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Effects of transglutaminase on the rheological and Mixolab thermomechanical characteristics of oat dough ☆

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ABSTRACT

The effects of added transglutaminase (TG) on the rheological and thermal properties of oat dough were evaluated. Mixolab, rheometer, and differential scanning calorimetry (DSC) were used to analyse the oat dough for changes in thermomechanical, rheological, and thermal properties. TG had distinct effects on dough water absorption, modified viscoelastic behaviour, and enhanced thermal stability. The dough also exhibited a decrease in the number of free amino groups after TG treatment, confirming protein cross-linking catalysed by TG. Electrophoresis of TG-treated oat protein fractions using SDS–PAGE, which was also used to analyse the effects of TG on the protein fractions of oat flour; it showed that both globulin and avenin were good substrates for TG.

1. Introduction

Although perhaps not as popular a breakfast food as many manufactured cereal alternatives, oats (*Avena sativa*) have received increased interest recently due to their nutritional value (e.g., high in soluble dietary fibre, proteins, unsaturated fatty acids, vitamins, minerals and other nutrients). A substantial amount of the dietary fibre of oat is β-glucan (mixed linkage (1–3)(1–4)-β-D-glucan), a cell wall polysaccharide that can reduce the concentration of serum cholesterol, attenuate blood glucose level, slow insulin response in the blood, and maintain the balance of intestinal flora (Krauss, Eckel, & Howard, 2000). Furthermore, the US Food and Drug Administration (FDA) reports a positive role of soluble dietary fibre from oats in reducing the risk for coronary heart diseases (FDA, 1996).

Utilisation of oats in baked products is limited due to the inability of oat flour to form cohesive, viscoelastic dough, such as the gluten network of wheat dough. Wheat prolamin proteins, gliadin and glutenin, make up approximately 80% of the seed storage proteins and are responsible for formation of the gluten network that imparts the unique properties of wheat dough. During mixing, the gluten network forms, endowing the dough with viscoelasticity and gas holding capacity, which is essential for production of bread products (Hoseney & Rogers, 1990).

Oats and rice are the two cereals in which the alcohol-soluble prolamins (avenins) are not the major storage proteins. Globulins belonging to the 11/12S family account for about 75% of the oat seed protein (Colyer & Luthe, 1984). In contrast, the alcohol-soluble avenins comprise 10–20% of total seed protein (Frey, 1951; Peterson & Smith, 1976).

The structural properties of food materials can be modified by introducing protein cross-links (Gerrard, 2002). The enzyme transglutaminase (TG) (EC 2.3.2.13) has been used in many industries including dairy, bakery, and meat processing (Jong & Koppelman, 2002; Kuraishi, Yamazaki, & Susa, 2001). TG, a γ-glutamyl transferase that is widely distributed in mammals, plants, fish, and microorganisms, can catalyse the reaction between an ε-amino group on protein-bound lysine residues and a β-carboxyamide group on protein-bound glutamine residues, which leads to covalent cross-linking of proteins (Folk & Chung, 1973; Yokoyama, Nio, & Kikuchi, 2004.). TG catalyses the formation of homologous and heterologous polymers among whey protein, soybean protein, rice protein, casein, and avenalin in order to increase the elasticity, water retention capability, and other functionality properties (Ahn, Kim, & Ng, 2005).

Many investigations into the cross-linking of wheat protein have demonstrated that the enzyme catalysis reaction not only affected the biochemical characteristics of the dough, but also the rheological properties (Autio et al., 2005; Köksel, Sivri, Ng, & Steffe,

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2001). The addition of TG to frozen wheat dough significantly improved the rheology of the dough as well as the specific volume and textural softness of the bread by re-establishing the damaged gluten network, which is a consequence of freezing and frozen storage of dough (Huang, Yuan, Kim, & Chung, 2008; Kim, Huang, Du, Pan, & Chung, 2008).

The aim of the present study was to investigate the effect of different amounts of TG on the rheological and thermal properties of oat dough using Mixolab, rheometer, and differential scanning calorimetry (DSC). In addition, the mechanism of the modification of TG on oat protein was investigated using SDS–PAGE and determination of the amount of free amino groups after TG treatment confirmed the protein cross-link catalysed by TG.

2. Materials and methods

2.1. Flour samples and composition

Commercial oat flour was used. The flour samples were analysed for proximate composition (AACC, 2000) and amylose value (Lv, 2005). The flour was analysed for moisture (9.6%), protein (7.6%), ash (0.9%), and amylose content (4.8%). Transglutaminase (100 U/g) was obtained from YiMing Fine Chemical Ltd. of Taizhou, China.

2.2. Oat dough preparation

TG was added to the base flour (20 g) at the levels of 0%, 0.5%, 1.0% and 1.5% (w/w oat flour basis) into a mixer (National Mfg., Lincoln, NE) and stirred uniformly; next, 70% (by volume) water was added and mixed for 3 min. The oat dough was packaged using a fresh-keeping film and allowed to rest for 25 min.

2.3. Thermomechanical measurements

Mixing and pasting behaviours of the oat flour dough were studied using a Mixolab analyser (Chopin Technologies, Villeneuve la Garenne, France), which was capable of evaluating rheological and enzymatic properties of the flour. The Mixolab analyser measures (in real time) the torque (Nm) that is produced by the passage of dough between two kneading arms, thus allowing the study of physicochemical behaviour. The instrument allows analysis of the quality of the protein network and the starch behaviour during heating and cooling. For the assays, 50 g of oat flour or oat flour-TG blends (i.e., using 0%, 0.5%, 1.0%, 1.5%, w/w, flour basis) were placed into the Mixolab analyser bowl and mixed. After tempering the solids, the water required for optimum consistency was added. Special attention was paid to the determination of the water absorption to ensure the complete hydration of all the components. The settings used in the test were 8 min at 30 °C with a temperature increase of 4 °C/min until the mixture reached 90 °C; at this point, there was an 8-min holding period at 90 °C, followed by a temperature decrease of 4 °C/min until the mixture reached 55 °C, and then 6 min of holding at 55 °C. The mixing speed during the entire assay was 73 rpm. The process was repeated twice for each blend as well as for the control.

The parameters that were obtained from the recorded curve were water absorption (%) or the percentage of water required for the dough to produce a torque of 1.1 ± 0.07 Nm; dough development time (min) or the time to reach the maximum torque at 30 °C; stability (min) or the elapsed time at which the torque produced is kept at 1.1 Nm; mechanical weakening (Nm) or the torque difference between the maximum torque at 30 °C and the torque at the end of the holding time at 30 °C; minimum torque (Nm) or the minimum value of torque produced by dough passage while being subjected to mechanical and thermal constraints; thermal weakening (Nm) or the difference between the torque at the end of the holding time at 30 °C and the minimum torque; peak torque (Nm) or the maximum torque produced during the heating stage; cooking stability (Nm), which is calculated as the ratio of the torque after the holding time at 90 °C and the maximum torque during the heating period; and setback (Nm), which is defined as the difference between the torque produced after cooling at 50 °C and the torque after the heating period. In addition, the slopes of ascending and descending torques and the angle between ascending and descending curves were calculated. Those angles were then used to determine α, β, γ, and δ, which correspond to the arc tangent of the four curve angles. The calculations were repeated two times for each blend.

2.4. Oscillatory measurements

Dynamic rheological measurements of the dough were determined on the AR1000 rheometer (TA Instruments, New Castle, DE) according to Marco and Rosell (2008). The measuring system consisted of parallel plate geometry (40 mm diameter, 1 mm gap). The dough was placed between the plates within 1 h after 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 while the measurements were being taken. 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') and loss modulus (G'') as a function of frequency. Two replicates of each measurement were made.

2.5. Thermal properties DSC measurements

The thermal properties of oat dough were examined using a Pyris-1 DSC thermal analyser (PerkinElmer, Norwalk, CT). Approximately 2.0 mg of dough samples were weighed with a microbalance into aluminium liquid pans, hermetically sealed, and heated from 30 to 110 °C at a rate of 10 °C/min. Onset (To), peak (Tp), and conclusion (Tc) temperatures with enthalpy (ΔH) were computed automatically; a sealed empty pan was used as a reference.

2.6. Quantification of free amino groups

Free amino groups were quantified in order to confirm the formation of TG-catalysed covalent bonds. This method is based on the reaction between primary amino groups and o-phthaldialdehyde (OPA) (Dinnella, Gargaro, Rossano, & Monteleone, 2002; Gujral & Rosell, 2004). Oat dough samples (0.2 g), to which different levels of TG had been added, were suspended in 2 ml 0.1 M HCl (pH 1.0), vortexed, and centrifuged for 10 min at 10,000*g*. To 0.1 ml of the clear supernatant, 2.5 ml OPA reagent was added. The mixture was allowed to react for 2 min and the absorbance was determined at 340 nm in an ultraviolet spectrophotometer. The process was repeated three times per sample.

To prepare the OPA reagent, the following compounds were diluted with water to 100 ml:80 mg OPA (dissolved in 2 ml 95% ethanol); 50 ml 0.1 M sodium tetraborate buffer solution pH 9.5; 5 ml 20% SDS; and 0.2 ml of 2-mercaptoethanol. The OPA reagent was prepared immediately before use and kept in a brown glass container at 4 °C.

2.7. SDS–PAGE

2.7.1. Protein extraction

Globulins and albumins were extracted from 0.5 g oat dough by the addition of 1.5 ml 400 mM NaCl, vortexing for 5 min and cen-

trifuged for 10 min at 10,000*g*. The supernatant was removed and stored at −10 °C. The precipitate was then washed using distilled water and was used for extracting the gliadin (avenin). Avenins were extracted from the pellet by the addition of 1.5 ml of 60% (v/v) ethanol; the sample was vortexed and centrifuged as described above. For extraction of glutelin, 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) was added to the above residue.

2.7.2. Electrophoretic analysis

Denaturing SDS–PAGE was used to analyse the effects of TG on the protein fractions of oat flour. The discontinuous gel was composed of a 12% separating gel (pH 8.8) and a 5% stacking gel (pH 6.8). The protein samples (20 μl) were dissolved in 10μL sample buffer (0.01 M Tris–HCl, pH 6.8, including 10% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 0.1% (w/v) bromphenol blue), heated for 5 min at 100 °C, and then centrifuged for 10 min at 4000*g*. Sample volumes of 15 μl were loaded into each well and electrophoresis was performed at 12 mA for the first 20 min then increased to 20 mA for the remainder of the run. The gel was stained with 0.25% Coomassie brilliant blue, and de-stained in 10% acetic acid.

2.8. Statistical analysis

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

3. Results and discussion

3.1. Effect of TG on the thermomechanical properties of oat dough

The Mixolab instrument measured the behaviour of both oat proteins and starch when subjected to a dual mechanical shear stress and temperature constraint. The early stages, (C1, C2, α), mainly represent the properties of the oat proteins; the latter shows the properties of the oat starch. Therefore, the effect of the protein sources and their possible cross-linking by TG on the dough mechanical changes due to mixing and heating could be registered. Fig. 1 shows a typical Mixolab curve to distinguish the different stages. The initial mixing (0–8 min) – when the hydration of the compounds occurred in combination with the stretching and alignment of the proteins – brought about the formation of a three-dimensional viscoelastic structure. The interactions between polymeric proteins resulted from disulphide-linked polymer proteins; hydrogen-bonding aggregates play the main role in this structure. In the 2nd stage (8–23 min), the combined effect of the mechanical shear stress and the temperature constraint induced a decrease in the torque due to the beginning of the protein destabilization and unfolding. As the temperature increased, the contribution of the proteins to the torque was masked by the starch changes (3rd stage). During this stage, the swelling and gelatinization of the starch granules occurred until the physical breakdown of the granules was accompanied by a reduction in the torque (4th stage). A further increase in the torque when the temperature decreased (5th stage) was associated with the recrystallization of the starch and was related to the retrogradation of the starch molecules. Fig. 1 shows four slopes (α, β, γ, δ). Slope α represents the protein weakening during a period of steady temperature rise; β represents the starch gelatinization; γ represents starch breakdown; and δ represents starch recrystallization during paste cooling.

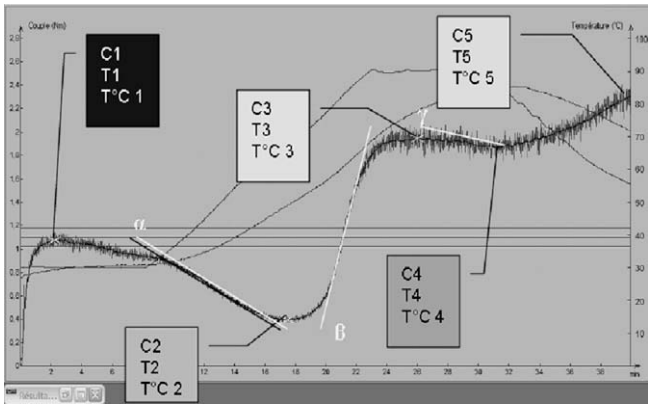

Fig. 1. Typical thermomechanical curve from Mixolab analysis of oat dough.

Mixolab parameters resulting from increasing TG had a distinct effect on the thermomechanical properties of oat dough (Table 1). Under the condition of C1 = 1.1 ± 0.07 Nm, the water absorption decreased as the level of TG was increased. Similar results were reported by Basman, Köksel, and Ng (2002a) on wheat dough analysed using the farinograph. The developing time and stability increase showed that the elasticity of the oat dough was increased by the TG action. In addition, the extensibility of paste increased due to modification of the cross-link between the oat proteins by TG. In turn, this corresponded with increased stability of the protein network. Gerrard et al. (1998) hypothesised that the cross-linking from the addition of TG results in increased water-holding capacity due to changes in secondary structure or, possibly, due to changes in protein hydrophobicity from the formation of glutamic acid residues from glutamine hydrolysis. Structural changes in TG-treated oat globulin have been reported by Siu, Ma, and Mine (2002).

After TG treatment, no significant difference was observed in the oat starch. TG did not result in any significant difference in the setback value and cooking stability of the oat flour. Only at the TG level of 1.5%, there an increase in cooking stability. Although the presence of TG induced an increased torque peak (C3), it may a result of TG's effect on the water absorption.

3.2. Effect of TG on the rheological properties of oat dough

A continuous protein phase is critical for the viscoelastic properties of dough; an increase in the average molecular weight of the protein matrix due to TG activity would lead to an increase in the elastic and viscous modulus. The viscoelastic properties of samples of oat dough containing different levels of TG were studied by dynamic oscillatory measurements. The mechanical spectra of all the samples showed the storage modulus (G') always higher than the loss modulus (G''), and both increased with increasing levels of TG (Fig. 2). This indicated that the presence of TG led to protein cross-linking and the formation of a network structure, which, therefore, modified the viscoelastic behaviour of the oat dough. Similar results have been reported in other studies (Renzetti, Dal Bello, & Arendt, 2008). However, when the oat dough was treated with 1.5% TG, the same tendency was observed in the storage modulus (G') when compared with the 1.0% sample. The loss modulus (G'') exhibited a higher increase in amplitude than did the storage modulus (G'). Therefore, in oat dough, the tanδ (G''/G') decreased with increasing TG concentration. It obtained the highest viscoelastic dough when the added TG was 1.0% whereas the tanδ increased when the concentration of the TG was increased (data not shown). Fig. 2 shows that the complex modulus $|G^*|$

Table 1
Comparison of the effects of increasing levels of transglutaminase (TG) on thermomechanical properties of oat flour.[A]

TG (%)	Water absorption (%)	Development time (min)	Thermal stability (min)	Torque peak- C3 (Nm)	Set-back value (Nm)	Cooking Stability (Nm)	Slope α	Slope β	Slope γ
0	66.1^{c}	0.65^{a}	4.53^{a}	1.98^{a}	0.38^{a}	0.71^{ab}	−0.043^{a}	0.301^{a}	−0.068^{b}
0.5	65.7^{b}	0.82^{b}	4.68^{ab}	2.03^{c}	0.38^{a}	0.70^{a}	−0.037^{a}	0.341^{a}	−0.072^{ab}
1.0	65.3^{a}	0.80^{b}	4.90^{bc}	2.03^{c}	0.37^{a}	0.72^{b}	−0.034^{a}	0.400^{b}	−0.076^{c}
1.5	65.2^{a}	0.82^{b}	5.03^{c}	2.00^{b}	0.38^{a}	0.74^{c}	−0.049^{a}	0.447^{b}	−0.048^{a}

[A] Different letters in each column shows statistically significant values ($p < 0.05$).

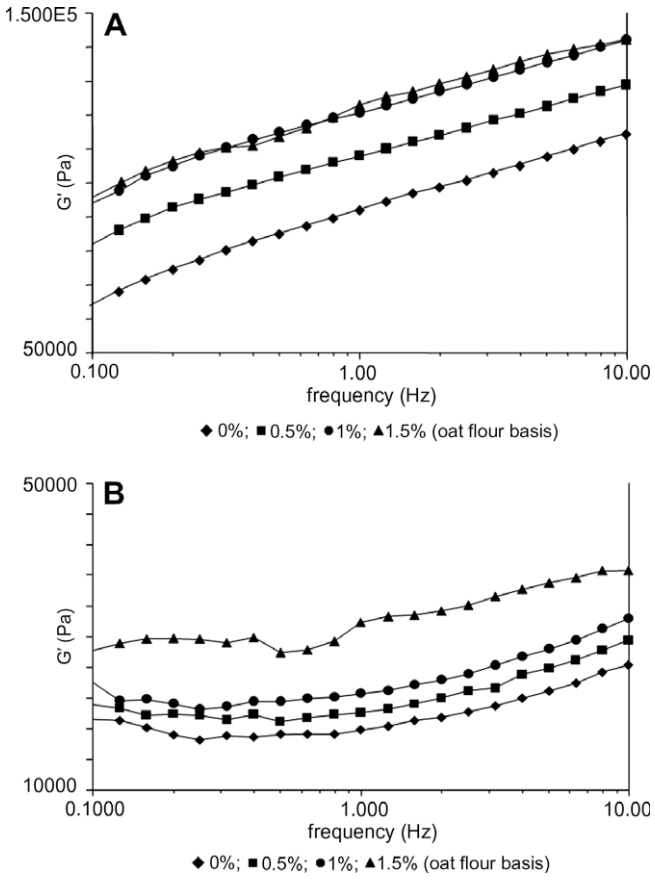

Fig. 2. The effect of increasing levels of Transglutaminase (TG) on the (A) storage (elastic) modulus (G'), and (B) loss (viscous) modulus (G'') of oat dough. Different letters indicate statistically significant values ($p < 0.05$).

$[(G^{*})^2 = (G')^2 + (G'')^2]$ increased with increasing TG concentration, which indicates that TG modified the anti-deformation ability of the oat dough. These results are consistent with several reports including that of Larré et al. (2000), who reported that the presence of TG can strengthen weak gluten and Köksel et al. (2001) who demonstrated the ability of TG to strengthen insect-damaged gluten.

3.3. Effect of TG on the thermal properties of oat dough

It has been reported that TG has an influence on protein denaturation temperature as measured by DSC (Tang, Chen, Li, & Yang, 2006), but no information is available for investigating the effects of TG on the thermal properties of a more complex system such as flour or dough. In the present study, the effect of TG on the thermal properties of oat dough was measured by DSC, and results are presented in Table 2. A single endothermic peak for oat dough was obtained between 60 and 70 °C. It has been reported that, as a result of the thermal properties of wheat flours in the presence of water as determined by the DSC, protein denaturation reactions might occur at temperatures near the gelatinization range of the starch; therefore, when examining flour samples using the DSC method, an endothermic peak due to protein denaturation might be superimposed on the gelatinization peak (Stevens & Elton, 1971).

A slight change in *To* (transition onset temperature) and *Tp* (transition peak temperature) was observed among the studied samples that had been treated with TG when compared with the control sample. This change indicated that the thermal stability of the sample had been enhanced. In addition, enthalpy (ΔH) of the dough samples significantly increased with TG treatment. Flours are heterogeneous materials, so the values for enthalpy reflect a combination of the transition of all components in the flour samples. These results are similar to those of Ahn et al. (2005) who reported that TG's effects on protein denaturation of pure protein samples led to a reduction in enthalpy due to protein unfolding; therefore, in soy flours or wheat–soy blends, the high protein content of soy may be responsible for the lower ΔH values. Similarly, Larré et al. (2000) reported that TG had significant effects on the thermal stability of gluten. This was mainly due to the covalent cross-linkage promoted by the network and enzyme, which made them insensitive to the temperature.

3.4. Effect of TG on the quantification of free amino groups

TG catalyses the reaction between an ε-amino group on protein-bound lysine residues and a γ-carboxyamide group on protein-bound glutamine residues leading to covalent cross-linking of the proteins. Because of the involvement of the amino groups in the

Table 2
Comparison of the effects of increasing levels of transglutaminase (TG) on thermal parameters of oat dough using differential scanning calorimetry.[A]

TG level (%)	Onset Temperature *To* (°C)	Peak Temperature *Tp* (°C)	Conclusion Temperature *Tc* (°C)	Denaturation Temperatures ΔTd (°C)	Enthalpy ΔH (J/G)
0	58.96^{a}	63.73^{a}	69.61^{a}	10.64^{a}	0.56^{a}
0.5	59.89^{a}	65.16^{a}	71.14^{a}	11.55^{a}	0.58^{ab}
1.0	59.29^{a}	64.32^{a}	70.81^{a}	11.53^{a}	0.60^{4b}
1.5	59.07^{a}	64.23^{a}	70.72^{a}	11.66^{a}	0.70^{c}

[A] Different letters show statistically significant values ($p < 0.05$).

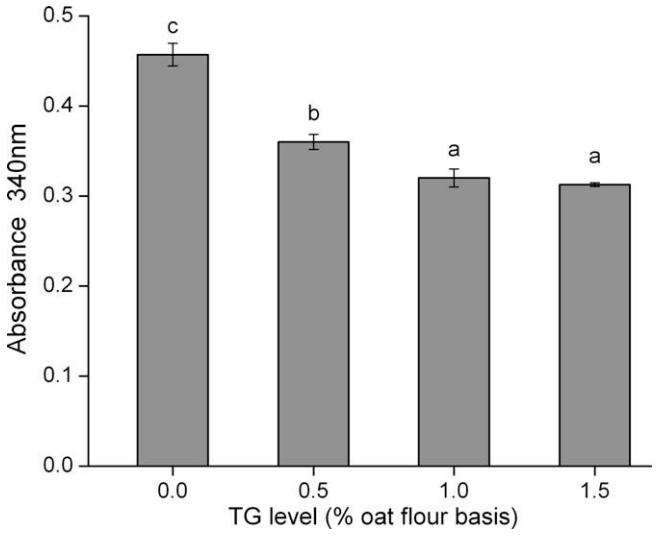

Fig. 3. Measurement of the effect of TG on the number of free amino groups in oat dough. * Different letters indicate statistically significant values ($p < 0.05$).

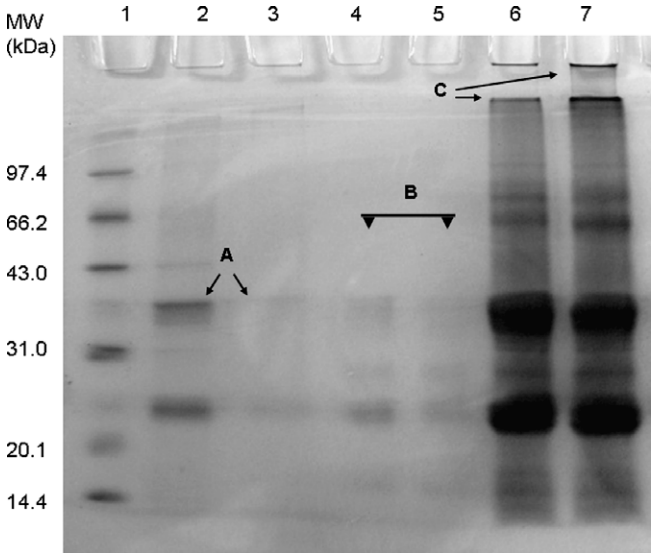

Fig. 4. SDS–PAGE analysis of protein fractions in oat dough prepared without (lanes 2, 4, 6) and with 1.0% TG (lanes 3, 5, 7). Lane 1, molecular weight standard; lanes 2 and 3, albumin and globulin; lanes 4 and 5 oat gliadin; lanes 6 and 7, glutelin.

cross-linking reaction, a decrease in the number of these groups would confirm that TG is catalysing this reaction. A decrease in the number of the free amino groups was reported (Gujral & Rosell, 2004) when cereal proteins were treated with TG. In order to evaluate the extent of the effect of TG, the free amino groups of the proteins from oat flour were quantified. The protein modification that was produced by the action of the TG (leading to the reduction of free amino group content) was measured by changes in the number of free amino groups before and after TG treatment (Fig. 3).

A progressive decrease in the number of free amino groups was observed when TG was added up to 1.0%, beyond which, no significant differences in the number of free amino groups were detected. Gujral and Rosell (2004) also reported similar results, but suggested that this could be due to the disappearance of the lysine groups that had been exposed to the enzyme reaction. The low amount of lysine limited the action of the additional TG. However, oat protein is rich in lysine; yet when the TG was 1.5%, there was no significant decrease in the number of free amino groups. This may have been due to the low protein content of the oat sample, which may have limited the effect of TG.

The results of the effect of TG on the rheological properties of oat dough were similar; when the addition of TG reached a critical level, the effects on the oat dough decreased.

3.5. The effects of TG on oat proteins

Numerous studies have demonstrated TG catalysed cross-linking of various proteins and formation of high molecular weight polymers by covalent bonds. This is observed in SDS–PAGE analysis through a loss of staining intensity or vanishing of protein bands and an increase in molecular weight and amount of proteins that enter the gel (Basman, Köksel, & Ng, 2002b; Renzetti, Behr, Vogel, & Arendt, 2008; Siu, Ma, Mock, & Mine, 2002). Fig. 4 shows the effect of 1.0% TG on the electrophoretic bands of different oat protein fragments. The figure shows that the molecular weights of globulins and albumins were distributed from 20–22 to 32–37 kDa (lanes 2 and 3), corresponding to the acidic and basic polypeptides in oat globulin monomers, respectively (Shotwell, Afonso, Davies, Chesnut, & Larkins, 1988). After treatment with TG, the band intensity of albumins and globulins changed dramatically. Staining of the two protein bands (Part A) was reduced so that they were scarcely detectable. Siu et al. (2002) also showed that TG-mediated polymerisation of oat globulins, as determined by the amount of protein remaining at the top of both the stacking and running SDS–PAGE gels, increased proportional to the increasing incubation time of globulins and TG. However, the results presented here differ. The intensity of gliadin bands decreased, albeit less obviously than the results of Siu et al. (2002) after adding TG (lanes 4 and 5); thus, the decrease mainly resulted from the reduction of oat gliadin, which only account for 10–20% of the total protein. High molecular weight protein polymers were formed with the reduction of low molecular weight protein fragments. After adding TG, the intensity of low molecular weight proteins (Part B) decreased (compare lanes 6 and 7). In contrast, the intensity of the bands on the top of the concentrated gel and the separated gel (Part C) increased due to the catalysis of TG, which polymerised low molecular weight protein fragments into high molecular weight protein polymers, because of their large molecular size, the polymers could not enter the gel.

4. Conclusions

Overall, the results indicate that the addition of TG changes the thermomechanical properties of oat dough. Parameters from the Mixolab analyser showed the effects on protein, which, in the context of oat protein, is the substrate of the TG. The viscoelastic properties of the oat dough containing different levels of TG were studied by dynamic oscillatory test, which found that the addition of TG induced significant effects on the storage modulus (G') and the loss modulus (G''). The test obtained the most viscoelastic dough when the addition of TG was 1.0%. TG had no significant effect on the thermal parameters of the samples studied, but the enthalpy (ΔH) of the dough samples significantly increased with TG treatment due to the TG-enhanced thermal stability of the protein. TG catalyses the cross-link of the oat proteins, avenalin and glutelin. After TG treatment, the number of free amino groups decreased, which confirmed the protein cross-linking catalysed by TG.

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