academicJournals

Vol. 10(9) pp. 157-163, September 2016
DOI: 10.5897/AJFS2016.1454
Article Number: FC8A93A59657
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

The composition and extractability of thermo - molded wheat gluten bio-plastics

Abrehet Fisseha

School of Nutrition, Food Science and Technology, Hawassa University, Hawassa, Ethiopia.

Received 19 April, 2016; Accepted 20 July, 2016

The study was aimed to evaluate the effect of thermo-molding on gluten and gluten fractions composition and its extractability with 1.0 ml 0.05 M sodium phosphate buffer (pH 6.8) with 2.0% sodium dodecyl sulfate (SDS) at 130 and 150°C for 5 and 25 min. The gluten, gliadin and glutenin composition as affected by thermo-molding was analysed with reverse phase high performance liquid chromatography (RP-HPLC) and amino acid was analysed with acid hydrolysis using high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD). The extractability and composition were significantly affected. Gluten extracts were found in equivalent proportions; gliadin (51%) and glutenins (49%). Gluten, gliadin and glutenin subunits (GS) extracts were significantly affected and a reduction with increased molding condition explains the occurrence of polymerization reactions due to SH-disulfide interchange reactions. The glutenin fraction contains 15% gliadin while the gliadin fraction contains 2.5% glutenin after extraction with 60% ethanol. The extractability of the gliadin types and GS decreased with increasing molding time and temperature.

Key words: Gluten, gliadin, glutenin, extractability, composition, thermo-molding, amino acids.

INTRODUCTION

Wheat is among the most important staple food crops in the world with different cultivars classified according to physical hardness, agronomic properties, color of the kernel and end-use characteristics. Wheat proteins are separated by the Osborne fractionation via successive extractions with solvents (Delcour and Hoseney, 2010; Veraverbeke and Delcour, 2002). Gliadins and glutenins are functional gluten proteins in wheat found in more or less equal amounts (Goesaert et al., 2005; Belitz et al., 2009). In contrast to non-gluten proteins, gluten proteins are poorly soluble in water or dilute salt solutions due to low amino acids content with ionizable side chains and high contents of non-polar amino acids. Cysteine, a minor amino acid in glutens is extremely important for its structure and functionality in disulfide bonds within a protein or between different proteins (Wieser, 2007).Glutenins,a heterogeneous mixture of disulfide-linked polymers (80,000 – Millions in MW) of GS is insoluble in alcohol. Glutenins are categorized into;

(1) HMW-GS (x- and y- types); a minor component (10%) of the total weight with three structural domains of N- and

E-mail: abifish3@gmail.com.

C-terminal and more than four cysteine residue of repetitive sequence of proline, glutamine and glycine (Delcour and Hoseney, 2010; Wieser, 2007; Veraverbeke and Delcour, 2002).
(2) LMW-GS (B-, C- and D-types); makes up 20% of the total protein, less characterized group than the HMW-GS. The B- and C-type LMW-GS are sulfur-rich prolamins, while the D-type LMW-GS are sulfur-poor prolamins (Veraverbeke and Delcour, 2002).

Gliadins represent a heterogeneous mixture of single-chained proteins (30,000-80,000 in MW) soluble in alcoholic media. Based on their mobility at low pH in gel electrophoresis, three gliadin groups that is, α-, γ- and ω- were identified (Wieser, 2007). The ω-Gliadins have a MW (39,000 to 55,000) composed of high level of glutamine, proline and phenylalanine, low level of methionine but lacked cysteine residues. The α- and γ-gliadins overlaps inMW (28,000 to 35,000) with lesser proline, glutamine than the ω-gliadins and increased level of cysteine residues in the C-terminal domain. The C-terminal domain contains non-repetitive sequences of glutamine and proline than the N-terminal domain (Wieser, 2007; Veraverbeke and Delcour, 2002). In general the α- and γ-gliadins exist in higher and equivalent proportion than ω-gliadins (Wieser, 2007). The N-terminal domain constitutes 40 to 50% of the protein with repetitive sequences of glutamine, proline, phenylalanine and tyrosine.

Proteins are cross-linked by covalent bonds between polypeptide chains within a single protein or between different proteins important for structural and functional properties. The protein cross links are either naturally occuring or formed during processing (Gerrard, 2002). Disulfide bonds between cysteine residues are the dominant crosslinks formed by oxidative coupling of two cysteine residues adjacent a protein matrix in the presence of an oxidant which accepts hydrogen atoms from the thiol groups (Veraverbeke and Delcour, 2002; Gerrard, 2002; Shewry and Tatham, 1997). An intramolecular disulfide bond can be converted into an intermolecular disulfide bond by a sulfhydryl (SH)-disulfide interchange reaction. Other cross-links include isopeptide bonds, dehydroprotein, Maillard reaction and tyrosine derived during thermal processing; chemical treatment in the formation of covalent bonds from the cysteine and lysine residue (Lagrain et al., 2010; Rombouts et al., 2009; Hanft and Koehler, 2005; Gerrard, 2002; Tilley et al., 2001; Singh, 1991).

The MW of gluten aggregates and protein extractability is dependent on the heating condition, hydrostatic pressure and moisture content attributed to oxidation of SH groups and SH-disulfide interchange reactions which forms intermolecular bonds (Kieffer et al., 2007; Lagrain et al., 2005; Singh and MacRitchie, 2004; Weegels et al., 1994). The formation or cleavage of disulfide bonds is stimulated by the application of redox agents (potassium bromate, potassium iodate and reducing agent dithiothreitol [DTT]) on the gluten proteins. During hydrothermal treatment, protein extractability was higher in the presence of oxidants thus suggesting a decreased cross-linking which hinders gluten polymerization. In the presence of reducing agents (DTT), protein extractability was lower suggesting increased crosslinking which promotes gluten polymerization due to increased levels of free SH groups and less flexibility of glutenin chains (Lagrain et al., 2008, 2007, 2005; Guerrieri et al., 1996).

Gluten bioplastic formation

The need for environmentally friendly, renewable and biodegradable plastics is increasing due to pollution and long term health impacts of synthetic polymers (Zhang et al., 2010; Sun et al., 2008); possess suitable gas barrier properties and nutritional value (Lagrain et al., 2010; Cao et al., 2007); poor moisture barrier properties due to its hygroscopic nature; binds with water through hydrogen bridges modified by thermal and chemical treatments (Sun et al., 2008; Carvalho et al., 2008; 2006; Brauer et al., 2007; Dicharry et al., 2006; Gallstedt et al, 2004; Domenek et al., 2004; Woerdeman et al., 2004). The effect of processing on gluten network formation is determined by the SDS extractability which measures the degree of polymerization: the lower the extractability the higher the degree of polymerization (Sun et al. 2008; Gallstedt et al., 2004; Domenek et al., 2004; Pommet et al., 2003; Micard et al., 2001; Cuq et al., 2000). Besides process conditions, the presence of additives influences extractability (Lagrain et al., 2010; Sun et al., 2007; Dicharry et al., 2006; Gallstedt et al., 2004; Woerdeman et al., 2004).

MATERIALS AND METHODS

Wheat gluten was fractionated into gliadin and glutenin with 60% ethanol and protein composition was analysed with RP-HPLC in the presence of redox agents (SDS) and thermo-molded at 130 and 150°C for 5 and 25 min in a Pinette Press Zenith 2 (Pinette Emidecau Industries, Chalon sur Saône, France) at 5 bars after conditioning for 2 days at 50% relative humidity and 20°C (the moisture was adjusted to 7%). Hydrophilic molecules elute first, followed by hydrophobic molecules which interact stronger with the column (Figure 1). Amino acid analysis was performed after acid hydrolysis of high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD) as described by Rombouts et al. (2009).

RESULTS AND DISCUSSION

Gluten composition and extractability

Thermo – molded gluten and gluten fractions composition and extractability with 1.0 ml 0.05 M sodium phosphate buffer (pH 6.8) and 2.0% sodium dodecyl sulfate (SDS) are presented in Tables 1 to 4. The gliadin (51%) and

Table 1. The extractability and composition of gluten protein before and after thermo - molding at 130 and 150°C for 5 and 25 min analysed by reverse phase –high performance liquid chromatography (RP-HPLC) (expressed in percentage).

Sample	Treatment				
	Control	130°C - 5 min	130°C - 25 min	150°C - 5 min	150°C - 25 min
Gliadin	**50.6 (0.7)**	**28.4 (2.7)**	**11.6 (0.9)**	**8.0 (0.3)**	**6.4 (0.1)**
ω-gliadin	6.2 (1.5)	6.2 (0.6)	3.6 (0.1)	3.3 (0.0)	4.0 (0.1)
α-gliadin	20.6(5.0)	13.3(1.3)	4.5 (0.0)	2.6 (0.0)	1.3 (0.1)
γ-gliadin	17.0 (3.9)	9.0 (0.9)	3.5 (1.0)	2.2 (0.2)	1.1 (0.1)
GS	**49.4 ((8.8)**	**70.8 (4.8)**	**66.5 (0.7)**	**65.6 (1.4))**	**50.8 (1.6)**
D-LMW-GS	2.7 (0.8)	2.5 (0.2)	2.1 (0.0)	2.2 (0.1)	2.1 (0.0)
HMW-GS	11.4 (1.2)	10.5 (0.9)	6.8 (0.1)	6.8 (0.2)	6.6 (0.1)
B/C-LMW-GS	35.7 (6.8)	57.8 (3.8)	57.6 (0.7)	56.6 (1.1)	42.1 (1.5)

The standard deviation is given in brackets

Table 2. The Extractability and composition of glutenin protein before and after thermo-molding at 130 and 150 °C for 5 and 25 min analysed by reverse phase-high performance liquid chromatography (RP-HPLC) (expressed in percentage).

	Treatment				
Sample	**Control**	**130°C - 5 min**	**130°C - 25 min**	**150°C - 5 min**	**150°C - 25 min**
Gliadin	**14.7 (0.0)**	**6.5 (0.2)**	**4.1 (0.1)**	**7.2 (0.1)**	**12.6 (1.5)**
ω-gliadin	2.3 (0.0)	2.2 (0.0)	2.1 (0.0)	3.8 (0.0)	7.6 (0.3)
α-gliadin	7.1 (0.0)	2.6 (0.1)	1.2 (0.0)	1.3 (0.0)	2.0 (0.2)
γ-gliadin	5.3 (0.1)	1.7 (0.1)	0.8 (0.1)	2.1 (0.0)	3.0 (1.0)
GS	**85.3 (0.8)**	**86.3 (2.3)**	**81.5 (1.2)**	**92.8 (4.8)**	**32.5 (1.4)**
D-LMW-GS	4.4 (0.1)	4.1 (0.2)	4.3 (0.0)	25.1 (1.7)	1.4 (0.6)
HMW-GS	23.4 (0.1)	19.4 (0.6)	16.5 (0.2)	52.6 (2.2)	13.8 (0.3)
B/C-LMW-GS	57.5 (0.8)	62.9 (1.5)	60.6 (0.9)	52.6 (2.2)	17.4 (0.5)

The standard deviation is given in brackets.

Table 3. The extractability and composition of gliadin protein before and after thermo-molding at 130 and 150 °C for 5 and 25 min analysed by reverse phase-high performance liquid chromatography (RP-HPLC) expressed in percentage.

Sample	Treatment				
	Control	130°C - 5 min	130°C - 25 min	150°C - 5 min	150°C - 25 min
Gliadin	**97.5 (1.3)**	**59.6 (0.8)**	**24.1 (0.1)**	**12.3 (0.5)**	**11.1 (0.1)**
ω-gliadin	14.5 (0.3)	9.4 (0.1)	6.3 (0.1)	5.6 (0.2)	6.5 (0.3)
α-gliadin	45.8 (1.1)	27.4 (0.4)	9.7 (0.3)	3.0 (0.1)	1.7 (0.1)
γ-gliadin	37.2 (0.2)	22.9 (0.3)	8.1 (0.4)	3.7 (0.2)	3.0 (0.3)
GS	**2.5 (0.4)**	**44.2 (2.1)**	**76.2 (3.0)**	**76.0 (6.4)**	**62.6 (0.3)**
D-LMW-GS	0.0(0.0)	1.0 (0.1)	1.3 (0.0)	1.5 (0.3)	1.6 (0.0)
HMW-GS	0.2 (0.0)	4.2 (0.2)	4.9 (0.2)	5.2 (0.5)	5.8 (0.1)
B/C-LMW-GS	2.3 (0.3)	39.0 (2.0)	70.0 (2.8)	69.3 (5.7)	55.3 (0.2)

The standard deviation is given in brackets.

glutenin extracts (49%) occurred in equivalent proportions in the SDS media (Goesaert et al., 2005; Belitz et al., 2009). Reverse phase – high performance liquid chromatography (RP-HPLC) profile showed that higher gliadin peak of gliadin fractions than gliadin peak of gluten explains that gliadins are more extractable than

Table 4. The lysine, LAN and cystine contents of gluten, gliadin and glutenin proteins (mole/10^5 g of protein) thermo-molded at 130 and 150 °C for 5 and 25 min.

Sample		Treatment				
		Control	**130°C - 5 min**	**130°C - 25 min**	**150°C - 5 min**	**150°C - 25 min**
Gluten	Lysine	19.4 (0.1)	14.2 (0.1)	13.9 (0.2)	13.0 (0.2)	11.7 (0.2)
	LAN	nd	nd	nd	0.1 (0.0)	0.6 (0.0)
	Cystine	5.6 (0.1)	5.4 (0.1)	5.4 (0.2)	5.5 (0.1)	4.7 (0.1)
Glutenin	Lysine	23.5 (3.3)	22.6 (1.6)	19.3 (0.5)	16.1 (0.6)	16.0 (0.6)
	LAN	nd	nd	0.3 (0.0)	0.5 (0.1)	1.5 (0.0)
	Cystine	4.7 (0.0)	4.8 (0.3)	4.6 (0.0)	3.5 (0.1)	3.1 (0.0)
Gliadin	Lysine	8.3 (0.3)	7.1(0.4)	5.8 (0.3)	4.9 (0.4)	2.3 (0.1)
	LAN	nd	nd	nd	0.1 (0.1)	0.5 (0.0)
	Cystine	9.1 (0.1)	9.4 (0.3)	9.4 (0.4)	8.6 (1.1)	8.4 (0.0)

The standard deviation is given in brackets

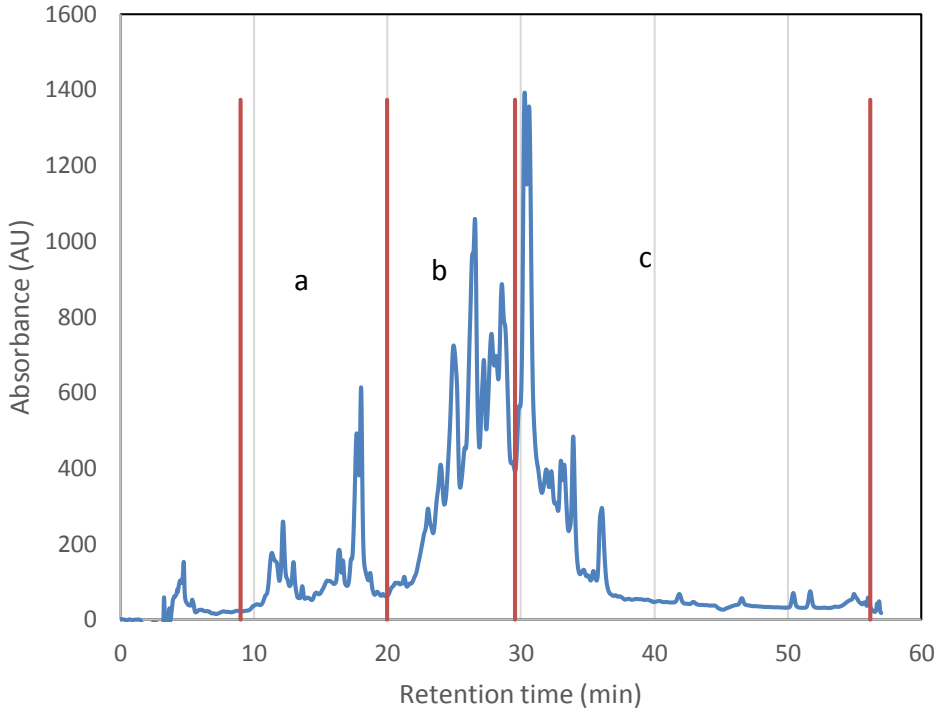

Figure 1. Typical reverse phase –High performance liquid chromatography (RP-HPLC) profiles of the Gliadin extracts (a) ω-gliadin, (b) α-gliadin and (c) γ-gliadin.

non-gliadin fractions (Abrehet, 2015). Indeed, all the gliadins are soluble in the SDS buffer, but not all glutenins are soluble. Small gliadin peak as observed in the glutenin fractions shows that pure fractions of neither gliadin nor glutenin was obtained with ethanol extraction (Abrehet, 2015). The three gliadin types exist in a variable proportion; α-gliadin (21%), γ-gliadin (17%) and ω-gliadin (6%) (Figure 1). The glutenin subunits; B/C-LMW-GS (36%), HMW-GS (11%) and D-LMW-GS (3%) were obtained from gluten protein (Delcour and Hoseney, 2010; Wieser, 2007; Veraverbeke and Delcour, 2002) (Figure 2). There was a reduction in the extractability of α-gliadin, γ-gliadin and ω-gliadin, HMW-GS and D-LMW-GS with increased molding condition thus explaining the occurrence of polymerization reaction due to the SH-disulfide interchange reactions (Sun et al. 2008; Kieffer et

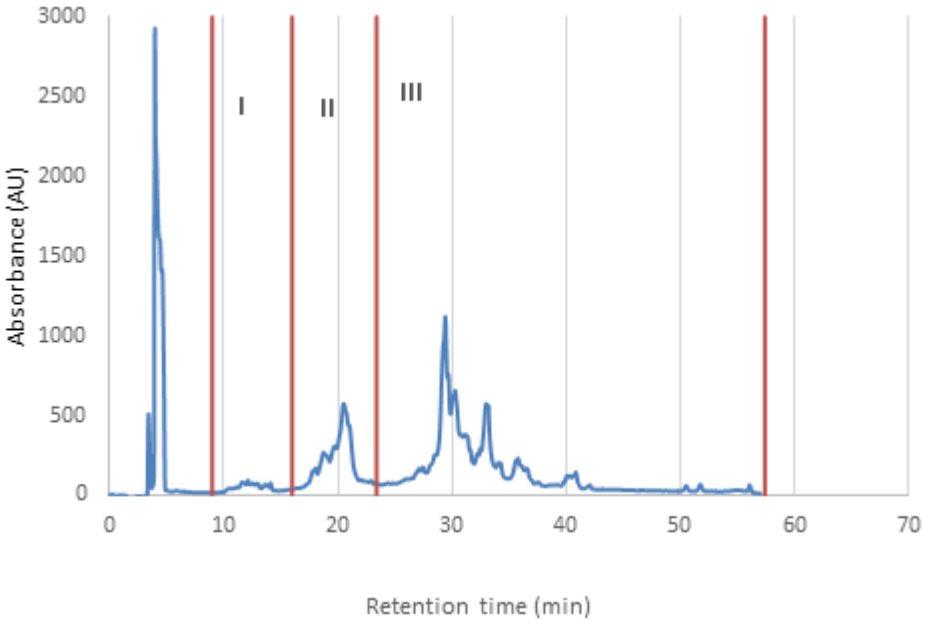

Figure 2. Typical reverse phase –High performance liquid chromatography (RP-HPLC) profiles of the glutenin subunit extracts; (I) D-LMW-GS. (II) HMW-GS and (III) B/C-LMW-GS.

al., 2007; Wieser, 2007; Lagrain et al., 2005; Singh and MacRitchie, 2004; Gallstedt et al., 2004; Domenek et al., 2004; Pommet et al., 2003;) while there was an increase in the extractability of B/C-LMW-GS with increased molding conditions thus explaining the oxidation and protein degradation (Veraverbeke and Delcour, 2002).

Glutenin composition and extractability

Upon extraction with 60% ethanol, the glutenin rich fractions contained 15% gliadins. The proportion of gliadin in the glutenin fractions indicates that the alcohol strength for the fractionation of gluten was not enough to separate the pure fractions of the gliadin and glutenins (Abrehet, 2015). The extractability of the GS decreases with increased molding condition which explains the occurrence of cross links (Abrehet, 2015). The distribution of ω- α- and γ-gliadins in the 15% gliadin of the glutenin enriched fractions was 2, 7 and 5% (Wieser, 2007), respectively, which further decreased with thermo-molding at 130 and 150°C in 5 and 25 min. GS extractability in the glutenin fraction was found to be 4, 23 and 57% for the D-LMW-GS, HMW-GS and B/C-LMW-GS, respectively (Delcour and Hoseney, 2010; Wieser, 2007; Veraverbeke and Delcour, 2002). With thermo-molding at 130 and 150°C in 5 and 25 min was a reduction and distribution in D-LMW-GS and HMW-GS as protein polymerization occurred due to the repetitive sequence of cysteine residue (Kieffer et al., 2007; Lagrain et al., 2005; Singh and MacRitchie, 2004; Weegels et al., 1994). There was increased extractabililty of B/C-LMW-GS with thermo-molding as the repetitive amino acid chains were broken down due to the thermal effects.

Gliadin composition and extractability

The gliadin fractions composed of 2.5% glutenin with higher proportion of B/C-LMW-GS while the D-LMW-GS and HMW-GS excites at lower concentration. The GS (B/C-LMW-GS, D-LMW-GS and HMW-GS) extractability was significantly affected with increased thermo-molding due to its hydrophobicity property similar to ω-gliadins in the gliadin enriched fractions. 97% of the gliadin fraction contained α-gliadin (46%) and γ-gliadin (37%) susceptible to covalent incorporation in the glutenins network formation as they are rich sources of cysteine residues than ω- gliadin (14.5%). Extractability was significantly reduced by more than 50% after molding at 130°C for 5 min due to the incorporation of gliadins in network formation and occurrence of non-disulfide bonds (Wieser, 2007; Veraverbeke and Delcour, 2002). The increased molding condition diminishes the extraction even though ω-gliadin exists in lesser amount and is unextractable with the application of thermo-molding than α- and γ-gliadins.

Amino acid composition

The three amino acids (cysteine, lysine and lanthionine (LAN)) were significantly affected by thermo-molding at

130 and 150°C for 5 and 25 min (Table 4). The reduction in cysteine and lysine amino acids with increased molding condition was due to sulfhydryl-disulphide interchange, oxidation and β-elimination reactions. LAN was undetectable after thermomolding at 130°C for 5 and 25 min for gluten and gliadin fraction and was detectable during thermomolding at 130°C for 25 min due to the occurence of repetitive sequence of cysteine due to oprotein agrigation. LAN formation increased with increased molding condition due to the sulfhydryl-disulfide interchange reaction and β-elimination reaction occurence. While cysteine and lysine amino acids were observed to be relatively similar with a slight reduction. The reduction in lysine with increased molding condition was due to oxidation and Maillard reactions (darkening of thermo molded plastic plates). Cysteine was converted to dehydroalanine (DHA) and atomic sulphur. Dehydroalanine was convereted in to lysinoalanine (LAL) (not detected) and lanthionine (LAN). The level of LAN formation is an indication of improved network formation.

Conclusion

Gluten proteins, the storage proteins of wheat and co-product of starch industry display interesting features for non-food applications. Understanding the composition and processing of gluten on network formation is important for biodegradable, renewable organic material formation. The fractionation of gluten with 60% ethanol yielded a glutenin- (49%) and a gliadin-rich (51%) fractions. Among the different amino acids; cystine, lysine and LAN were significantly affected by thermo-molding; the level of cysteine and lysine amino acids decreased with increased modling condition due to protein polymerization reactions. While the LAN increased with molding at 150°C. The reduction of extrability with increased thermo-molding condition improves protein polymerization and mechanical properties.

Conflict of Interests

The author has not declared any conflict of interests.

ACKNOWLEDGMENTS

The Laboratory of Food Chemistry and Biochemistry, KU Leuven and Hawassa University, School of Nutrition, Food Science and Technology are duly acknowledged.

REFERENCES

Abrehet F (2015). Effect of redox agents and thermo molding on the network formation and stability of wheat gluten bio-plastics. Int. J. Food Sci. Nutr. Eng. 5(5):203-208.

Belitz HD, Grosch W, Schieberle P (2009). Food Chemistry, Amino Acids, Peptides, Proteins. 4th ed., pp. 8-92.

Brauer S, Meister F, Gottlober R, Nechwatal A (2007). Preparation and thermoplastic processing of modified plant proteins. J. Macro Mol. Mater. Eng. 292:176-183.

Cao N, Fu Y, He J (2007). Preparation and physical properties of soy protein isolate and gelatine composite films. Food Hydrocolloids 21:1153-1162.

Carvalho RA, Grosso CRF, Sobral PJA (2008). Effect of chemical treatment on the mechanical properties, water vapor permeability and sorption isotherms of gelatin-based films. J. Packag. Technol. Sci. 21:165-169.

Carvalho RAD, Grosso CRF (2006). Properties of chemically modified gelatin films. Braz. J. Chem. Eng. 1(23):45-53.

Cuq B, Boutrot F, Redl A, Lullien-Pellerin V (2000). Study of the temperature effect on the formation of wheat gluten network: Influence on mechanical properties and protein Solubility. J. Agric. Food Chem. 48:2954-2959.

Delcour JA, Hoseney RC (2010). Principle of cereal science and technology. 3rd ed. pp. 1-69.

Dicharry RM, Ye P, Saha G, Waxman E, Asandei AD, Parnas RS (2006). Wheat gluten-thiolated poly (vinyl alcohol) blends with improved mechanical properties. Biomacromolecules 7:2837-2844.

Domenek S, Feuilloley P, Gratraud J, Morel MH, Guilbert S (2004). Biodegradability of wheat gluten based bio-plastics. J. Chemosphere 54:551-559.

Gallstedt M, Mattozzi A, Johansson E, Hedenqvist MS (2004). Transport and tensile properties of compression-molded wheat gluten films. J. Biomacromolecules 5:2020-2028.

Gerrard JA (2002). Protein–protein cross linking in food: methods, consequences, applications. Trends Food Sci. Technol. 13:391-399.

Goesaert H, Brijs K, Veraverbeke WS, Courtin CM, Gebruers K, Delcour JA (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. Trends Food Sci. Technol. 16:12-30.

Guerrieri N, Alberti E, Lavelli V, Cerletti P (1996). Use of spectroscopic and fluorescence techniques to assess heat induced molecular modification of gluten. J. Cereal Chem. 73(3):368-374.

Hanft F, Koehler P (2005). Quantitation of dityrosine in wheat flour and dough by liquid chromatography-tandem mass Spectrometry. J. Agric. Food Chem. 53:2418-2423.

Kieffer R, Schurer F, Kohler P, Wieser H (2007). Effect of hydrostatic pressure and temperature on the chemical and functional properties of wheat gluten: Studies on gluten, gliadin and glutenin. J. Cereal Sci. 45:285-292.

Lagrain B (2007). Heat-induced gluten polymerisation and its importance in bread making. PhD dissertation, Katholieke Universiteit Leuven, Leuven, Belgium.

Lagrain B, Brijs K, Veraverbeke WS, Delcour JA (2005). The impact of heating and cooling on the physic – chemical properties of wheat gluten-water suspensions. J. Cereal Sci. 42:327-333.

Lagrain B, Goderis B, Brijs K, Delcour JA (2010). Molecular basis of processing wheat gluten toward bio-based materials. J. Biomacromolecules 11:533-541.

Lagrain B, Thewissen BG, Brijs K, Delcour JA (2008). Mechanism of gliadin–glutenin cross-linking during hydrothermal treatment. J. Food Chem. 107:753-760.

Micard V, Morel MH, Bonicel J, Guilbert S (2001). Thermal properties of raw and processed wheat gluten in relation with protein aggregation. J. Polym. 42:477-485.

Pommet M, Redl A, Morel MH, Domenek S, Guilbert S (2003). Thermoplastic processing of protein-based bioplastics: chemical engineering aspects of mixing, extrusion and hot molding. In. Macromolecular symposia. WILEY-VCH Verlag. 197(1):207-218.

Rombouts I, Lamberts L, Celus I, Lagrain B, Brijs K, Delcour JA (2009). Wheat gluten amino acid composition analysis by high-performance anion-exchange chromatography with integrated pulsed amperometric detection. J. Chromatogr. 1216(29):5557-5562.

Shewry PR, Tatham AS (1997). Disulphide bonds in wheat gluten proteins. J. Cereal Sci. 25:207-227.

Singh H (1991). Modifications of food proteins by covalent cross linking. Trends Food Sci. Technol. 2:196-200.

Singh H, MacRitchie F (2004). Changes in proteins induced by heating gluten dispersions at high temperature. J. Cereal Sci. 39:297-301.
Sun S, Song Y, Zheng Q (2008). Thermo-molded wheat gluten plastics plasticized with glycerol: effect of molding temperature. J. Food Hydrocoll. 22:1006-1013.
Sun S, Song Y, Zheng Q (2007). Morphologies and properties of thermo-molded biodegradable plastics based on glycerol-plasticized wheat gluten. J. Food Hydrocoll. 21:1005-1013.
Tilley KA, Benjamin BE, Bagorogoza KE, Okot-Kotber B, Prakash O, Kwen H (2001). Tyrosine cross-Links: molecular basis of gluten structure and function. J. Agric. Food Chem. 49:2627-2632.
Veraverbeke WS, Delcour JA (2002).Wheat protein composition and properties of wheat glutenin in relation to Bread making functionality. Crit. Rev. Food Sci. Nutr. 42(3):179-208.
Weegels PL, Verhoek JA, DeGroot AMG, Hamer RJ (1994). Effect of gluten of heating at different moisture contents. I. Changes in functional property. J. Cereal Sci. 19:31-38.
Wieser H (2007). Chemistry of gluten proteins. Food Microbiol. 24:115-119.
Woerdeman DL, Veraverbeke WS, Parnas RS, Johnson D, Delcour JA, Verpoest I, Plummer CJG (2004). Designing new materials from wheat protein. J. Biol. Macromol. 5:1262-1269.
Zhang X, Do MD, Kurniawan L, Qiao G (2010). Wheat gluten-based renewable and biodegradable polymer materials with enhanced hydrophobicity by using epoxidized soybean oil as a modifier. J. Carbohydr. Res. 345:2174-2182.