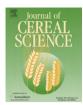
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# Effects of transglutaminase on the rheological and noodle-making characteristics of oat dough containing vital wheat gluten or egg albumin<sup>†</sup>

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#### ABSTRACT

Incorporating exogenous proteins into food production is a common practice for improving processing characteristics. In the present study, oat dough containing 15% (w/w, blends of protein-oat flour basis [POB]) vital wheat gluten (VWG) or 15% (w/w, POB) egg albumin (EA) was used to produce noodles with or without gluten (i.e., gluten-free). The rheological and noodle-making characteristics of oat dough containing exogenous proteins and the effects of added transglutaminase (TGase) were examined. The results indicate that the extent of TGase's modification of the thermomechanical and dynamic rheological characteristics (G' and G'') is dependent on the source of exogenous proteins in the oat dough. By adding 1.0% (w/w, POB) TGase, the cooking qualities of the resulting noodles (i.e., those containing VWG and EA) were significantly elevated with lower cooking loss; the elasticity of both types of noodles increased. The effects of TGase in different dough systems were analyzed by SDS-PAGE. In oat dough prepared with VWG, TGase was shown to catalyse the cross-linking of both oat protein and gluten protein; however, oat protein acted as the only substrate of TGase in the noodles that had been prepared with EA.

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#### 1. Introduction

The addition of exogenous proteins to food formulae is often used to improve the quality, texture, and storage stability of products (Bonet et al., 2006; Lodi et al., 2007). Oats (Avena sativa L.), have received increased interest in human foods due to the dietary benefits associated with  $\beta$ -glucans (FDA, 1997). However, the use of oats in baked products has been limited due to the inability of oat flour to form cohesive, viscoelastic dough that can retain gas, as that found in the gluten network of wheat dough. Addition of wheat gluten to oat flour improves the processing properties of the dough and the quality of the final product (Flander et al., 2007; Salmenkallio-Marttila et al., 2004). Other proteins (e.g., soybean and egg albumin) have been applied in food processing for their

good gelling and emulsifying properties (Marcoa and Rosell, 2008; Singh et al., 2008).

Enzymes are widely applied in food processing to improve the

Enzymes are widely applied in food processing to improve the textural properties and qualities of product and are generally recognized as safe. Transglutaminase (TGase, EC2.3.2.13) catalyses an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors) (Folk and Chung, 1973; Yokoyama et al., 2004). The covalent bond of  $\varepsilon$ –( $\gamma$ -Glu)–Lys is formed when the ε-amino group of lysine residues acts as acyl acceptor and crosslinks with other proteins. The formation of homologous and heterologous polymers among different proteins (e.g., whey, soybean, rice, casein, avenin, etc.) results from the addition of TGase. The enzyme increases the elasticity, water-holding capability, and other functional properties of food products (Guiral and Rosell, 2004; Tang et al., 2006; Truong et al., 2004). The TGase mediated cross-linked protein could (a) re-stabilize the damaged gluten network, which is a consequence of freezing and frozen storage, and (b) provide improved rheological and bread-making properties to the frozen dough (Huang et al., 2008; Kim et al., 2008). The application of TGase in gluten-free products has also attracted increased research and industry interests in recent years (Gujral and Rosell, 2004; Moore et al., 2006).

*Abbreviations*: TGase, transglutaminase; VWG, vital wheat gluten; EA, egg albumin; POB, blends of protein-oat flour basis; SDS—PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; HPMC, hydroxy propyl methyl cellulose.

<sup>†</sup> Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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The effects of TGase and effects of exogenous proteins on the rheological and Mixolab thermomechanical characteristics of oat dough have been previously discussed (Huang et al., 2010; Wang et al., 2009). In this study, oat dough containing 15% vital wheat gluten (VWG) and oat dough containing 15% egg albumin (EA) were used to produce oat noodles with or without gluten (gluten-free), respectively. TGase was added to examine the effects on the noodlemaking characteristics and overall quality of the oat noodles.

#### 2. Materials and methods

#### 2.1. Materials

Commercial oat flour was used, and protein supplements of vital wheat gluten (VWG) and egg albumin (EA) were purchased from Ruixiang Biological Technology Co. and Kangde Biological Products Co., Nantong, China, respectively. Microbial transglutaminase (TGase, 100 U/g) was obtained from Yiming Fine Chemical Ltd. (Taizhou, China), and hydroxypropyl methyl cellulose (HPMC) was purchased from Hope Top Co. (Huzhou, China).

#### 2.2. Analysis of oat flour and exogenous proteins

The proximate composition of oat flour and exogenous proteins (VWG and EA) was analyzed using approved methods of the AACCI (2000). The  $\beta$ -glucan content of oat flour was determined according to the method described by Lv (2005).

#### 2.3. Dough preparation

TGase was added to the oat flour (20 g) that contained 15% (w/w, POB) exogenous proteins at the levels of 0.0%, 0.5%, 1.0%, and 1.5% (w/w, POB). These were placed in a mixer (National Mfg., Lincoln, NE) and stirred uniformly; then 90% (v/w, POB) water was added and mixed for 4 min. The oat dough was packaged using a fresh-keeping film and allowed to rest for 25 min.

#### 2.4. Thermomechanical measurements of oat dough

Thermomechanical measurements of the oat dough were obtained using a Mixolab analyser (Chopin Technologies, Villeneuve-la-Garenne, France) according to the method reported by Huang et al. (2010) with slight modifications, i.e. a total weight of 90 g of oat dough was used for the assays rather than 50 g as reported previously. The composition of oat flour (containing 15% VWG or EA) with various concentrations of TGase (0.0%, 0.5%, 1.0%, and 1.5% w/w, blends of protein-oat flour basis, POB) was calculated by the corresponding Mixolab analysis software on a 14%-moisture basis. Thereafter, the water required for optimum consistency was added automatically to produce a dough torque of 1.1  $\pm$  0.07 Nm. The processes for each blend were repeated twice.

#### 2.5. Rheological measurements

Dynamic rheological measurements of the dough were determined using an AR1000 Rheometer (TA Instruments, New Castle, DE) as described previously by Huang et al. (2010). The measuring system consisted of parallel plate geometry (40 mm diameter, 1 mm gap). The dough was placed between the plates as soon as possible within 1 h of mixing, and the test was started after the dough had rested for 5 min. The rim of the sample was coated with Vaseline to prevent evaporation during measurement. Measurements were performed at 30 °C. The linear viscoelastic zone was determined by stress sweeps at 1 Hz frequency. Frequency sweep tests were performed from 0.01 to 10.00 Hz to determine the storage modulus (*G'*)

loss modulus (G'') and loss factor ( $\tan \delta$ ) as a function of frequency. Three replicates of each measurement were made.

### 2.6. Protein extraction and sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS—PAGE)

Protein fractions were extracted as described by Huang et al. (2010) from dough prepared as described above with 1.5 ml solvent. Salt-soluble proteins were extracted from 0.5 g oat dough by the addition of 1.5 ml 400 mM NaCl, alcohol-soluble proteins were extracted using 1.5 ml 60% (v/v) ethanol and glutelin with 1.5 ml SDS buffer (62.5 mM Tris—HCl, pH 6.8, 2.3% [w/v] SDS, 10% [v/v] glycerol, 5% [v/v] 2-mercaptoethanol).

Denaturing SDS—PAGE was performed using 12% separating gel (pH 8.8) and 5% stacking gel (pH 6.8). Samples (20  $\mu$ l) were mixed with 10  $\mu$ l sample buffer (0.01 M Tris—HCl, pH 6.8, 10.0% [w/v] SDS, 5.0% [v/v] 2-mercaptoethanol, 10.0% [v/v] glycerol, and 0.1% [w/v] bromophenol blue). Samples were heated in a boiling water bath for 5 min, and then centrifuged for 10 min at 4000  $\times$  g. Samples (15  $\mu$ l) were loaded into each lane and electrophoresis was carried out at 12 mA for the first 20 min then increased to 20 mA for the remainder of the run. The gel was stained with Coomassie Brilliant Blue 0.25% (w/v) in 50% methanol, 10% acetic acid, and de-stained in 10% acetic acid.

#### 2.7. Quantification of free amino groups

Quantification of free amino groups was conducted using the OPA reagent (Huang et al., 2010). 0.2 g of oat dough was suspended in 2 ml 0.1 M HCl (pH 1.0), vortexed, and centrifuged for 10 min at  $10,000 \times g$ . Then, 2.5 ml OPA reagent was added to 0.1 ml of the clear supernatant. The mixtures were allowed to react for 2 min; the absorbance was determined at 340 nm in an ultraviolet spectrophotometer. The values presented represent the means of the three replicates.

#### 2.8. Noodle preparation and quality analysis

#### 2.8.1. Noodle preparation

The basic ingredients in the oat-flour noodles are listed in Table 1. Water with corresponding amounts of salt (2%, w/w, POB) was added to the exogenous protein-oat flour blends, and the dough was mixed for 5 min by hand. Using a noodle press (Ohtake Noodle Machine Manufacturing Co., Ltd., Tokyo, Japan), the dough was sheeted between rollers set with a 2.5 mm gap. The sheet was folded, rolled twice, and rested for 30 min at 30 °C. The dough sheet was folded and rolled through three times each through successively decreasing roller gaps of 2.04 mm, 1.65 mm, and 1.10 mm. The final dough sheet was cut into 2.0 mm-wide noodles with a roller cutter, air dried at room temperature (ca. 22–24 °C) for 48 h. and then cut into 22 cm long strips and stored at room temperature for further use.

**Table 1**Basic formulation of four oat-flour blends for noodle production with salt level of 2% (w/w, POB).

Flour blends	Oat (%)	VWG (%)	EA (%)	TGase (%)	HPMC <sup>a</sup> (%)	Water <sup>b</sup> (%)
FI	85	15	_	_	_	54
FII	85	15	_	1	_	54
FIII	85	_	15	_	4	56
FIV	85	-	15	1	4	56

a w/w, POB.

<sup>&</sup>lt;sup>b</sup> Determined through repeated trials focusing on formation and processing characteristics of the dough during noodle making. VWG, vital wheat gluten; EA, egg albumin; TGase, transglutaminase; HPMC, hydroxypropyl methyl cellulose.

#### 2.8.2. Cooking characteristics and texture analysis

The optimum cooking time and the cooking loss of noodles were determined according to approved method 66-50 (AACCI, 2000). Cooking yield was determined as described by Wu and Corke (2005) with minor modifications. Cooking time was performed using 25 g of dried noodles that were cooked in 500 ml of boiling water, and following the 2-min time point, a sample was removed from the water and squeezed between two pieces of glass. The optimum cooking time was the time recorded when the white hard-core line disappeared. The process was duplicated to obtain the mean values of cooking time for each replicate.

Cooking yield and cooking loss were determined on 25 g of dried noodles cooked in 500 ml of boiling water to the optimum cooking times determined as described above. Following cooling in 20 °C water, cooked noodles were drained for 5 min and were then weighed. Cooking yield was calculated from the wet weight of cooked noodles divided by the dry weight of the raw noodles and expressed as a percentage. The cooking water was evaporated at 125 °C for 5 h, and the residue was reported as percent cooking loss based on the dry weight of the uncooked noodles. All samples were analyzed in triplicate.

The textural properties, hardness, springiness, and cohesiveness of a set of 3 strands of optimally cooked noodles were determined following the method of Baik and Lee (2003) using a TA-XT2 texture analyzer (Stable Micro Systems, Ltd., Godalming, UK). Four replicates of each treatment were evaluated.

#### 2.9. Statistical analysis

Means, standard error of the means, and t-tests were calculated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA), and one-way analysis of variance (ANOVA) results were calculated using SAS statistical software package (SAS Institute, Cary, NC).

#### 3. Results and discussion

#### 3.1. Elementary composition of oat flour and exogenous proteins

Moisture, protein, ash, total starch, lipids and  $\beta$ -glucan contents of oat flour used in this study were 9.6%, 7.6%, 0.9%, 69.3%, 6.5% and 4.8%, respectively. The moisture, protein, total starch and ash contents were 9.0%, 73.4%, 16.1% and 0.9% for VWG and 8.2%, 79.3%, 0% and 7.8% for EA.

## 3.2. Effects of TGase on the thermomechanical characteristics of oat dough

The effects of TGase on the thermomechanical properties of oat dough varied with the addition of different exogenous proteins. Water absorption decreased as the proportion of TGase increased in both samples (i.e., VWG- and EA-containing types) (Table 2). This is consistent with results reported by Basman et al. (2002) in wheat dough treated with TGase, who reported a decrease in Farinograph water absorption in TGase-treated wheat dough. The mechanism has been suggested to be the result of acyl-transfer reactions that introduce new groups into proteins and thus changes in charge, hydrophobicity and structure (DeJong and Koppelman, 2002; Gerrard et al., 1998). Wu and Corke (2005) speculated that the action of TGase leads to an increased water-holding capacity due to the increased hydrophilicity of gluten proteins by the hydrolysis of glutamine residues to glutamic acid.

In the oat dough with VWG, TGase treatment did not significantly affect the dough development time but did decrease the degree of protein softening and increased the stability, which may have been the result of the promoted protein cross-links that were modified by TGase, which, in turn, enhanced and stabilized the gluten network. However, the addition of TGase to the oat dough with EA resulted in no significant variation within the parameters present in Table 2, except water absorption and dough development time.

Mixolab parameters (Table 2) such as peak torque C3, cooking stability, and setback were associated with changes in the starch gelatinization and retrogradation. The effects of TGase on the dough starch properties were less significant than the effects on protein. The presence of TGase induced a decreased torque peak (C3) of the oat dough containing VWG during heating. Wu and Corke (2005) similarly reported that the addition of 0.5% TGase in wheat resulted in a decrease in hot paste viscosity. It was most likely because part of the starch was embedded in the protein network that had been formed by the TGase induced cross-linking resulting in a decrease in the degree of starch gelatinization and viscosity. Bonet et al. (2006) concluded that increasing protein content can alter starch gelatinization. Additionally TGase results in an increase in protein water absorption which is available to starch during gelatinization (Salmenkallio-Marttila et al., 2004).

Wu and Corke (2005) reported a decrease in hot paste and final viscosity in wheat dough treated with 0.5% TGase. However, Marcoa and Rosell (2008) reported no significant difference in RVA parameters in a TGase rice flour—egg-white blend. Using a wheat dough system supplemented with different protein sources, Bonet et al. (2006) reported an increase in peak torque only in dough containing egg albumin.

As these parameters were related to the dough protein properties, it is clear that TGase had a greater effect in protein systems containing oat protein and VWG than that with oat protein and EA. Han and Damodaran (1996) suggested that cross-link formation between two heterologous proteins depends on the thermodynamic compatibility. TGase has been shown to increase quality parameters of oat—wheat mixed dough (Salmenkallio-Marttila et al., 2004).

**Table 2**Effect of TGase and different proteins on the thermomechanical properties of oat flour.

Sample	TGase addition (%)	Water absorption (%)	Dough development time (min)	Protein softening degree (Nm)	Stability (min)	C3 (Nm)	Setback (Nm)	Cooking stability (min)	β	γ
VWG	0.0	76.2 <sup>a</sup>	8.2 <sup>a</sup>	0.61 <sup>a</sup>	6.6 <sup>b</sup>	1.90 <sup>a</sup>	0.39 <sup>a</sup>	0.70 <sup>a</sup>	0.367 <sup>a</sup>	$-0.130^{b}$
	0.5	76.1 <sup>a</sup>	8.2 <sup>a</sup>	0.51 <sup>c</sup>	9.3 <sup>a</sup>	1.87 <sup>a</sup>	0.39 <sup>a</sup>	0.68 <sup>a</sup>	$0.449^{a}$	$-0.049^{a}$
	1.0	75.8 <sup>a</sup>	8.4 <sup>a</sup>	0.53 <sup>c</sup>	10.4 <sup>a</sup>	1.83 <sup>ab</sup>	0.38 <sup>a</sup>	$0.70^{a}$	$0.432^{a}$	$-0.063^{a}$
	1.5	73.6 <sup>b</sup>	8.5 <sup>a</sup>	0.57 <sup>b</sup>	10.3 <sup>a</sup>	1.78 <sup>b</sup>	0.41 <sup>a</sup>	0.73 <sup>a</sup>	0.477 <sup>a</sup>	$-0.036^{a}$
EA	0.0	45.3 <sup>a</sup>	1.5 <sup>a</sup>	0.56 <sup>a</sup>	7.5 <sup>b</sup>	2.53 <sup>c</sup>	0.87 <sup>c</sup>	1.12 <sup>a</sup>	0.438 <sup>a</sup>	$-0.058^{a}$
	0.5	43.9 <sup>b</sup>	1.3 <sup>ab</sup>	0.61 <sup>a</sup>	8.7 <sup>a</sup>	3.34 <sup>b</sup>	1.21 <sup>ab</sup>	0.95 <sup>b</sup>	0.263ab	$-0.063^{a}$
	1.0	43.5 <sup>b</sup>	1.3 <sup>b</sup>	$0.64^{a}$	8.5 <sup>a</sup>	$3.46^{a}$	1.14 <sup>b</sup>	0.95 <sup>b</sup>	$0.245^{ab}$	$-0.085^{a}$
	1.5	43.3 <sup>b</sup>	1.4 <sup>ab</sup>	$0.59^{a}$	8.9 <sup>a</sup>	3.47 <sup>a</sup>	1.30 <sup>a</sup>	0.96 <sup>b</sup>	$0.138^{b}$	$-0.080^{a}$

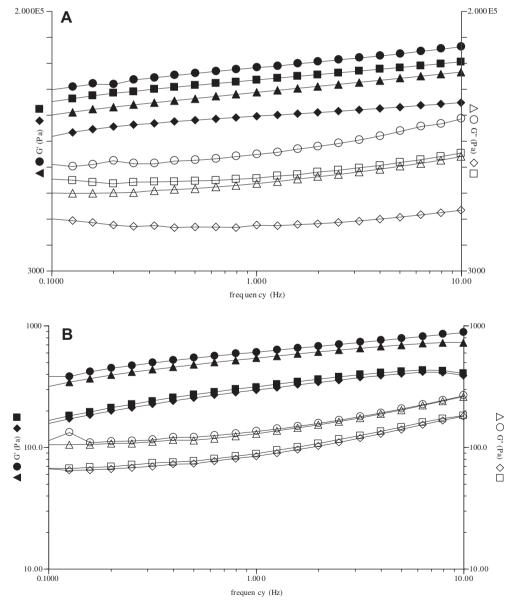
Different letters in each column show statistically significant differences (p < 0.05) in oat dough that contains the same protein. EA, egg albumin; VWG, vital wheat gluten.

#### 3.3. Effects of TGase on the rheological properties of oat dough

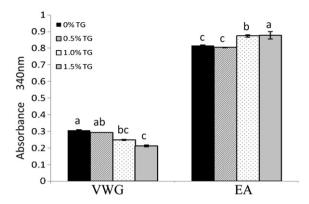
Rheological characteristics, which are related to machining properties, processing conditions, and final product quality, are critical in food manufacturing. The rheological properties of oat dough containing different levels of TGase were studied by dynamic oscillatory measurements (Fig. 1A and B).

The mechanical spectra of oat dough containing VWG showed an increase in both storage modulus (G') and loss modulus (G'') with increasing levels of TGase (Fig. 1A). This indicated that TGase led to protein cross-linking and the formation of a network structure, which, in turn, modified the viscoelastic behavior of the oat dough. Similar results have been reported by Larré et al. (2000) who noted that the main impact of TGase on gluten was formation of protein cross-linking through formation of new covalent bonds. However, when the oat dough was treated with 1.5% TGase, the opposite trend was observed. This may be attributed to the limited reactivity of TGase because there was enough glutamine but relatively lower content of lysine in gluten and oat flour. With

TGase addition,  $\tan \delta$  presented similar variation trends as that observed with G' and G'' (data not shown). The storage modulus (G') and the loss modulus (G'') exhibited a lower level in oat dough containing EA than in the oat dough with VWG due to the lower water absorption of the former. The addition of TGase at the level of 0.5% and 1.0% (w/w, POB) resulted in an increase in G' and G''values. However, the dynamic rheological properties were reduced when the TGase addition increased from 1.0% to 1.5% (w/w. POB). This result was in agreement with the results of Huang et al. (2010) who studied the effects of TGase on the rheological characteristics of non-supplemented oat dough. In the oat dough with EA,  $\tan \delta$ increased concomitantly with the increase in TGase (data not shown). Water absorption for dough making is critical when the rheological properties of dough are examined and is directly influenced by the amount of water added (Marcoa and Rosell, 2008). In this study, constant water absorption (90%) was used to ensure all ingredients mixed were fully hydrated, in order to obtain results comparable with the previous study (Huang et al., 2010).



**Fig. 1.** The effect of TGase on the rheological properties (storage modulus G', solid symbols and loss modulus G'', open symbols) of oat dough with (A) 15% (w/w, POB) vital wheat gluten (VWG) and (B) 15% (w/w, POB) egg albumin (EA). ( $\blacklozenge$ ,  $\diamondsuit$ 0%;  $\blacksquare$ ,  $\Box$ 0.5%;  $\blacklozenge$ ,  $\bigcirc$ 1.0%;  $\blacktriangle$ ,  $\triangle$ 1.5%; w/w, POB).



**Fig. 2.** Effects of TGase on the number of free amino groups in oat dough with 15% (w/w, POB) exogenous proteins. Error bars indicate the standard deviation of replicates. Different letters above the histogram bars show significant differences (p < 0.05) in oat dough that contains the same protein.

## 3.4. Effects of TGase on the quantification of free amino groups in oat dough

TGase mediated protein modification in dough has been measured by determining the free amino acid content (Gujral and Rosell, 2004). The number of free amino groups varied in oat dough that contained different exogenous proteins (Fig. 2). The greatest increase was induced by the addition of EA. In a previous study (Huang et al., 2010), we observed that increasing TGase results in a decrease in the number of free amino groups in oat dough. In this study, TGase treatment caused different changes in oat dough depending on the proteins contained in the dough. The number of free amino groups in oat dough containing VWG decreased significantly when TGase was increased from 0.0% to 1.5% (w/w, POB), which indicated that the free amino group of VWG was consumed during the protein cross-linking reaction. Dough containing EA displayed a higher amount of free amino groups than that of the VWG supplemented dough. Contrary to the effect observed in the VWG containing dough, TGase had a minimal effect on oat dough containing EA, in that a non-statistically significant decrease was observed at 0.5% (w/w, POB) and a slight increase when higher levels were used. The results may be a result of the processing conditions used in the preparation of EA. TGase mediated protein cross-linking is determined by the molecular structure and the accessibility of glutamine and lysine residues (Matsumura et al., 1996). Thermal denaturation and protein aggregation of egg white protein during processing may have resulted in smaller peptide chains as well as amino acids that are inaccessible to enzymatic cross-linking (Lim et al., 1998).

#### 3.5. Cooking time, loss, and yield of oat noodles

Cooking characteristics such as cooking time, loss, and yield of different noodle preparations are listed in Table 3. The cooking time of noodles containing EA was longer than that of those with VWG. No significant effect (p < 0.05) of TGase on cooking time was observed.

**Table 3**Cooking properties and texture profiles of cooked oat noodles.

Flour Blends <sup>a</sup>	Cooking time (min)	0	_	Hardness (g)	Springiness	Cohesiveness
NI	12 <sup>c</sup>	261.2 <sup>bc</sup>	11.4 <sup>b</sup>	262.0 <sup>c</sup>	0.87 <sup>c</sup>	0.52 <sup>b</sup>
NII	12 <sup>c</sup>	245.6 <sup>d</sup>	8.1 <sup>c</sup>	271.1 <sup>c</sup>	0.97 <sup>b</sup>	0.55 <sup>b</sup>
NIII	14.5 <sup>b</sup>	264.4 <sup>b</sup>	7.4 <sup>cd</sup>	352.4 <sup>b</sup>	0.82 <sup>cd</sup>	0.41 <sup>c</sup>
NIV	14 <sup>b</sup>	255.2 <sup>c</sup>	6.7 <sup>d</sup>	363.1 <sup>b</sup>	0.89 <sup>c</sup>	0.42 <sup>cd</sup>

Different letters in each column show statistically significant values (p < 0.05).

Cooking yield and loss were influenced by noodle formulations. In the absence of TGase, no significant difference was observed in cooking yield of oat noodles containing EA or VWG, whereas cooking loss was lower in the EA noodles which could be attributed to the gelatinization of EA and the addition of HPMC. Both cooking yield and loss of oat noodles containing different exogenous proteins were reduced with the addition of TGase. Specifically, cooking yield and loss were decreased by 6.0% and 28.9%, respectively, when TGase was added at a level of 1.0% (w/w, POB) in oat noodles containing VWG. Cooking loss is attributed to starch properties (Bhattacharya et al., 1999). However, in the case of oat noodles containing either VWG or EA, it is likely that this is due, in part, to the protein network because the level of starch in samples with or without TGase was the same. Starch embedded in the cross-linked protein network that formed as a result of the addition of TGase, resulted in limited starch loss as reflected by a reduction in cooking loss. The degree of this effect was determined mainly by the protein composition of the samples. Previously, Wu and Corke (2005) found that cooking loss of white salted noodle was not significantly influenced by TGase treatment. The diversity between the properties of oat starch and wheat starch may be responsible for difference.

#### 3.6. Noodle texture analysis

Oat noodles containing EA (NIII and NIV) were characterized by greater hardness than those containing VWG (Table 3). It has been previously reported that noodles produced with higher protein flour exhibit a harder texture (Shiau and Yeh, 2001). Noodle hardness could be influenced by the different proteins in flour-exogenous protein blends; however, the main reason for the degree of hardness is certain characteristics (in particular gelatinization properties) of the exogenous proteins. Liang and Kristinsson (2007) reported that albumin tends to form a network, thus the gel network developed by albumin condensation during heating could endow the dough with a solid texture and consequently, increase the hardness of cooked noodles. The hardness of TGase-treated noodles was slightly but not significantly higher.

The springiness of noodles containing VWG (NI) was higher than that of the noodles with EA. Especially when TGase was added, the springiness value of noodle NI increased due to TGase enhanced elasticity of the gluten (Wu and Corke, 2005). The gelatinization properties and protein cross-linking resulting from TGase could benefit the textural properties of noodles with some elastic properties, which is similar to the behavior of gluten.

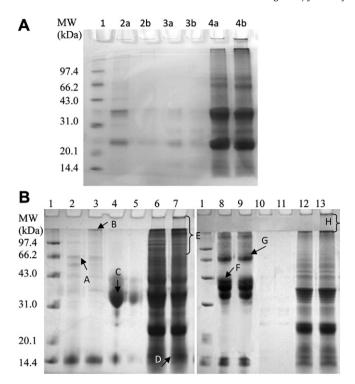
Generally speaking, the cohesiveness of cooked noodles is a measurement of the starch properties (Epstein et al., 2002). However, in this experiment, cohesiveness was affected by the properties of exogenous proteins. The addition of TGase had no significant effect on noodle cohesiveness.

#### 3.7. Effects of TGase on protein fractions in oat dough

The effects of TGase on protein fractions from oat dough containing 15% (w/w, POB) VWG or EA treated with 1.0% (w/w, POB) TGase were analyzed by SDS-PAGE (Fig. 3).

A comparison of Fig. 3A and B shows that the addition of VWG to oat dough led to a slight increase in salt-soluble proteins with high-molecular-weight (marked as A). However, a decreased intensity was observed in the oat derived electrophoretic bands. Following treatment with TGase, a band (B) was detected at the top of the separation gel indicating the formation of high-molecular-weight protein polymers formed by TGase that were too large to enter the separating gel. The intensity of alcohol-soluble proteins (marked as C) was increased significantly by adding VWG. The molecular weight in the range of 25–43 kDa indicates that proteins were

<sup>&</sup>lt;sup>a</sup> Noodle preparation used the formulation listed in Table 1.



**Fig. 3.** A: SDS–PAGE analysis of protein fractions in oat dough prepared without (lanes 2a, 3a, 4a) and with 1.0% TGase (lanes 2b, 3b, 4b). Lane 1, molecular weight standard, lane 2, salt-soluble proteins; lane 3, alcohol-soluble proteins; lane 4, SDS-soluble proteins. B: SDS–PAGE analysis of protein fractions of oat-protein dough (VWG–oat dough: lanes 2–7; EA–oat dough: lanes 8–13) without (lanes 2, 4, 6, 8, 10, 12) and with 1.0% (w/w, POB) TGase (lanes 3, 5, 7, 9, 11, 13). Salt-soluble proteins (lanes 2, 3, 8, 9), alcohol-soluble proteins (lanes 6, 7, 12, 13).

wheat gliadins. Treatment with TGase dramatically reduced the intensities of all alcohol-soluble protein bands. Compared to the SDS-soluble bands in lane 4 of Fig. 3A, a new intensive band of SDS-soluble protein was observed near 43 kDa as were a series of new bands (around 97 kDa) in the gluten-oat dough due to the gluten addition (Fig. 3, lane 6). The band intensities of highmolecular-weight SDS-soluble protein with molecular weight higher than 60 kDa in the gluten-oat dough were reduced after TGase treatment, while a high concentration of proteins was aggregated at the top of the stacking gel (marked as E), which may be attributed to the fact that the high-molecular-weight protein polymers were formed with the low-molecular-weight protein fragments and, for this reason, may not enter the gel. Larré et al. (2000) and Gerrard et al. (2001) reported that all constituents of gluten may be good substrates for TGase, yet high-molecularweight glutenin subunits are optimal.

Comparatively, the effects of TGase on protein fractions in oat dough with EA were less than those in the dough with VWG. In EA, water-soluble protein was the main component, which caused intensive bands (the ovalbumin [F: MW 45 kDa] and ovotransferrin [G: MW 70–78 kDa] in salt-soluble protein fraction) whereas oat globulin/albumin was diluted (Liang and Kristinsson, 2007). No significant change was observed after TGase treatment. The treatment with EA had no distinct effect on alcohol- and SDS-soluble proteins. However, an increase in the concentration of the protein band (marked as H, lanes 12 and 13) was observed on the top of the stacking gel. A similar result was obtained when oat glutelin was treated with TGase (Huang et al., 2010). This might suggest that a cross-link of oat protein and gluten could be catalyzed by the addition of TGase to oat dough with VWG, whereas oat protein should be the main substrate for TGase acting in EA—oat dough.

#### 4. Conclusion

In conclusion, the rheological and processing properties of oat dough could be improved by the addition of TGase and exogenous proteins. This research has shown that it is possible to produce oat noodles both with and without gluten (gluten-free) and has provided an essential foundation for development of other oat products.

TGase treatment modified the thermomechanical properties and rheological characteristics of oat dough containing 15% (w/w, POB) exogenous proteins: specifically, that with 15% (w/w, POB) gluten protein. The number of free amino groups also varied according to the protein cross-linking interaction that was induced by TGase. Adding 1.0% (w/w, POB) TGase to the noodles (with or without gluten) that had been produced with oat dough containing 15% (w/w, POB) VWG or EA improved the cooking quality, reduced the cooking loss, and enhanced the hardness and springiness of the final product. The cross-link of TGase analyzed by SDS-PAGE showed that there were significant influences of TGase on each protein fraction from gluten-oat dough, which indicated TGase could catalyze the cross-linking of both oat protein and gluten. However, this effect was not so obvious in EA-oat dough in which oat protein might be the only substrate for TGase.

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