Rheological and functional properties of composite sweet potato – wheat dough as affected by transglutaminase and ascorbic acid

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Abstract Effect of transglutaminase (TGM) and ascorbic acid (AA) on composite sweet potato – wheat dough functional and rheological properties was studied. Partial substitution of wheat flour with sweet potato flour at the level of 20 % significantly ($P \leq 0.05$) reduced glutenin, gliadin, dough stability, protein weakening, storage modulus (G') and viscous modulus (G''). Mixolab revealed that both TGM and AA treated dough had stability and protein weakening closed to wheat dough (control), with TGM treated dough having the highest values. TGM Introduced new cross-link bonds as shown by the change of amino acid concentration, leading to an increase in storage modulus (G') and viscous modulus (G''), with G' being higher at all levels of TGM concentration. The opposite was observed for composite dough treated with AA as measured by controlled - stress rheometer. TGM treatment increased glutenin and gliadin content. Compared with the control, dough treated with AA exhibited high molecular weight

of polymers than TGM treated dough. The results indicate that the TGM and AA modification of the mixolab and dynamic rheological characteristics (G' and G'') dependent on the changes of GMP, glutenin, gliadin and protein weakening in the composite dough. TGM and AA treatment could improve functional and rheological properties of sweet potato – wheat dough to levels that might be achieved with normal wheat bread. However, it's extremely important to optimize the concentrations of both additives to obtain the optimum response.

Keywords Sweet potato flour · Wheat flour · Transglutaminase · Ascorbic acid · Composite dough

Introduction

Bread making potential of wheat flour is largely related to the gluten proteins. It is a portion of wheat flour constituted by alcohol - soluble gliadins and alcohol - insoluble glutenins (dough forming) with the elasticity characteristics necessary for the structure of most baked products. Both are unique in terms of their amino acid compositions, which are characterized by high contents of glutamine (30–35 %) and proline (10–15 %) and by low contents of amino acids with charged side groups. It has been stated that gluten proteins play an important role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesiveness, viscosity and elasticity on dough (Rasiah et al. 2005). Although the amount of protein can be determined precisely, its quality is extremely complex and very difficult to measure especially when the overall composition of bread formulation has been modified (Seravalli et al. 2011). Indeed, The molecular weight distribution (MW) of glutenins has been long recognized as one of the main determinant of dough properties

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and baking performance. For example, glutenin macropolymers (GMP) are believed to make the largest contribution to dough properties and their amount in wheat flour has significantly correlated with dough strength and loaf volume (Mazzeo et al. 2013; Steffolani et al. 2010). Naturally HMW glutenin subunits are not present in flour and dough as monomers. It is therefore generally assumed that they form interchain disulphide bonds through a reduction process. Partial and total reduction of intermolecular disulphide bonds lowers both MW and elasticity of gliadins/glutenins ratio and the size of glutenin polymers, and yet these have a significant effect on the rheological properties of dough. Steffolani et al. (2010) reported that transglutaminase (TGM: protein-glutamine γ -glutamyl transferase, EC 2.3.2.13) creates new covalent bonds and raise the molecular weight of gluten thereby producing high quality dough with less viscosity and higher dough stability. However, incorporation of sweet potato flour could be highlighted unless wheat protein covalently cross-linked effectively, either intramolecularly or intermolecularly to produce a stable network in composite TGM treated sweet potato – wheat dough to yield dough with improved rheological properties. TGM application has been extensively used to improve wheat dough properties and bread quality (Collar et al. 2005; Hong and Xiong 2012; Joye et al. 2009b; Onyango et al. 2010; Pongjaruvat et al. 2013; Wang et al. 2007). Therefore, this highlights TGM's potential in improving the dough rheological properties and the baking performance of dough with low protein content like sweet potato – wheat dough (Bovell-Benjamin 2007). Furthermore, TGM could mimic a low viscoelastic dough formed when sweet potato flour is kneaded with water leading not only to adequate gases retention produced during proofing, but also the resulting product has acceptable specific volume and texture (Jean Bernard et al. 2014). Various studies have proved sweet potato's potential for utilization in processed products due to it high nutritional benefits, therefore, the incorporation of sweet potato flour for bread making is now gaining momentum (Lu and Gao 2011; Rodriguez-Amaya et al. 2011), despite bad rheological properties and poor breadmaking characteristics previously reported (Wu et al. 2009; Trejo-González et al. 2014). On the other hand, ascorbic acid (AA) has been widely used as an improving agent for the production of various baked products. Patel et al. (2012) postulated that under normal dough mixing conditions, ascorbic acid can exert considerable effects on both dough and bread properties through the mechanism by which ascorbic acid oxidizes reduced glutathione (GSH) to oxidized glutathione (GSSG) in wheat dough (Maforimbo et al. 2007). As previously reported by Maforimbo et al. (2008), the exchange of this bond (S-S) between polypeptide chains provides stability to dough by immobilizing parts of the protein chains, thereby facilitating hydrogen bonding and hydrophobic interactions in low gluten protein content dough. Although the effects of enzymes and

reducing agents in strengthening and improving bread-making qualities in wheat dough have been reported (Caballero et al. 2005; Marco et al. 2008; Huang et al. 2008; Patel et al. 2012), deep understanding of non amylolytic enzyme, TGM and reducing agent, AA in low gluten protein content sweet potato – wheat dough system may give an insight into different ways to improve rheological and functional properties of composite sweet potato – wheat dough. The aim of this study was to investigate the effect of TGM and AA on dough rheological and functional properties in composite sweet potato-wheat dough.

Materials and methods

Material

Sweet potato flour (SPF) with starch, moisture, viscosity, ash, protein content of 78 %, 12.5 %, 200 BU/Min, 8.2 %, and 0.8 % respectively was purchased from Qingdao province, (Mckinnon Natural Products Co.Ltd.,) No information was available on the variety, exact location, harvest batch and transportation, but flour was stored in an air conditioned room during both processing and analysis time. High-gluten wheat flour (WF) was purchased from China Oil and Food Import and Export Corporation Ltd. Instant dry yeast, salt, sugar and butter were purchased from wuxi local market. Enzyme transglutaminase, TGM (Novozymes, China) and Ascorbic Acid or vitamin C (North China, Kang Pharmaceutical co., LTD China).

Proximate analysis of composite sweet potato – wheat flour

The proximate analysis of composite sweet potato – wheat flour was conducted using approved methods in terms of moisture, ash, protein and total starch and were found to be 12.6, 9.8, 12.8 and 64.4 % respectively.

Experimental design

Experiment was designed as follows: control (100 % WF), described as target; T1 (80 % WF + 20 % SPF), described as baseline; T2 (80 % WF + 20 % SPF + 0.5 % TGM); T3 (80 % WF + 20 % SPF + 1 % TGM); T4 (80 % WF + 20 % SPF + 1.5 % TGM); T5 (80 % WF + 20 % SPF + 0.1 % AA); T6 (80 % WF + 20 % SPF + 0.3 % AA) and T7 (80 % WF + 20 % SPF + 0.5 % AA). The addition of TGM was calculated on the basis of the amount of protein content of GMP present in each sample. The recommended level of use for food is generally 1 U of TGM/g of protein. Levels of AA addition were selected based on multiple preliminary experimental run.

Determination of mixing properties of composite sweet potato – wheat dough

The mixing properties of composite sweet potato – wheat dough was determined using Mixolab (Chopin, Tripette and Renaud, Villeneuve-la-Garenne, France). (Abdel-Samie et al. 2010). A certain amount of composite flour was calculated on a 14 %-moisture basis by Mixolab analysis software and placed into the Mixolab analyzer bowl, mixed to obtain a dough approximately of 75 g. Different concentrations of TGM (0.5, 1 and 1.5 %) and AA (0.1, 0.3 and 0.5 %) were added immediately during mixing. The water required for the dough to produce a torque of 1.1 N.m (C1) was automatically added by the Mixolab system. Five parameters (C1, C2, C3, C4, and C5) are obtained from a Mixolab curve. C1 represents the maximum point of the first mixing stage, while points C2–C5 represent the end points of the corresponding mixing stages. The curve is separated into five stages. In the first stage, dough mixing characteristics such as stability, elasticity, and water absorption can be determined at temperature range of 29.7–33.9 °C. In this stage, an increase in the torque is observed until a maximum is reached. Consistency of the dough decreased with mixing at 52.2–57.7 °C dough temperature, which is an indication of protein weakening (stage 2). As the temperature increases, first a decrease and then an increase in consistency is observed and is attributed to starch gelatinization (stage 3) at temperature range of 75.2–86.2 °C. In stage 4, consistency decreases with temperature increase from 83.5 to 88.7 °C, attributed to amylolytic activity. Finally, in stage 5, the decrease in temperature from 88.7 to 60.6 °C caused an increase in the consistency, attributed to starch gel formation. This stage is also related to the retrogradation of starch. Key steps used to compare mixolab, farinograph and RVA are: first one consist of dough mixing during 30 min at 29.7–33.9 °C and gives similar information on dough behavior to that one carried out with farinograph (Codinah and Mironeasa 2013; Moreira et al. 2011). The second one, after a short (8 min) mixing step, involves a heating-cooling cycle (37 min) and gives similar information to that one carried out with RVA (Moreira et al. 2011). The composite doughs from Mixolab were immediately frozen and then freeze-dried, powdered, and stored at –19 °C. To minimize natural oxidative effects, analysis was performed within one week of preparation.

Quantification and isolation of glutenin and gliadin

Freeze dried dough from Mixolab in the form of flour (1 g) was defatted twice with petroleum ether (5 ml) for 30 min. Albumin and globulin were extracted by adding to the air dried defatted flour 0.4 M NaCl in 0.067 M NaH₂PO₄ buffer, pH 7.6 (4 ml). The suspension was shaken for 10 min and centrifuged for 10 min at 400 rpm to prevent dough or gluten

formation in the pellet. Subsequently, the supernatant was centrifuged for 15 min at 15,500×g. The pellets were combined and extracted for a second time using the same procedure as described by Weegels et al. (1995). Glutenin macropolymer (GMP) and glutenin/gliadin ratio was isolated as previously described by Steffolani et al. (2010).

Determination of free and total amino acid of composite sweet potato – wheat dough

Amino acids were determined as previously reported by Eric et al. (2013) and Huang et al. (2011). The amino acids in the samples with and without TGM and AA were analyzed using an Agilent liquid chromatography 1100 (Agilent Technology, Palo Alto, CA, USA) with a UV detector operated at 338 nm. The column used was ODS Hypersil (250 mm × 4.6 mm), while the mobile phase; consisted of 20 mM sodium acetate and 1:2 (v/v) methanol-acetonitrile, delivered at a flow rate of 1 ml/min. The column temperature was 40 °C. An appropriate pretreatment of the sample was done before amino acid analysis. For the determination of free amino acids, an equivalent volume of trichloroacetic acid (TCA) was added to 1 g of freeze dried dough for proteins precipitation. For the total amino acid determination, 0.150 g of sample was hydrolyzed at 110 °C for 24 h with 6 M hydrochloric acid in evacuated sealed tubes. A calibration curve was obtained with a standard amino acid mixture (Sigma Chemical Co., St. Louis, MO, USA) and qualitative analysis was made on the basis of retention time and peak area of standard compounds. Analysis was carried out on the following amino acids as total and free amino acids respectively: aspartic acid, glutamic acid, serine, histidine, glycine, threonine, alanine, tyrosine, cysteine, valine, methionine, phenylalanine, isoleucine, lysine and proline.

Estimation of molecular weight distribution of polymers of composite sweet potato – wheat dough

The molecular weight distribution (MWD) profiles of the samples were estimated by high – performance gel – filtration chromatography according to the method of (Eric et al. 2014). Waters 600 liquid chromatography system (Waters Co., Milford, MA, USA) equipped with a 2487 UV detector and an Empower work station was used for this experiment. The column used was a TSK gel 2000 SWXL 7.8 i.d. × 300 mm (Tosoh Co., Tokyo, Japan), while the mobile phase consisted of acetonitrile/water/trifluoroacetic acid (45/55/0.1, v/v/v) and was delivered at a flow rate of 0.5 ml/min. The column temperature was 30 °C, and 10 µl of sample was injected into the HPLC system. A MW calibration cuve was obtained from the following standards from Sigma cytochrome C (12,500 Da), aprotinin (6500 Da), bacitracin (1450 Da), tetrapeptide GGYR (451 Da), and tripeptide GGG (189 Da). The results were

obtained using a UV detector (220 nm), and the data analysis was performed using a gel permeation chromatography software.

Viscoelastic properties

Viscoelastic properties of composite doughs were measured using a controlled – stress rheometer (Physica MCR 301, Anton Paar, Graz, Austria) as previously described by Murekatete et al. (2014) and Wang et al. (2011). A small sample of composite sweet potato – wheat flour dough was deposited on the inset plate. After setting the module into a measurement position, the sample was pre-sheared at a preselected shear rate of 500/s to avoid any bias due to the structural memory, and subsequently rested for 5 min to allow for a structural rebuilding. The newly formed structure would be governed by the system composition. The sample was then subjected to a small amplitude oscillatory measurement. The magnitude of strain was verified prior to experiments by conducting an amplitude test to ensure that the test was performed in the linear viscoelastic (LVE) domain. First, frequency sweeps oscillatory tests were performed using a frequency ramp from 1 to 100 Hz at a constant strain of 0.5 % to ascertain viscoelastic properties and then performed at variable angular frequency (ω) over the range 0.1–100 rad/s, keeping the amplitude stress at a constant value (3 Pa). Changes in storage modulus (G'), viscous modulus (G''), phase angle (δ) and complex modulus (G^*) were recorded and used in the analysis of oscillatory rheological data. Exponents n' and n'' (both dimensionless) denoted the influence degree of ω on both moduli.

$$G' = G' \omega^{n'} \quad (1)$$

$$G'' = G'' \omega^{n''} \quad (2)$$

Analysis of oscillatory rheological data was performed according to the method described by Gabriele et al. (2001) and Murekatete et al. (2014) with slight modifications. A power model was used to characterize the angular frequency (ω) dependence to G' and G'' using equations (Eqs. 2 and 3). As established by Gabriele et al. (2001). The weak gel model parameter z is the coordination number, which is the number of flow units interacting with each other to give the observed flow response, and the flow regime is characterized by the following equation (Eq. 3)

$$G^* = \left(\left[G'(\omega)^2 + G''(\omega)^2 \right] \right)^{1/2} = A \omega^{1/z} \quad (3)$$

Where A is a constant that can be interpreted as the interaction strength between the flows rheological units; these are basic conformational structures that are connected by small threats and made up of flow units with a certain degree of coordination. The three – dimensional structure characterizing

dough is described in terms of A , which is related to the overall stiffness or resistance to deformation with the LVE region at angular frequency of 1 rad/s, whereas z can be used as a convenient measure for strength of interaction of dough. In turn, $1/z$ was taken as index of viscoelastic nature of the material (a measure of physical cross-links in a protein network).

Statistical analysis

All measurements were performed in duplicate unless otherwise stated. The data was subjected to analysis of variance (ANOVA) and Duncan's test ($p \leq 0.05$) using Minitab statistical software (State College, PA). Pearson correlation coefficient (r) was also carried out for determining relationship between different variables by Minitab statistical software (State College, PA). Results were graphically represented using OriginPro Data analysis and Graphing Software (Northampton, MA, USA).

Results and discussion

Effect of TGM and AA on mixolab characteristics of composite sweet potato – wheat dough

Mixolab properties of composite sweet potato – wheat flour are shown in Table 1. Substituting part of wheat flour with sweet potato flour significantly decreased dough development time, dough stability, C2, C3, C4, and C5 although increased water absorption of the composite dough., revealing that the composite dough was less tolerant to mixing compared with the control. This is similar with farinographic results reported by Trejo-González et al. (2014) Despite report that farinograph water absorption decreased with increasing sweet potato paste in composite dough, it was not the case in the current study (Wu et al. 2009). Treatment with TGM and AA, however, brought significant changes in composite sweet potato – wheat dough mixing behavior. Thus, water absorption of composite dough increased and remained constant with increased concentration of TGM compared with AA. Stability value is an indicator of the dough strength. Therefore, higher values should be obtained to indicate high strength of the dough. Long dough development time is related to high energy input, therefore is not desired in baking industries. TGM-treated composite dough significantly reduce dough development time and, surprisingly increased dough stability time during the first phase of mixing at constant temperature, this is presumably due to the protein cross-link promoted by TGM, resulting in a more stable and resistible to mechanical mixing composite dough due to stable protein network. Inversely, AA-treated composite dough slightly increased dough development time and reduced stability of composite dough. As consistency decrease C2, defined as a measure of protein weakening increased with TGM

Table 1 Effect of transglutaminase (TGM) and ascorbic acid (AA) on mixolab characteristics of composite sweet potato – wheat dough

	WA (%)	DDT (min)	Stability (min)	C2 (N.m)	C3 (N.m)	C4 (N.m)	C5 (N.m)
Control	61.4 ± 0.00c	1.82 ± 0.01a	10.87 ± 0.05a	0.47 ± 0.01a	1.77 ± 0.02a	1.63 ± 0.01a	2.50 ± 0.0a
T1	63.6 ± 0.03a	0.97 ± 0.00e	5.05 ± 0.20f	0.25 ± 0.00f	1.22 ± 0.05d	0.95 ± 0.00e	1.09 ± 0.00e
T2	62.0 ± 0.00b	0.76 ± 0.00f	8.82 ± 0.00c	0.41 ± 0.00bc	1.13 ± 0.00bc	0.96 ± 0.00e	1.11 ± 0.00d
T3	62.0 ± 0.00b	0.71 ± 0.01 g	10.53 ± 0.05b	0.42 ± 0.00b	1.30 ± 0.01 dc	0.91 ± 0.01f	0.97 ± 0.01f
T4	62.0 ± 0.00b	1.05 ± 0.01d	10.66 ± 0.06ab	0.40 ± 0.00c	1.30 ± 0.00cdc	0.83 ± 0.00g	0.87 ± 0.01g
T5	61.0 ± 0.00d	0.76 ± 0.02f	7.72 ± 0.01d	0.41 ± 0.01bc	1.32 ± 0.00bbc	1.03 ± 0.01d	1.21 ± 0.02b
T6	61.23 ± 0.05 cd	1.22 ± 0.01b	6.37 ± 0.01e	0.38 ± 0.02d	1.33 ± 0.00bc	1.06 ± 0.00c	1.22 ± 0.00b
T7	61.0 ± 0.00d	1.15 ± 0.01c	6.37 ± 0.00e	0.35 ± 0.00e	1.35 ± 0.00b	1.11 ± 0.00b	1.13 ± 0.00c

Control (100 % WF), described as target; T1 (80 % WF + 20 % SPF), described as baseline; T2 (80 % WF + 20 % SPF + 0.5 % TGM); T3 (80 % WF + 20 % SPF + 1 % TGM); T4 (80 % WF + 20 % SPF + 1.5 % TGM); T5 (80 % WF + 20 % SPF + 0.1 % AA); T6 (80 % WF + 20 % SPF + 0.3 % AA) and T7 (80 % WF + 20 % SPF + 0.5 % AA). Mean values within a column followed by different letters are significantly different ($P \leq 0.05$). Mean ($n = 2$) ± standard deviation

concentrations compared with AA, indicating that TGM strengthen protein network of composite despite weak composite dough obtained after sweet potato incorporation. TGM treatment improved the rheological and thermal properties of insect damaged wheat flour (Caballero et al. 2005) and rheological properties and baking performance through the formation of continuous protein matrix of rye dough (Beck et al. 2011). Various concentrations of added TGM and AA did not significantly modified starch gelatinization (C3) but their values were higher compared with the controle, implying higher tendency of starch retrogradation (C4) as reported by Marcoa and Rosell (2008). Our findings agreed with those reported by Pongjaruvat et al. (2014) that RVA-setback of jasmine rice breads increased with TGM treatment, since setback of RVA-setback is compared with starch gelatinisation. The effectiveness of TGM depends on the amount of substrate available in the composite dough as Mixolab properties are related to gluten protein in composite dough. Clearly, TGM improved mixola properties as a result of formation of continuous protein network in composite sweet potato wheat dough.

Effect of TGM and AA on protein content of GMP, glutenin and gliadin of composite sweet potato- wheat dough

The addition of different TGM levels necessary to make noticeable changes for network formation largely depend on the protein source (substrate) available to the enzyme. In the current study, the effect of substitution of WF with SPF significantly ($p \leq 0.05$) reduced protein content of GMP in composite dough from 1.6 (control) to 1.42 T1 (20 % SPF). Results are summarised in Fig. 1. GMP significantly ($p \leq 0.05$) reduced with the enzyme concentration, from 1.42 to 0.8 % in T1 and T4 respectively in Fig. 1a. These results are in agreement with Seravalli et al. (2011) and Basman et al. (2004) who reported that TGM catalyze the formation of non - disulfide covalent

crosslinks between peptide-bound glutaminy residues and ϵ -amino groups of lysine residues in protein, thereby decreasing the protein aggregates in the gluten network. Doughs treated with AA showed a significant increase in protein content of GMP compared with the control Fig. 1b. The current study is in agreement with the literature that TGM reduced the protein content of GMP in dough by inducing cross-linking into gluten proteins (Steffolani et al. 2010). Therefore, this highlights the potential of TGM to improve the protein functional properties of composite sweet potato-wheat dough. TGM catalyze a protein cross-linking reaction through an acry - transferase mechanism involving protein-bound glutamunyl residues (acyl donor) and primary amines (acyl receptors), including ϵ -amine group of lysine residues in certain proteins (Wang et al. 2007). Moreover, TGM yield intra and intermolecular ϵ -N-(γ -glutamyl)-lysine crosslinks between proteins reinforcing gluten network, thereby increasing dough strength and tenacity (Joye et al. 2009b). The role of different ingredients during dough formation can be monitored if the chemical and physical changes during the bread making process are clearly understood. Glutenin macropolymers (GMPs) are the largest polymers which makes the largest contribution to dough properties and their amount in wheat flour is strongly correlated with dough strength and bread qualities (Wieser 2007). Therefore, changes that occur when ingredients are used have to be evaluated especially when the normal bread formulation has been modified. In the current study, substitution of wheat flour by sweet potato flour significantly ($p \leq 0.05$) reduced glutenins and gliadins as shown in Fig. 1c, d. That is from 28.35 to 24.41 (control) and from 28.15 to 27.31 T1 respectively, before TGM and ascorbic acid treatments. This was in agreement with results reported by Steffolani et al. (2010). Both glutenins and gliadins significantly ($p \leq 0.05$) increased with the increase of TGM up to 1 % (% flour basis), beyond which, a slight reduction was observed Fig. 1c. This was due to the presence of the TGM which catalyzes the formation of cross-links, thereby reducing protein

Fig. 1 Effect of transglutaminase (TGM) and ascorbic acid (AA) on: **a** protein content of gluten macropolymers (GMP) for dough treated with TGM; **b** protein content of gluten macropolymers (GMP) for dough treated with AA; **c** glutenins and gliadins of dough treated with TGM; **d** glutenins and gliadins of dough treated with AA of composite sweet potato – wheat dough. Control (100 % WF), described as target; T1 (80 % WF + 20 % SPF), described as baseline; T2 (80 % WF + 20 % SPF + 0.5 % TGM); T3 (80 % WF + 20 % SPF + 1 % TGM); T4 (80 % WF + 20 % SPF + 1.5 % TGM); T5 (80 % WF + 20 % SPF + 0.1 % AA); T6 (80 % WF + 20 % SPF + 0.3 % AA) and T7 (80 % WF + 20 % SPF + 0.5 % AA). Bars represent $\pm$ SD for triplicate

aggregates. These results are similar with those reported by Seravalli et al. (2011) who worked on the effects of TGM in wheat proteins during the production of bread. According to their report, the enzyme modified chemical and functional properties of glutenins fraction. Dough treated with ascorbic acid showed high glutenins content but significantly reduced gliadins content when ascorbic acid was added at 0.3 % (T6) to 0.5 % (T7) Fig. 1d. AA works as an oxidizing agent in the presence of oxygen, either from the atmosphere or from other oxidants; if oxygen is limited, the AA acts as a reducing agent because dehydroascorbic acid (DHA) is not formed (Faccio et al. 2012; Maforimbo et al. 2007). It has been suggested that protein disulfide isomerase (PDI) is involved in the AA improver mechanism by oxidizing directly glutenin SH groups (Joye et al. 2009a). In addition, this reaction happens after conversion of endogenous (glutathione) GSH to (oxidized glutathione) GSSG by DHA formed after oxidation of added AA (Koehler 2004), and this may result in reduction of protein content in dough. Ours results are surprising and are different from the AA mechanism; therefore, we recommend further study.

Effect of TGM and AA on changes of amino acids group of protein in composite sweet potato – wheat dough

As a relatively new enzyme in baking, TGM catalyses the reaction between an ϵ - amino group on protein-bound glutamine residues leading to covalent crosslinking of protein (Motoki and Seguro 1998; Renzetti et al. 2008;

Renzetti et al. 2012; Volken de Souza et al. 2011; Zhu and Tramper 2008). During enzymatic catalyzed reaction of polymers, polymers undergo mainly two chemical reactions; being that polymers can be degraded to smaller fractions and amino acids (de-polymerization) or they can crosslink directly with protein residues (polymerization) to form high molecular weight polymers. Changes in free and total amino groups of proteins from dough with and without TGM and AA were done in order to evaluate whether these two additives catalyze this reaction. Substitution of wheat flour with sweet potato flour resulted in significant reduction of both free and total amino groups of protein (Fig. 2a–d). This was attributed to the reduction of protein content of GMP in corresponding dough. As expected, the amount of free amino groups significantly decreased with the addition of TGM, while total amino group increased compared with AA treated dough. The results are in agreement with those found by Marcoa and Rosell (2008) who evaluated the effect of TGM on functionality of rice flour protein. According to these authors, free amino group of protein in samples significantly decreased with the addition of TGM. The extent of TGM catalyzed reaction was also confirmed by Huang et al. (2010) who observed a progressive decrease in the amount of free amino groups. According to Gujral and Rosell (2004), this could be because of involvement of amino acids groups of protein in the crosslinking reaction, and this change from free to total amino groups in terms of their concentrations, clearly showed that TGM catalyses this reaction.

Fig. 2 Effect of TGM and ascorbic acid (AA) on change in amino acid content of composite dough: **a** free amino groups ($\mu\text{g}/\text{mg}$) for dough treated with TGM; **b** total amino groups ($\mu\text{g}/\text{mg}$) for dough treated with TGM; **c** free amino group for dough treated with AA; **d** total amino groups ($\mu\text{g}/\text{mg}$) for dough treated with AA. control (100 % WF), described as target; T1 (80 % WF + 20 % SPF), described as baseline; T2 (80 % WF + 20 % SPF + 0.5 % TGM); T3 (80 % WF + 20 % SPF + 1 % TGM); T4 (80 % WF + 20 % SPF + 1.5 % TGM); T5 (80 % WF + 20 % SPF + 0.1 % AA); T6 (80 % WF + 20 % SPF + 0.3 % AA) and T7 (80 % WF + 20 % SPF + 0.5 % AA)

Effect of TGM and AA on molecular weight distribution of polymers of composite sweet potato – wheat dough

The molecular weight distribution (MWD) of polymers in composite dough was estimated by high – performance gel – filtration chromatography. Results are presented in Table 2. The MW distribution of polymers could be changed by several factors such as thermal treatment, maillard reaction as well as enzymatic hydrolysis. In the case of gluten protein, all these factors alter mainly elasticity behavior by changing the molecular weight of glutenin or its solubility. The MW distribution of polymers in the control sample were higher

than those obtained after wheat flour substitution (T1) and ranged from 13KDa (99.78 %) to below 1 KDa (1.06 %), whereas the MW distribution of polymers in the sample with 20 % SPF (T1) before TGM and AA treatment ranged from 2 to 3 KDa (20.20 %) to below 1 KDa (0.09 %). Larroque et al. (2004) observed that the X-glutenin subunits have a slower electrophoretic mobility in SDS-PAGE and had higher MW than the Y-type glutenin subunits. Thus, the drastic decreases in MW distribution were due to the fact that, part of gluten protein were reduced by substitution of wheat flour, and most of them were, presumably, derived from high molecular weight (HMW) glutenin subunits as clearly shown by the

Table 2 Effect of transglutaminase (TGM) and ascorbic acid (AA) on change of molecular weight distribution (MWD) in kilo - Dalton (KDa) of polymers in composite sweet potato-wheat dough

	12–13 KDa (%)	11–12 KDa (%)	7–8 KDa (%)	5–6 KDa (%)	4–5 KDa (%)	3–4 KDa (%)	2–3 KDa (%)	1–2 KDa (%)	<1 KDa (%)
Control	99.78 ± 0.00a	93.54 ± 0.00a		41.18 ± 0.01a		30.07 ± 0.00a	18.32 ± 0.00b	57.03 ± 0.00c	1.06 ± 0.00a
T1						20.20 ± 0.00c	20.10 ± 0.00a	79.78 ± 0.00a	0.09 ± 0.00g
T2					27.29 ± 0.00b	22.32 ± 0.00b		46.57 ± 0.00e	0.35 ± 0.01b
T3				30.28 ± 0.01c	44.51 ± 0.00a			55.47 ± 0.01d	0.34 ± 0.00c
T4			22.26 ± 0.00a	32.08 ± 0.00b				57.36 ± 0.01c	0.11 ± 0.00ef
T5	97.26 ± 0.00c		1.91 ± 0.00c		1.72 ± 0.00d		0.43 ± 0.00d	0.39 ± 0.00 g	0.12 ± 0.00e
T6		92.61 ± 0.01b	5.05 ± 0.03b		4.85 ± 0.00c		1.07 ± 0.00c	1.05 ± 0.00f	0.32 ± 0.01d
T7	97.53 ± 0.00b		1.70 ± 0.02d		1.62 ± 0.00e		0.41 ± 0.01e	0.34 ± 0.00 h	0.10 ± 0.00f

Control (100 % WF), described as target; T1 (80 % WF + 20 % SPF), described as baseline; T2 (80 % WF + 20 % SPF + 0.5 % TGM); T3 (80 % WF + 20 % SPF + 1 % TGM); T4 (80 % WF + 20 % SPF + 1.5 % TGM); T5 (80 % WF + 20 % SPF + 0.1 % AA); T6 (80 % WF + 20 % SPF + 0.3 % AA) and T7 (80 % WF + 20 % SPF + 0.5 % AA). Mean values within a column followed by different letters are significantly different ($P \leq 0.05$). Mean ($n = 2$) ± standard deviation

reduction of protein content of GMP Fig. 1. Interestingly, in spite of minor variations in MW distribution of polymers of samples treated with 0.5 % (T2), 1 % (T3) and 1.5 % (T4) TGM, major variations were observed in MW distribution of polymers for dough treated with 0.1 % (T5), 0.3 % (T6), and 0.5 % (T7) AA. That is, 4–5 KDa (27.27 %), 5–6 KDa (30.28 %), 7–8 KDa (22.26 %) and 12–13 KDa (97.26 % and 97.53 %), 11–12 KDa (92.26 %) respectively. This is assumed to be due to the polymerization of glutenin polymers into the GMP induced by the action of TGM. Polymers with low MW distribution drastically decreased with an increase in both TGM and AA concentration. This was attributed to the cross-linking of small polymers which resulted in formation of larger polymers of HMW in all samples treated with TGM and AA. Joye et al. (2009b) and Zoccatelli et al. (2004) observed that endogenous glutathione (GSH) during mixing preferentially reacts with the intermolecular SS bonds responsible for the covalent linkages between low molecular weight glutenin subunits (LMW-GS). Several authors (Skerritt et al. 1999; Joye et al. 2009a, b; Stephen and Roger 2000) reported that, during mixing, a gluten network is formed by depolymerization and (re-) polymerization process, eventually resulting in a buildup rather than a breakdown of the polymeric structure.

Effect of TGM and ascorbic acid on dynamic rheological properties of composite sweet potato – wheat dough

The effects of TGM and AA on composite sweet potato – wheat dough rheological properties are recorded in Table 3. As shown, substitution of wheat flour by sweet potato flour significantly affected dough visco-elasticity behavior, both G' (storage modulus) and G'' (viscous modulus) were higher in the control (wheat flour dough) than T1 (20 % SPF), this indicated that the dough became less elastic and more rigid.

From technological point of view, this was expected since the increase in G' indicates rigidity of the sample associated with the formation of elastic material structure and G'' indicates the extension of the viscous element in the sample. TGM and AA treatment changed composite dough visco-elastic behavior, though the effect was different from one additive to another. Thus, when TGM was used, G' and G'' increased with increasing levels of TGM, and G' was always higher than G'' in all frequency ranges studied, while the results were opposite when AA was used. These results revealed that the increase in both molecular weight distribution and a decrease of protein aggregates of GMP of protein matrix as a result of TGM treatment led to an increase in both elasticity and viscous modulus. In accordance with the results discussed above, the comparison of G' and G'' showed that TGM treated dough had high G' than AA treated dough, and this is usually attributed to protein crosslinking and the formation of a network structure, which, consequently, changed the viscoelasticity properties of composite dough. This was expected considering that TGM treated dough had higher n' and n'' than AA treated dough, ranging from 0.184 to 0.174 and 0.183 to 0.173 and from 0.203 to 0.183 and 0.185 to 0.187 for G' and G'' respectively. The current study showed that for all TGM and AA treated dough, the resulting values of n' and n'' were below 0.21, meaning that dough became more elastic since the increase values of n indicate an increase fraction of uncross linked material (Gabriele et al. 2001). Several studies reported that in wheat based system where gluten protein and starch are the main dough forming components, enzyme such as TGM (Collar et al. 2005; Renzetti et al. 2008; Renzetti et al. 2012; Seravalli et al. 2011) and additive like AA (Every et al. 2008; Faccio et al. 2012; Maforimbo et al. 2006; Maforimbo et al. 2007) facilitate the interaction between lipids, proteins and starch (Sciarini et al. 2012) likely as a result of their ability

Table 3 Effect of transglutaminase (TGM) and ascorbic acid (AA) on storage modulus (G'); viscous modulus (G'') of composite sweet potato – wheat dough

Sample	G'			G''		
	($G'O \pm SD$)(Pa.s ^{n'})	n'	R^2	($G''O \pm SD$)(Pa.s ^{n'})	n''	R^2
Control	7598.8 $\pm$ 44.75d	0.184 $\pm$ 0.01a	0.996 $\pm$ 0.00a	3218.3 $\pm$ 17.1c	0.202 $\pm$ 0.00a	0.962 $\pm$ 0.00a
T1	6496.3 $\pm$ 35.33e	0.184 $\pm$ 0.08a	0.994 $\pm$ 0.00a	3144.1 $\pm$ 54.3c	0.203 $\pm$ 0.00a	0.96 $\pm$ 0.00a
T2	7652.8 $\pm$ 25.2d	0.186 $\pm$ 0.00a	0.995 $\pm$ 0.00a	3751.6 $\pm$ 43.2b	0.202 $\pm$ 0.00a	0.956 $\pm$ 0.00b
T3	9039.7 $\pm$ 12.8b	0.174 $\pm$ 0.03b	0.995 $\pm$ 0.00a	4441.7 $\pm$ 33.5a	0.193 $\pm$ 0.00b	0.951 $\pm$ 0.00c
T4	9499.6 $\pm$ 39.4a	0.173 $\pm$ 0.01b	0.995 $\pm$ 0.00a	4459.9 $\pm$ 18.7a	0.186 $\pm$ 0.00b	0.95 $\pm$ 0.00c
T5	8847.7 $\pm$ 18.1b	0.183 $\pm$ 0.02a	0.996 $\pm$ 0.00a	4629.9 $\pm$ 32.8a	0.185 $\pm$ 0.00b	0.957 $\pm$ 0.00b
T6	8167.4 $\pm$ 32.4c	0.171 $\pm$ 0.02b	0.996 $\pm$ 0.00a	3892 $\pm$ 20.3bb	0.186 $\pm$ 0.00b	0.959 $\pm$ 0.00a
T7	7803.3 $\pm$ 24.5 cd	0.173 $\pm$ 0.09b	0.996 $\pm$ 0.00a	3864.5 $\pm$ 25.8b	0.187 $\pm$ 0.00b	0.959 $\pm$ 0.00a

Control (100 % WF), described as target; T1 (80 % WF + 20 % SPF), described as baseline; T2 (80 % WF + 20 % SPF + 0.5 % TGM); T3 (80 % WF + 20 % SPF + 1 % TGM); T4 (80 % WF + 20 % SPF + 1.5 % TGM); T5 (80 % WF + 20 % SPF + 0.1 % AA); T6 (80 % WF + 20 % SPF + 0.3 % AA) and T7 (80 % WF + 20 % SPF + 0.5 % AA). Mean values within a column followed by different letters are significantly different ($P \leq 0.05$). Mean ($n = 3$) $\pm$ standard deviation

Table 4 Power law parameters of Eq. (3) from frequency sweep tests, for composite sweet potato – wheat dough

Sample	G*		
	(A ± SD)(Pa.s ^{1/z})	Z (intercepts)	R ²
Control	8914.4 ± 34.00	5.22 ± 0.03b	0.987 ± 0.00b
T1	7219.5 ± 6.36c	5.31 ± 0.23b	0.988 ± 0.00b
T2	8828.3 ± 8.42b	5.26 ± 0.00b	0.990 ± 0.00ab
T3	9661.7 ± 5.12ab	5.58 ± 0.08ab	0.99 ± 0.00b
T4	10,501.0 ± 12.33a	5.67 ± 0.04a	0.99 ± 0.00ab
T5	10,082.4 ± 15.41a	5.42 ± 0.00ab	0.993 ± 0.00a
T6	9052.25 ± 11.81b	5.73 ± 0.06a	0.991 ± 0.00ab
T7	9029.2 ± 21.16b	5.66 ± 0.26a	0.991 ± 0.00ab

Control (100 % WF), described as target; T1 (80 % WF + 20 % SPF), described as baseline; T2 (80 % WF + 20 % SPF + 0.5 % TGM); T3 (80 % WF + 20 % SPF + 1 % TGM); T4 (80 % WF + 20 % SPF + 1.5 % TGM); T5 (80 % WF + 20 % SPF + 0.1 % AA); T6 (80 % WF + 20 % SPF + 0.3 % AA) and T7 (80 % WF + 20 % SPF + 0.5 % AA). Mean values within a column followed by different letters are significantly different ($P \leq 0.05$) Mean ($n = 3$) ± standard deviation

TGM Transglutaminase, AA ascorbic acid

to crosslink with gluten strand. The same mechanism could have taken place in sweet potato – wheat composite dough, where gluten protein and starch are the major dough forming components. Table 4 shows the computation using Eq. (3) of the parameters A and z, which can describe the material

functions of the food system in the LVE regime (Murekatete et al. 2014). It is clear that the strength of the network, A value was higher in the control (wheat flour) than in T1 (20 % SPF). However, A values increased and decreased as a function of dosages in TGM and AA treated dough respectively. The power law exponent, 1/z (Eq. 3), which is inversely proportional to z can be used as a convenient measure of viscoelasticity of dough, therefore should be near zero for material exhibiting an ideal elastic behavior and increase with the increasing degree of viscoelasticity. Our study revealed that increasing TGM and AA doses in composite dough increased the dough viscoelasticity compared with the control.

Correlation coefficients of dough functional properties and rheological characteristics of composite dough

Multiple data handling of analytical dough variables provided beneficial information on the significantly correlated dough functionalities and rheological properties of formulated composite sweet potato – wheat dough Table 5. Pearson correlation coefficient showed that for GMP, TGM and AA concentrations had mostly strong to very strongly significant linear relationship with gliadin, G', G'', dough stability and protein weakening ability. For glutenin; TGM concentration had mostly very strong significant positive linear relationship with gliadin, G', G'', dough stability and protein weakening ability

Table 5 Pearson correlation coefficients between gluten functional properties; rheological properties and breadmaking

	GMP	Glutenin	Gliadin	G'	G''	Stability	C2	C3	C4
Composite sweet potato – wheat dough treated with TGM									
GMP	1								
Glutenin	−0.89	1							
Gliadin	−0.98	0.84	1						
G'	−0.99	0.92	0.96	1					
G''	−0.98	0.95	0.96	0.99	1				
ST	−0.93	0.99	0.87	0.95	0.97	1			
C2	−0.75	0.96	0.68	0.80	0.84	0.94	1		
C3	−0.73	0.61	0.82	0.67	0.64	0.45	0.14	1	
C4	0.83	−0.55	−0.80	−0.82	−0.74	−0.64	−0.36	−0.77	1
Composite sweet potato – wheat dough treated with AA									
GMP	1								
Glutenin	0.75	1							
Gliadin	−0.83	−0.26	1						
G'	−0.99	−0.69	0.87	1					
G''	−0.97	−0.88	0.68	0.95	1				
ST	−0.98	−0.90	0.65	0.93	0.99	1			
C2	−0.96	−0.56	0.94	0.98	0.88	0.86	1		
C3	0.90	0.39	−0.99	−0.93	−0.77	−0.75	−0.98	1	
C4	0.92	−0.43	−0.98	−0.95	−0.80	−0.78	−0.99	0.99	1

Storage (G') and viscous (G'') moduli; gluten macropolymers (GMP). * $p \leq 0.05$, ** $p \leq 0.005$

and moderate positive linear relationship with starch gelatinization in composite dough. Inversely, for glutenin; AA concentration had mostly moderate negative linear relationship with gliadin, G' , G'' , dough stability and protein weakening ability, some of these correlations appear to be rather low although they are statistically significant. The differences in linear relationship for glutenin shows the dependence of viscoelastic (G' and G'') of composite dough promoted by TGM than AA. For dough stability and protein weakening ability, both TGM and AA concentration had strong significant linear relationship with composite dough viscoelastic properties. Additional experiments in our lab showed strong negative linear relationship between crumb hardness and bread specific volume of breads resulting from composite sweet potato – wheat dough ($r = 0.70$, $P \leq 0.05$), the softer the crumb, the larger the specific volume. This investigation revealed that both added TGM and AA significantly improved functional properties and rheological characteristics of composite sweet potato wheat dough; however, caution should be paid when normal dough formulation have been modified.

Conclusion

To sum up, substitution of wheat flour with sweet potato flour significantly affected the rheological properties of the dough with lower elasticity and dough stability as a result of reduction of protein content of GMP, glutenin and gliadin. The little protein content of GMP left after substitution was modified through cross – linking and extent of modification was evaluated by change in amino acid groups of protein. Clearly, the new cross – link introduced by TGM treatment progressively increased elastic (G') and viscous (G'') moduli with increase in TGM concentration. Composite dough treated with AA seems to have antagonistic effect on rheological properties and can therefore control the strengthening effect of TGM. However, composite dough without TGM and AA had poor mixing behavior, especially poor dough stability, poor dough development time and lower protein weakening when compared with composite sweet potato – wheat dough treated with TGM and AA. At lower concentration, AA showed a promising effect on dough stability and increased protein weakening. Both additives modified protein functionality by shifting molecular weight distribution of glutenin content of GMP. Therefore, we are strongly recommending the use of AA and TGM in production of sweet potato – wheat bread.

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