Effect of Transglutaminase on the Quality of Dried Alkaline Noodle from Rice Flour

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***Abstract*—The aim of the present study was to improve quality of dried alkaline noodle made from rice flour by utilization of transglutaminase at difference concentrations (0, 1, 2, 3 and 4 g/ 100 g flour). The transglutaminase activated protein crosslinking according to SDS-PAGE protein patterns and the decrease amount of free amino groups. The increase amount of transglutaminase increased cooking yield, hardness, and tensile strength, while decreased cooking loss and adhesiveness of alkaline rice noodle. According to the optimum of cooking properties, textural properties and sensory characteristics, the 3 and 4 % of transglutaminase was recommended for quality improvement of alkaline noodle from rice flour.**

***Index Terms*—transglutaminase, alkaline noodle, rice flour**

I. Introduction

Celiac disease is an autoimmune disorder in which the body mistakenly reacts to gluten. The gluten stimulates an immune response that is not normal. This damages the inside of small intestine so that it can’t absorb nutrients from food. Gluten is a type of protein, which found in the grains wheat [1]. Gluten consists of gliadin and glutenin, which is mainly responsible for the quality of food product from wheat, including elasticity and chewability of noodle [2].

Rice flour is raw material instead of wheat flour in many type of gluten-free product owing to its benefits of high digestibility and hypoallergenic properties [3]. Rice flour is reported as raw material for gluten-free food products such as bread [4], cake [5], snack [6], including noodle. For instance, the use of rice and legumes blend in gluten-free pasta decreased cooking loss, hardness, while increased firmness, adhesiveness [7]. Rice noodle is gluten-free noodle cause quality problems. Utilization of rice flour instead of wheat flour reduced the quality of noodle. Thus, the addition of gluten replacement ingredient to improve gluten-free noodle structure such as pregelatinized flour (e.g. pregelatinized corn starch [8], pregelatinized rice flour [1], hydrocolloids (e.g. xanthan gum [9], guar gum [2], locust been gum [1], protein and transglutaminase [10].

Transglutaminase (EC 2.3.2.13) is enzyme that catalyse the formation of an isopeptide bone between ε-amino groups of lysine residues side chains and γ-carboxyamide groups of glutamine residues, leading to the covalent crosslinking of the protein [11], [12]. Transglutaminase is a covalent cross-linking enzyme which confirmed by SDS-PAGE and free amino groups determination. Pea proteins [13] peanut protein [14] the mixer soy protein isolate and durum wheat protein [15] were found protein cross-linking by the action of transglutaminase determined by SDS-PAGE and free amino groups reduction. Due to protein-crosslink catalyze ability, transglutaminase has been used to improve the textural and cooking properties of gluten-free noodles. The quality improvement of gluten-free rice noodle with rice protein isolate and transglutaminase was reported. The rice protein isolate supplementation with cross-linking reaction catalysed by transglutaminase induced the changes in dough properties, quality of rice noodle, cooking quality, and microstructure of rice noodle [10]. The combination of transglutaminase, whey protein concentrate and sodium caseinate led to decreased cooking loss of gluten free pearl millet-based noodle [16]. However, there is a lack of information regarding the effect of transglutaminase on alkaline noodle properties, especially gluten-free noodle from rice flour. The objectives of the present study were investigated the effect of transglutaminase on protein crosslinking, microstructure, cooking properties, texture and sensory characteristics of alkaline noodle from rice flour.

II. Materia and Methods

A. *Materials and Chemical*

Rice flour (Double Bear Brand), salt (Prung Thip Brand) were purchased from local market (Chonburi, Thailand). Pregelatinized rice flour was a by-product from extruded rice pasta production donated by Family Tree Food Co., Ltd. (Rayong, Thailand). The food grade soy protein isolate was purchased from Solae, LLC (USA). Guar gum was purchased from Asia Pacific Specialty Chemicals Ltd. (Switzerland). Transglutaminase was purchased from Taixing Yiming Fine Chemicals Co., Ltd. (China). Sodium carbonate and potassium carbonate was purchased from Ajax Finechem Pty. Ltd. (New Zealand). All chemicals used were analytical grade.

B. *Alkaline Noodle from Rice Flour Preparation*

Alkaline noodle from rice flour formulated with different levels of transglutaminase (0, 1, 2, 3 and 4 g/ 100 g flour) was prepared. The basic recipe of noodle calculated based on flour weight including 70% rice flour, 30% pregelatinized rice flour, 10% soy protein isolate,

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1.5% salt, 3% guar gum, 0.9% sodium carbonate, 0.1% potassium carbonate and 85% water were mixed for 10 min using mixer (Kitchen Aid Professional 5 Plus, USA) and rested at ambient temperature for 1 hours. The dough was passed through the pasta machine (Atlas Regina Marcarto Manual Pasta Maker, Italy) to made 2 mm diameter noodle strands. Fresh noodles were dried at 55 °C by using tray dryer (EK Food Tech Ltd., Thailand) to 12% moisture content. Dried noodle was kept in polyethylene plastic bags.

C. SDS-PAGE Protein Electrophoresis

The SDS-PAGE protein patterns of dough, fresh noodle and dried noodle samples were investigated modified according to the method of [10] and [11]. The samples (0.05 g) were dissolved in 1 mL of extraction buffer solution. The buffer containing 0.5 mL 0.5 M tris-(hydroxymethyl) aminomethane (Tris-HCl, pH 6.8), 0.9 mL 10% sodium dodecyl sulphate, 1 mL glycerol, and 2.5 mL 1% bromophenol blue was added under non-reducing conditions. For reducing condition, the buffer solution also contained 0.1 mL β-mercaptoethanol. Suspensions were vortexed for 2.5 hours, heated in boiling-water bath for 5 min and cooled at room temperature. Then, they were centrifuged at 3,000 ×g for 5 min, and the proteins in the supernatants extracted under non-reducing and reducing conditions were loaded onto the well of gel slab. SDS-PAGE analysis was performed in 7.5% separating gel with 4% stacking gel. APC-001 AccuProtein Chroma (16-250 kDa) was used as molecular weight marker (Enzmart biotech Co.,LTD, Thailand).

D. Quantification of Free Amino Groups

Free amino groups were quantified in the dough, fresh noodle and dried noodle samples in order to confirm the formation of TGase-catalysed covalent bonds. This method is based on the reaction between primary amino groups and o-phthaldialdehyde (OPA) [12]. According to the method of [13], [17], the samples (0.2 g) were suspended in 2 mL 0.1 M HCl (pH 0.1), vortex, and centrifuged at 5,000 ×g for 20 min. The 2.5 mL OPA reagent was added to 0.1 mL supernatant. The mixture was allowed to react for 2 min and the absorbance was determined at 340 nm.

E. Microstructures of Noodle

The microstructures of dried noodle were examined by using scanning electron microscope (SEM) (Leo 1450 VP, UK). The dried noodle samples were fractured and followed by mounting on a specimen stubs. The mounted noodle samples were coated with gold by using sputter coater (Polaron Range, SC7620, England). The surfaces of noodles were observed with SEM at 20x, 75x and 350x magnification. The cross-sections of noodles were observed with SEM at 35x, 150x and 350x magnification.

F. Cooking Properties of Alkaline Rice Noodle

Cooking qualities were determined according to AACC standard methods [18]. The 5 g of dried rice noodle of was boiled in 200 ml distilled water. Optimum cooking time of rice noodle was determined at 30-sec intervals. The noodle stand was squeezed between two pieces of clear glasses. When center core disappeared, cooking time was record. The cooking water was collected in a tared beaker and dried in a hot air oven at 105 °C to a constant weight. Cooking loss (%) and cooking yield (%) of noodle sample were calculated as follows;

Cooking loss (%) = [(Weight of cooking water after drying)/ Weight of uncooked noodle] x100

Cooking yield (%) = (Weight of cooked noodle / Weight of uncooked noodle) x100

G. Textural Properties of Alkaline Rice Noodle

Tests of hardness, adhesiveness and tensile strength were performed using TA-XT2 Texture Analyzer following the method of Stable Micro Systems. Two strands of cooked noodle samples (approximately 60 mm length) were placed parallel on the middle of the compression plate, and two continuous compressions under cylindrical probe (diameter 35 mm). The measurements were performed at 2mm/s pre-test speed, test speed, and post-test speed, 70 % strain height. From the force/time curve of the texture profile, textural parameters including hardness and adhesiveness were obtained. For tensile measurement, Spaghetti/Noodle Tensile Rig, 3 mm/s pre-test speed, test speed, and 5 mm/s post-test speed, 50 nm distances were used. The maximum force referred to the resistance to breakdown of the noodle was named as tensile strength.

H. Sensory Analysis of Alkaline Rice Noodle

The alkaline noodle from rice flour were cooked to optimum cooking time and served to 30 untrained panelists. The panelists evaluated noodle samples for color, flavor, texture, taste and overall acceptability on 9-points hedonic scale, where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely).

I. Statistical Analysis

All experimental results were reported as means ± SD (standard deviation). The results were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's HSD post hoc test to determine the significance between treatment ($p < 0.05$). Minitab17 software (Minitab Pty. Ltd., Australia) was used to perform the statistical analysis of the data.

III. RESUZLTS AND DISSCUSSION

A. Protein Electrophoretic Patterns by SDS-PAGE

In order to characterize the possible protein crosslinking formed the protein patterns of dough, fresh noodle and dried noodle from rice flour were investigated. Under non-reducing conditions, the protein bands with molecular weight of 16-21, 51, 57, 69-79, 79-127 and

127-175 kDa were presented, while the protein bands with molecular weight of 16-21, 27-41, 51-57, 57-69, 69-79 and 79 kDa in reducing conditions were appeared (Fig 1). As reported in [12], the molecular weight of soybean protein isolate at 51.4, 76.2 and 85 kDa corresponding to the β-conglycinin, at 22.5 and 37.4 kDa corresponding to the glycinin, and the molecular weight of rice protein at 15.1, 22.3 and 32.7 kDa. From our result the protein bands with molecular weight of 27-41, >79 kDa that appeared in reducing conditions was not observed under non-reducing conditions. This evident showed that protein in noodle sample presented the existence of disulfide bonds. Due to disulfide bonds were reduced by a reducing agent, inducing the new lower molecular weight subunit of protein to be clearly observed. The soy protein bands with molecular weight of 32 and 36 (glycinin) [19], and rice protein bands with molecular weight of 51 [11] and 60 [20] are reported that they are existed in disulfide linkages. According to [11], they reported that protein electrophoretic patterns of rice dough adding 5% soy protein isolate in reducing condition showed the decreasing of high molecular protein of 53 kDa compared with non-reducing conditions due to the breaking of disulfide bond existing in this protein fraction.

However, the protein patterns of dough, fresh noodle and dried noodle in the same extraction conditions were not different. This evident implied that noodle making process such as noodle extrusion and drying at 55 oC did not affect to protein structure change in alkaline rice noodle. [21] reported that the protein molecular weight of wheat protein in kernel and fresh noodle were not different, but the protein molecular weight of cooked noodle was increased greatly after cooking (95 ℃) due to heat treatment is damage to the intramolecular disulfide bonds.

Moreover, it was observed that the increase amount of transglutaminase decreased in the intensity of 69-79 and 79-127 kDa bands (non-reducing conditions), 69-79 and 79 kDa (reducing conditions) might be due to polymerized low molecular weight protein fragments into high molecular weight polymers, because of their large molecular size, the polymers could not enter the polyacrylamide gel. According, [22] reported that the transglutaminase treatment of soy protein isolate occurred the higher molecular weight band at 180 and 310 kDa. [11] reported that the 1 % transglutaminase treatment caused a decreased in the protein molecular weight of 47 and 74 kDa bands and increased the protein molecular weight on the top of separating gel of rice protein. These indicated that transglutaminase induced cross-linking of proteins and led to significant polymerization of rice protein.

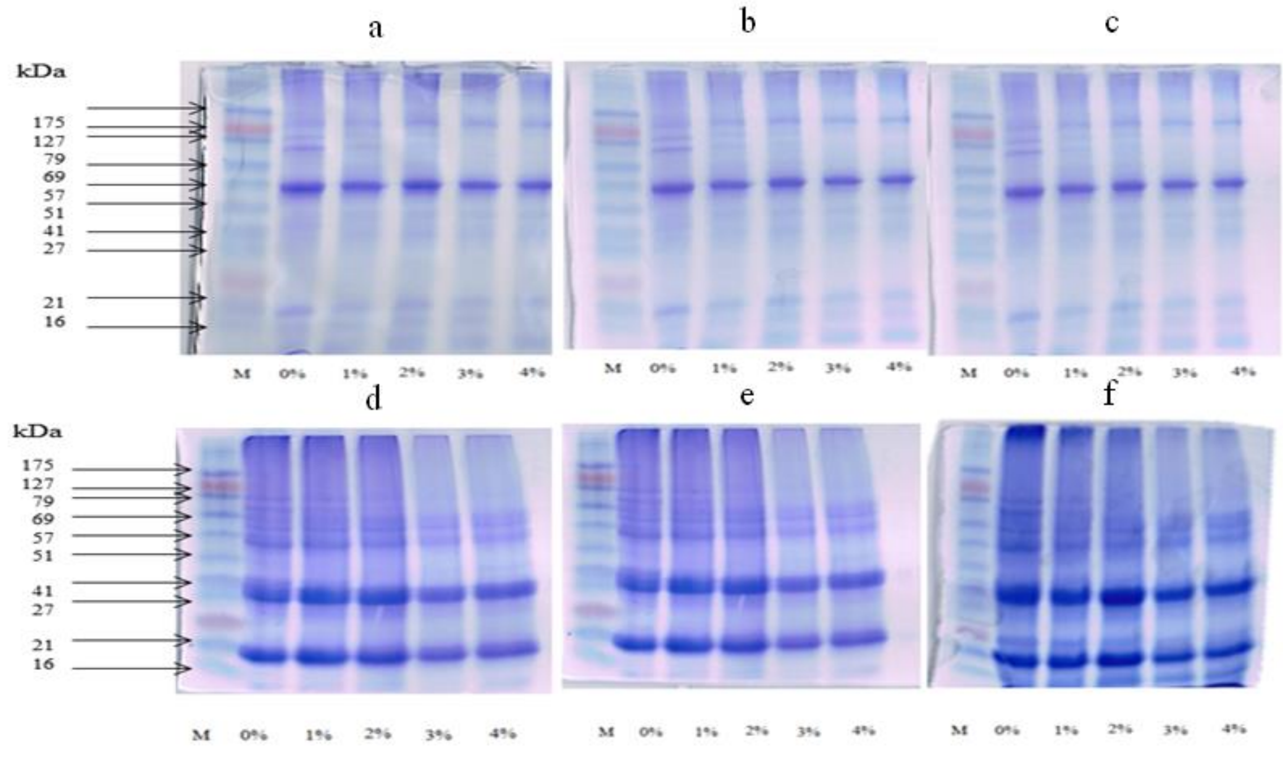


Figure 1. Protein electrophoretic patterns of (a) dough, (b) fresh noodle, (c) dried noodle in the non-reducing conditions and (d) dough, (e) fresh noodle, (f) dried noodle in the reducing conditions from rice flour added with different concentrations of transglutaminase (0, 1, 2, 3 and 4 g/ 100 g flour).

B. Quantification of Free Amino Groups

Effect of transglutaminase of dough, fresh noodle and dried noodle on the quantification of free amino groups was presented in Table I. The result showed that the amount of free amino groups of dough, fresh noodle and dried noodle were not different ($p \geq 0.05$). It can be understood that noodle extrusion and drying at low temperature did not affect to the amount of free amino group in alkaline rice noodle. The traditional drying process from wheat flour at low temperature (< 60 ℃) caused a less organized protein network produced [23].

The increase amount of transglutaminase decreased the content of free amino groups of dough, fresh noodle and dried noodle from rice flour. In a previous study, [17] also reported that the oat dough exhibited a decreased quantification of free amino groups after transglutaminase treatment. Transglutaminase catalyzed covalent cross-linking of protein between an ε-amino group on protein-bound lysine residues and a γ-carboxyamide group on protein-bound glutamine residues. [12] also reported about a decreased of free amino acid group by addition of 1% transglutaminase in rice flour and soy protein blend. The increasing transglutaminase resulted in a decrease in the content of free amino groups in oat dough and also affected oat dough rheology in noodle [24].

TABLE I. Free Amino Groups of Dough, Fresh Noodle and Dried Noodle with Different Concentrations of Transglutaminase (0, 1, 2, 3 and 4 g/ 100 g Flour)

Transglutaminase (g/ 100 g flour)	Free amino groups (µg Ser/mg sample)		
	Dough	Fresh noodle	Dried noodle
0	$0.39\pm0.03^{a/A}$	$0.41\pm0.03^{a/A}$	$0.41\pm0.03^{a/A}$
1	$0.33\pm0.02^{b/A}$	$0.33\pm0.02^{b/A}$	$0.32\pm0.02^{b/A}$
2	$0.28\pm0.01^{bc/A}$	$0.28\pm0.01^{bc/A}$	$0.27\pm0.01^{bc/A}$
3	$0.26\pm0.01^{c/A}$	$0.26\pm0.01^{c/A}$	$0.27\pm0.01^{c/A}$
4	$0.23\pm0.02^{c/A}$	$0.25\pm0.02^{c/A}$	$0.24\pm0.00^{c/A}$

a,b,.. Different superscript letters at each column indicate significantly different ($p < 0.05$); A = Not significantly different at each row ($p\geq0.05$).

TABLE II. Cooking Properties and Textural Properties of Alkaline Noodle from Rice Flour with Different Concentrations of Transglutaminase (0, 1, 2, 3 and 4 g/ 100 g Flour).

Transglutaminase (g/ 100 g flour)	Cooking time ns (min)	Cooking loss (%)	Cooking yield (%)	Hardness (kg.f)	Adhesiveness (g.f-s)	Tensile strength (g.f)
0	6.50 ± 0.50	17.97 ± 1.41^{a}	515.00 ± 94.30^{c}	3.08 ± 0.46^{b}	31.34 ± 1.99^{a}	10.50 ± 0.90^{b}
1	6.50 ± 0.50	15.98 ± 1.35^{ab}	611.70 ± 27.50^{bc}	3.37 ± 0.54^{b}	26.63 ± 0.98^{b}	11.12 ± 0.16^{ab}
2	6.50 ± 0.50	13.66 ± 1.06^{b}	714.40 ± 74.10^{ab}	3.66 ± 0.88^{b}	19.16 ± 1.20^{c}	11.57 ± 0.65^{ab}
3	6.30 ± 0.29	10.77 ± 1.08^{c}	745.40 ± 62.80^{ab}	4.72 ± 0.76^{ab}	18.44 ± 0.99^{c}	12.31 ± 1.05^{ab}
4	6.30 ± 0.29	9.06 ± 0.29^{c}	803.60 ± 44.60^{a}	5.70 ± 0.42^{a}	18.68 ± 0.50^{c}	13.03 ± 0.76^{a}

a,b,.. Different superscript letters at each column indicate significantly different ($p < 0.05$); ns = Not significantly different ($p\geq0.05$).

TABLE III. Sensory Characteristics of Alkaline Noodle from Rice Flour with Different Concentrations of Transglutaminase (0, 1, 2, 3 and 4 g/ 100 g Flour).

Transglutaminase (g/ 100 g flour)	Color ns	Flavor ns	Texture	Taste ns	Overall acceptability
0	6.23 ± 1.38	6.13 ± 1.55	6.03 ± 1.07^{b}	6.40 ± 1.38	6.03 ± 0.85^{c}
1	6.43 ± 1.17	6.27 ± 1.34	7.00 ± 1.02^{a}	6.43 ± 1.19	6.83 ± 0.87^{b}
2	6.67 ± 0.96	6.50 ± 1.06	7.00 ± 1.02^{a}	6.63 ± 1.06	7.20 ± 0.66^{ab}
3	6.83 ± 0.91	6.60 ± 1.00	7.00 ± 1.02^{a}	6.77 ± 0.89	7.37 ± 0.56^{a}
4	7.00 ± 1.02	6.73 ± 1.01	7.40 ± 0.67^{a}	6.83 ± 0.91	7.47 ± 0.57^{a}

a,b,.. Different superscript letters at each column indicate significantly different ($p < 0.05$); ns = Not significantly different ($p\geq0.05$).

C. Morphological Properties of Alkaline Rice Noodle

The appearance of dried alkaline noodle from rice flour with different concentrations of transglutaminase is shown Fig. 2. The microstructure of surface and cross-section of uncooked alkaline noodle from rice flour is illustrated in Fig. 3 and Fig. 4. Alkaline noodle sample with transglutaminase addition showed smooth surface and homogenous structure due to catalyzing the formation of non-disulphide covalent cross-linking between glutamine residues and lysine residues of peptides [10]. The addition of cross-linking enzyme transglutaminase increased thickness of the protein layer covering the starch granules in yellow alkaline noodles and contributed to the slick surface of noodles [25]. The 10 % rice protein isolate and 1 % transglutaminase treatment increased the continuity of the protein coating on the rice noodle surface and smoothed hollows [10].

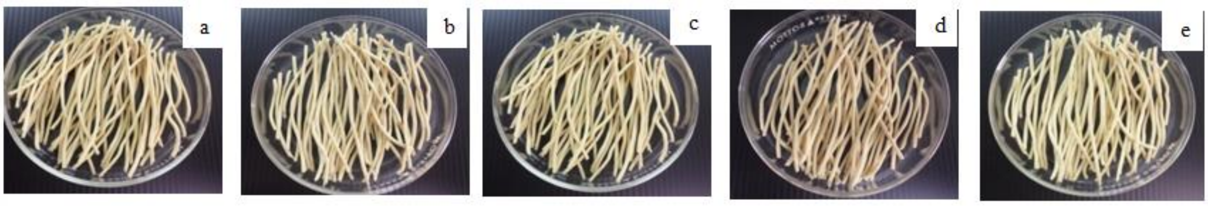


Figure 2. Dried alkaline noodle from rice flour with different concentrations of transglutaminase (a) 0, (b) 1, (c) 2, (d) 3 and (e) 4 g/ 100 g flour, respectively.

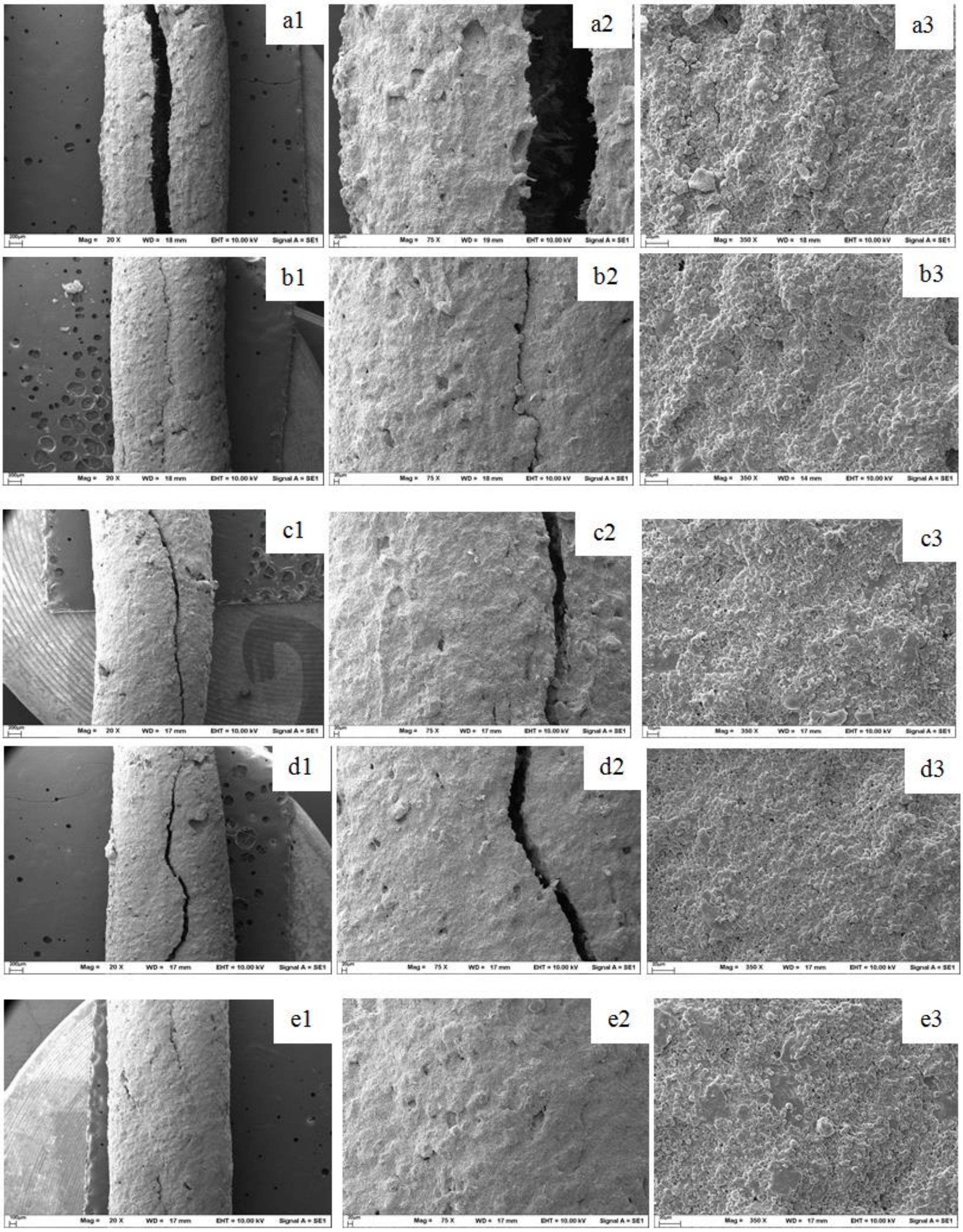
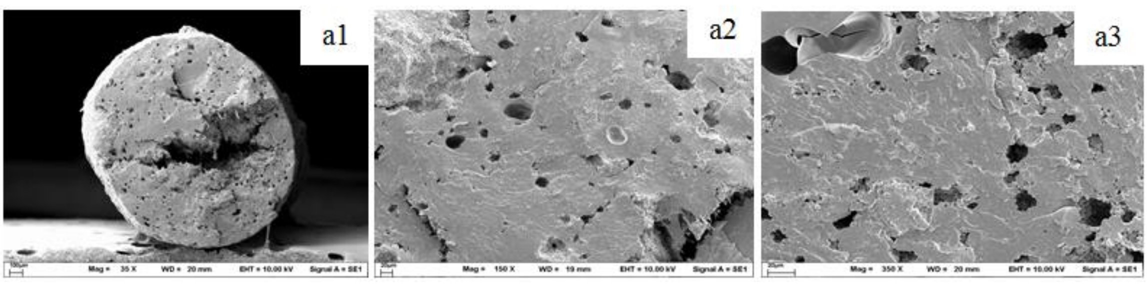


Figure 3. Surface of dried alkaline noodle from rice flour with different concentrations of transglutaminase (a) 0% (b) 1% (c) 2% (d) 3% and (e) 4%, at (1) 20x, (2) 75x and (3) 350 x magnification.



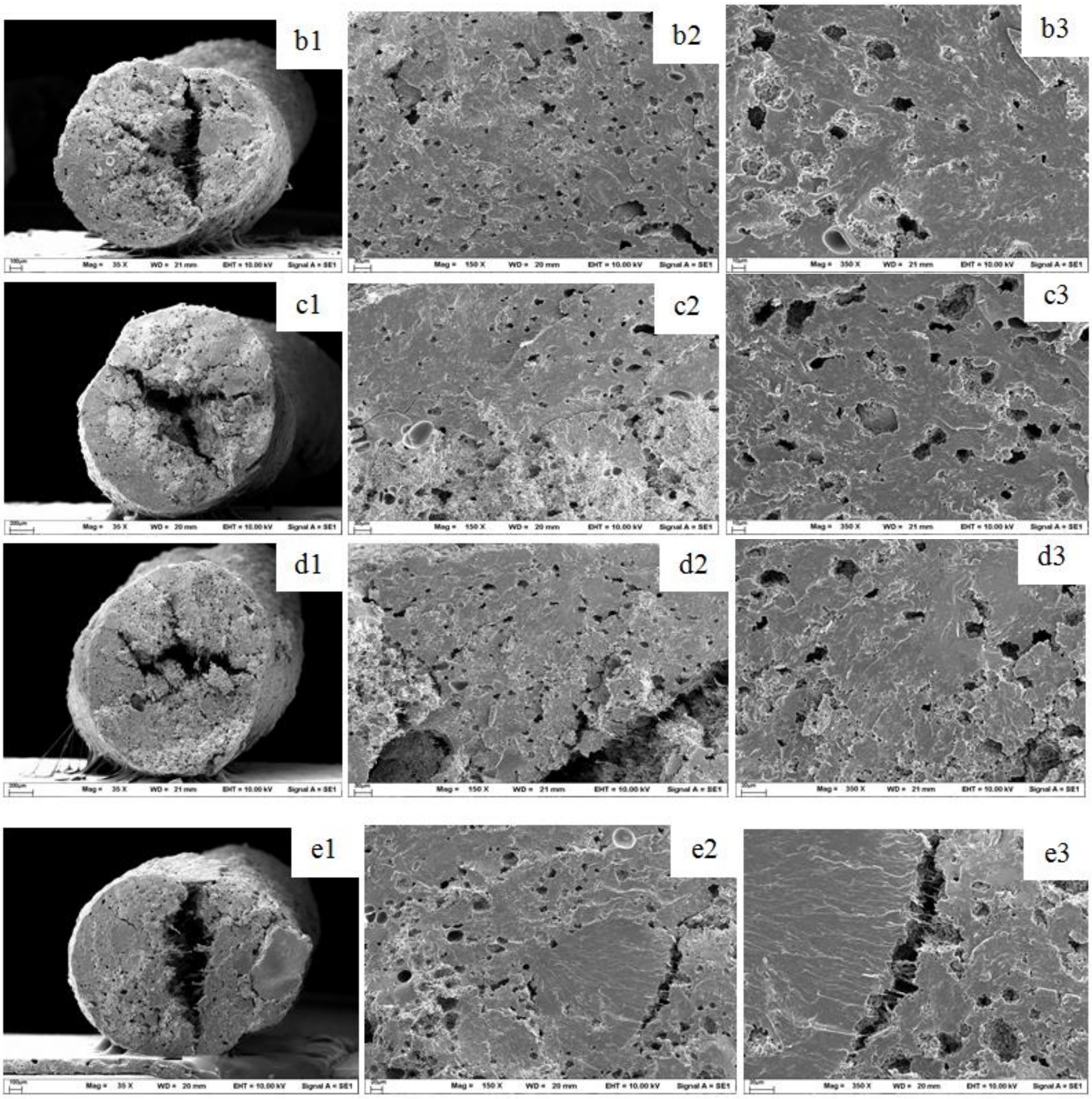


Figure 4. Cross-section of dried alkaline noodle from rice flour with different concentrations of transglutaminase (a) 0% (b) 1% (c) 2% (d) 3% and (e) 4%, at (1) 35x, (2) 150x and (3) 350x magnification.

D. Cooking Properties of Alkaline Rice Noodle

The cooking properties of alkaline noodle from rice flour prepared at different concentrations of transglutaminase level are shown in Table II. The optimum cooking time of all rice noodle samples were not significantly different at 6.30-6.50 min ($p \geq 0.05$). Its might be due to all rice noodles sample was not different of size, shape, and thickness. The increase amount of transglutaminase decreased cooking loss while increased cooking yield. The wheat-based pasta with transglutaminase decreased cooking loss compared to untreated pasta [26]. The decreased cooking loss of rice flour noodle supplemented with rice protein and transglutaminase due to the cross-linking in rice noodle structure with transglutaminase catalysing reaction led to more expensive protein network being capable of holding starch components [10].

E. Textural Properties of Alkaline Rice Noodle

Effect of transglutaminase on the textural properties of cooked alkaline noodle from rice flour was determined using a Texture Analyzer. The result showed that hardness, adhesiveness and tensile strength values were affected by the addition of transglutaminase (Table III). The increase amount of transglutaminase increased hardness and tensile strength while decreased adhesiveness. It might be due to the cross-linking of protein of cooked alkaline noodle from transglutaminase treatment caused strengthen noodle strand. The gluten-free faba bean pasta increased hardness after transglutaminase (20 nkat/g flour dm) addition [27]. The 0.05 % transglutaminase treated whole wheat noodle increased the hardness value [28].

F. Sensory Analysis of Alkaline Rice Noodle

The sensory evaluation of cooked alkaline noodle from rice flour samples is presented in Table III. The results showed that increase amount of transglutaminase was not affected to the liking score of color, flavor and taste of cooked rice noodle ($p \geq 0.05$), while texture and overall acceptability were increased ($p < 0.05$). The addition of 3 and 4 % of transglutaminase exhibited the highest scores of texture and overall acceptability.

IV. Conclusions

According to the result, the increase of cooking yield, hardness, and tensile strength and the decrease of cooking loss and adhesiveness of alkaline rice noodle were affected by transglutaminase treatment. The addition of 3 and 4 % transglutaminase could be used to improve the quality of alkaline noodle from rice flour.

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