteins from various plant sources. Corm germ proteins seem to be a good alternative for soy proteins and some workers have published their works on the extraction procedure and functional properties evaluation of corn germ proteins (Mila and Hojilla-Evangelista, 2012).

The mechanism of protein gelation is described as a three-dimensional network formation through the cross-linking of its polypeptide chains. Cross linking of protein polypeptide chains is driven by different molecular forces which may include disulphide bonds, hydrophobic interactions, hydrogen bonds, ionic interactions, or a combination of the all. O'Riordan et al. (1988) indicated that when electrostatic interactions are involved in whole plasma protein gel formation, gel strength is affected by salts and pH. The molecular forces that exist in the gel network are dependent on the protein and its structure, which can be influenced by the method used for protein extraction (Utsumi and Kinsella, 1985).

Urea and guanidine hydrochloride (GuHCl) can destabilize hydrogen bonds and hydrophobic interactions in proteins. A protein molecule can be denatured by urea through preferential adsorption (Wallqvist et al., 1998). Some workers proposed that Sulfhydryl/disulfide interchanges are involved in soy protein gelation due to the reaction of the gel to certain reagents: β-mercaptoethanol (β-ME or 2-ME) (Utsumi and Kinsella, 1985), and N-ethylmaleimide (NEM) (Utsumi and Kinsella, 1985; Wang and

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Damodaran, 1990).

Hofmeister (1888) observed that the solubility of proteins in water could be affected to a certain degree by salts. Generally, salts may either reduce or enhance the hydrophobicity of a solute in water. The "Hofmeister series" is a series of ions ranked in the sequence of how strongly they influence the hydrophobicity. Case et al. (1992) reported that Na_2SO_4 facilitated the gelation of konjac glucomannan, whereas NaSCN and $NaNO_3$ suppressed it. We hypothesize these salts also have the same effects in CGP gelation thus providing evidence of hydrophobic interaction. The effects of salts, pH, reducing agents and dissociating agents may be employed to determine the involvement of different interaction forces in forming the structure of protein gels (Utsumi and Kinsella, 1985). Techniques employed to identify various molecular forces are summarized in Table 1.

Plant protein concentrate is one type of protein product on the market. We intend to explore the molecular forces involved in this particular product and confirm the involvement of these forces since no study has been conducted to investigate such forces involved in CGP concentrate. This is fundamental for the future development and application of CGP concentrate in foods.

The objective of this work was to investigate the impacts of salts, chemicals that target non-covalent and covalent interactions, and reheating and recooling on gelation properties of CGP gels to better understand the importance of different molecular forces involved in network formation, particularly gels formed by CGP concentrate. This will enable us to elucidate the driving molecular forces presented in CGP concentrate gel network formation and maintenance.

2. Materials and methods

2.1. Commercial corn germ and corn germ protein concentrate

Commercial corn germ was purchased from a local corn milling plant and the corn germ was separated via a dry milling process. CGP was extracted following a typical isoelectric precipitation procedure. The corn germ was first ground to flour by a grinder (Philips Electronics, HR2168, China); then passed through a 80 mesh screen, sealed in plastic bags and stored in a freezer for the further extraction. A sample of 1000 g corn germ flour was suspended in 3000 mL of 0.1 M NaOH solution (pH 9.3) and stirred for 30 min to enhance solubility of the globular protein in corn germ, followed by centrifugation at 3000 rpm for 30 min. The suspension was collected and the pH value was adjusted to 4.5 (Nielsen et al., 1973) using 2 M HCl to reach the isoelectric point of corn germ proteins. The suspension was then centrifuged at 4000 rpm for 30 min, followed by sediment collection and freeze drying. The dry powder was finally defatted for 2 h with 10-fold petroleum ether two times. The recovered CGP contained 55.6% (w/w) of protein as determined by the Kjeldahl method, and an N to protein conversion factor of 6.25 was employed (AACC, 1982). GuHCl and β-mercaptoethanol (Nuotai Chemical Co. Ltd., Shanghai, China), PG (Jusheng Technology Co. Ltd., Hubei, China), Na_2SO_4 and NaSCN (Ziyi Chemical Co. Ltd., Shanghai, China), and N-ethylmaleimide (Klamar Shanghai Puzhen Biotech. Co. Ltd., Shanghai, China) were of analytical grade.

2.2. Determination of crude fat, ash, total starch, and total dietary fiber

Crude fat (method 960.39) and ash (method 920.153) of commercial corn germ and CGP were determined in duplicate using AOAC (1990) procedures. The Megazyme (Megazyme International Ltd., Wicklow, Ireland) total starch analysis kit (K-TSTA/05/06) and total dietary fiber kit (K-TDFR/12/05) were used for determining the total starch and dietary fiber contents, respectively.

2.3. Rheology

The CGP was mixed with 0.3 M NaCl to obtain a suspension with protein concentration of 15% (w/v) as a good gel can form at this salt concentration. The samples were mixed by a stirring rod for 1 min to achieve complete suspension. The samples were then loaded into a TA Discover HR-1 rheometer (TA Instruments, Newcastle, Del. USA) equipped with a 4 cm diameter parallel plate geometry. Rheological properties were then determined as previously described by Sun and Arntfield (2012).

Approximately 1 mL of the CGP suspension was loaded to the lower plate of the parallel plate geometry of the rheometer for each test. Then, the upper plate was lowered to reach a gap width of 1.00 mm and a thin layer of light mineral oil was applied to the well of the upper plate geometry. A solvent trap cover was used to prevent sample drying during heating and cooling. A water-saturated atmosphere can be maintained at the sample's surface through this method.

The following heating and cooling procedures were employed. First, samples were equilibrated at 25 °C for 2 min and then heated and cooled over a temperature range of 25–95–25 °C at a controlled rate (4 °C/min). Rheological data was collected every 30 s during heating and cooling. This was followed by a frequency sweep over a range of 0.01–10 Hz at 25 °C. Data during gel formation was collected along with data of the final gel.

The values of storage modulus (G′) and loss modulus (G″) were collected as a function of frequency. The tan delta (tan $\delta = G''/G'$), a measure of the energy lost compared to the energy stored in a single deformation cycle, was also determined. The input amplitude strain of 0.02, a value found to be in the linear viscoelastic

Table 1
Impact of different reagents on molecular driving forces exist in proteins.

	Non-covalent bonds			Covalent bond	References
	Electrostatic interaction	Hydrophobic interaction	Hydrogen bond	Disulfide bond	
NaCl	Disrupt		Disrupt		Kauzmann (1959)
Na_2SO_4			Promote		
NaSCN			Disrupt		
GuHCl		Disrupt	Disrupt		Tanford (1968)
2-ME				Disrupt	Wall and Huebner (1981)
NEM				Disrupt	Creighton (1993)
PG	Promote	Disrupt	Promote		Tanford (1962)
Urea		Disrupt	Disrupt		Gordon and Jencks (1963)

Abbreviations: Sodium chloride, NaCl; Sodium sulfate, Na_2SO_4; Thiocyanate, NaSCN; Guanidine hydrochloride, GuHCl; β-mercaptoethanol, β-ME or 2-ME; N-ethylmaleimide, NEM; Propylene glycol, PG.