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ชื่อโครงการ ผลของการเชื่อมข้ามของโปรตีนข้าวโดยเอนไซม์ทรานส์กลูตามิเนสต่อคุณสมบัติของเจลข้าว

Effect of Rice Protein Crosslinking by Transglutaminase on Rice Flour Gel Properties

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บทคัดย่อ

คุณภาพของเจลแป้งข้าวถูกปรับปรุงได้โดยการเชื่อมข้ามของโปรตีนข้าวโดยเอนไซม์ทรานส์กลูตามิเนส (TGase) ร่วมกับการใช้โปรตีนสกัดจากถั่ว (Pea protein isolate; PPI) เพื่อเพิ่มปริมาณไลซีน เจลแป้งข้าวเตรียมจากพันธุ์ข้าว 3 พันธุ์คือ ชัยนาท 1 (แอมิโลสสูง) หอมดอกมะลิ 105 (แอมิโลสต่ำ) และ กข 6 (ข้าวเหนียว) ทำการเชื่อมข้ามโดยการเติมเอนไซม์ TGase 1% และ 2% (น้ำหนัก/น้ำหนัก ของส่วนผสมแป้งทั้งหมด) และ โปรตีนสกัดจากถั่ว 5% (น้ำหนัก/น้ำหนัก ของส่วนผสมแป้งทั้งหมด) ผลการวิเคราะห์ค่าความหนืดและค่าเซ็ตแบคแสดงว่าเอนไซม์ TGase มีผลต่อคุณภาพของเจลข้าวมากกว่า PPI แต่ PPI ให้ผลเสริมกับเอนไซม์ TGase โดยเฉพาะอย่างยิ่งในเจลแป้งข้าวแอมิโลสสูง เนื่องจากเจลแป้งข้าวแอมิโลสสูงจะอัดตัวกันแน่นกว่าจึงเกิดการเชื่อมข้ามได้ง่ายกว่า การใช้เอนไซม์ TGase ร่วมกับ PPI ส่งผลต่อสมบัติทางเนื้อสัมผัสโดยเฉพาะค่าความแข็งที่สูงขึ้น โปรตีนข้าวที่สกัดโดยวิธีของออสบอร์นถูกนำมาบ่มร่วมกับเอนไซม์ TGase พบว่าการเกิดโพลีเมอไรเซชันของโปรตีนข้าวเกิดขึ้นมากในส่วนของกลอบูลินและอัลบูมิน แสดงว่าโปรตีนทั้งสองส่วนนี้เป็นส่วนที่เกิดปฏิกิริยาได้ง่ายต่อเอนไซม์ TGase. การวิเคราะห์ SDS-PGAE ของโปรตีนที่ถูกโพลีเมอไรซ์ดังกล่าว แสดงถึงการเชื่อมข้ามด้วยพันธะไดซัลไฟด์และที่ไม่ใช่พันธะไดซัลไฟด์ โดยสังเกตจาก ε-(γ-glutamyl) ไลซีน ที่เกิดขึ้นทั้งในส่วนของกลอบูลินและอัลบูมิน การเปลี่ยนแปลงสมบัติทางเนื้อสัมผัสของเจลแป้งข้าวเป็นผลร่วมระหว่างการเชื่อมข้ามด้วยพันธะไดซัลไฟด์ และที่ไม่ใช่ พันธะไดซัลไฟด์ตลอดจนการเสริมไลซีนจากโปรตีนถั่ว เอนไซม์ทรานส์กลูตามิเนสและไลซีนจากโปรตีนถั่วสามารถพัฒนาสมบัติทางเนื้อสัมผัสและกระแสวิทยาของเจลแป้งข้าว

คำสำคัญ: การเชื่อมข้าม เอนไซม์ทรานส์กลูตามิเนส โปรตีนข้าว โปรตีนถั่วสกัด กระแสวิทยา

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ABSTRACT

Rice flour gel quality was expected to be improved by protein crosslinking using transglutaminase (TGase) altogether with pea protein isolate (PPI) for lysine enrichment. Rice flour gels of three cultivars; Chai nat 1 (high amylose), KDML 105 (low amylose) and RD6 (waxy rice), were prepared with 1% and 2% TGase (w/w composited flour) and/or 5% PPI (w/w composited flour). Analyses of viscosity and setback showed that TGase exerted more effect on gel forming properties than PPI did. PPI showed a synergistic effect with TGase especially in high amylose rice flour gel. Gels of high amylose flour with TGase and PPI were highly packed which ease enzymatic crosslinging. TGase treatment in the presence of PPI altered textural property of rice flour gel especially hardness. The rice proteins extracted by the Osborne method was incubated with TGase. The polymerization of rice proteins occurred only in globulin and albumin fractions, suggesting that the globulin and albumin are accessible and susceptible to TGase. SDS-PGAE analysis of the polymerized proteins showed that disulfide crosslinking together with non-disulfide crosslinking such as ε-(γ-glutamyl) lysine were formed between rice globulin and albumin. Thus, the texture properties of rice flour gel with TGase and PPI might have been altered through the formation of disulfide and non-disulfide crosslinking among rice globulin, albumin and lysine-rich PPI. TGase and PPI could improve certain textural and rheological properties of rice flour gel.

Key words: Crosslinking; Transglutaminase; Rice protein; Pea protein isolate; Rheology

INTRODUCTION

Rice flour has bland taste, colourless, hypoallergenic, and easy digestible carbohydrate (Marco *et al,* 2007; Marco and Rosell, 2008a). Rice is applied in many kinds of products such as rice noodles, cakes, puddings, infant formulas and breakfast cereal. The products sold in the market are usually in the form of gel or gel sheets (Lin *et al,* 2009). Although rice flour is widely used, the utilization of rice flour still limited, because of its poor viscoelastic property. Modification of rice flour can be performed by physical and chemical method. Recently, the crosslinking of cereal protein in order to improve its properties had been increasing interested. Protein in rice, even has a small amount (7%), played an important role in structural, functional and nutritional property quality of food product.

Protein crosslinking relates to the formation of covalent bonds between polypeptide chains. It could be occured both within (intramolecular crosslinks) or between protein (intermolecular crosslinks). Protein crosslinking could be performed by physical, chemical, and enzymatic means. The production of protein crosslinking promotes changes in the structure of protein, resulting in changes of functional and nutritional properties of the final products (Gerrard, 2002; Gujral and Rosell, 2004).

Use of enzyme to induce protein crosslinking of protein was interested in our study because it was perceived as natural and non-toxic food components. Enzyme, which is widely used, is Transglutaminase (TGase) (EC 2.3.2.13) a GRAS enzyme. It has been used in various countries to improve quality of foods including flours and their products (Gerrard *et al,* 2001, Gujral and Rosell, 2004). TGase catalyses the acyl-transfer reaction between γ-carboxylamide groups on protein-bound glutamine amino residues and ε-amino groups on protein-bound lysine residues. This lead to a covalent crosslinking by the formation of ε-(γ-glutamyl)lysine bond (G-L bond) (Gerrard *et al.*, 2001; Gerrard, 2002; Joye *et al.*, 2009, Motoki and Seguro, 1998; Rastall, 2007). The action of TGase also led indirectly to a conversion of soluble proteins to insoluble high-molecular-weight protein polymers through formation of disulfide covalent crosslinking in wheat protein (Gujral and Rosell, 2004). The addition of protein from legume, such as pea protein, to rice flour is not only increase the nutritional value of product but also increase the possibility for crosslinking by TGase, since pea protein has a high content of lysine (Nunes *et al*, 2006), which is substrate for this enzyme.

This work tried to modify properties of rice flour gel from various rice cultivars by using TGase to stimulate the crosslinking of rice proteins. The addition of pea protein isolate was also investigated. The protein fractions which related to rice protein crosslinking by TGase were studied. And changes of physicochemical properties of rice flour gel were determined.

OBJECTIVES

1. To modify properties of rice flour gel from various rice cultivars using TGase to stimulate the crosslinking of rice protein

2. To investigate effect of lysine enrichment to promote protein crosslinking by the addition of pea protein isolate

3. To monitor protein fractions and related bonding of rice protein crosslinking caused by TGase

4. To determine changes of physicochemical properties of rice flour gel

LITERATURE REVIEW

1. Rice flour

Rice flour is widely used in the manufacture of food products such as rice noodles, rice cakes, rice puddings, infant formulas, puffed grains and breakfast cereals. Rice products in the market are usually in the form of gel sheets (Lin *et al.*, 2009). Rice flour is usually produced from the broken kernels during the milling process because of its lower cost. Rice flour can be classified due to amylose content as waxy (0-5%), very low (5-12%), low (12-20%), intermediate (20-25%), and high (25-33%) amylose according to Juliano (1992).

Flour from rice (Oryza sativa) has unique nutrition, hypoallergenic, colourless and bland taste. It has low levels of sodium and is an easily digestible carbohydrate (Marco *et al.*, 2007). Compared with other cereals, rice has higher lysine content and its glutelin has a more balanced amino acid profile than wheat prolamin, which is deficient in lysine and tryptophan (Gujral and Rosell, 2004). Since rice possesses these unique properties, its use in baby foods, and foods for gluten intolerant patients has been increased (Gujral and Rosell, 2004). Recently, rice flour has been found to be one of the most suitable cereal flours for preparing foods for celiac patients accounted for its low levels of prolamins (2.5-3.5%), Peptides released from the breakdown of the prolamins act as toxins for those suffering from celiac disease. As a result, cereals containing high prolamins (wheat, rye, barley and oats) cannot be consumed by the celiac patient and the only preventive measure is to keep the diet as gluten free as possible (Gujral and Rosell, 2004).

However, low levels of prolamins in rice flour consequently caused a poor viscoelastic rice flour dough. Rice proteins lack the ability to form necessary network for holding the gas produced during fermentation and baking (Gujral and Rosell, 2004). As a consequence, the application of rice flour is limited.

1.1 Composition of rice flour

In general, rice flours have the same chemical composition as parent-milled kernels. Carbohydrate and protein are the two major component in rice. The characteristics of rice flours are governed by inherent cultivar's variations, environmental variation, the grinding methods, and their previous treatments (Rosell and Marco, 2008).

1.1.1 Carbohydrates

Carbohydrates are the most component in rice with approximately 80% of starch content. Rice starch is a glucose polymer composed of amylose and amylopectin in different proportions depending on its variety. Starch content in rice grain increases from its surface to core, and thus milled rice is rich in starch. Rice starch is considered not allergenic due to its hypoallergenic proteins. Physical and functional properties of rice starch are greatly related to the amylose/amylopectin ratio of starch (Rosell and Marco, 2008).

1.1.2 Rice protein

The protein content of rice, approximately 7%, is relatively low compared with that of other cereal grains. However, it plays a significant role in determining the functional properties of starch, which makes up approximately 80% of the rice kernel. Rice protein has significant influence on the structural, functional, and nutritional properties of rice. It is a major factor in determining texture, pasting properties, and sensory characteristics of rice. Recently, rice protein has been perceived as uniquely nutritious and hypoallergenic, which makes rice increasingly popular for use in foods (Shih, 2004).

Protein content in rice is calculated as N x 5.95, lower than the factor employed for other cereals but higher than that for wheat. Some literature stated that the usual coefficient of 5.95 was an overestimate and a factor of 5.5 to 5.6 seems more realistic. The amino acid composition is relatively well-balanced. In comparison with other cereals, the amino acid composition of the total protein of the rice grain is generally characterized by a higher lysine content (about 3.5% of the total protein) and lower glutamic acid (glutamine) content (<20%). Lysine is still the first limiting amino acid, followed by threonine. As with other cereal grains, the protein contents of the embryo and the aleurone layer are higher (up to 20 to 25%) than that of the endosperm. Nevertheless, the greatest part of total protein is located in the endosperm, and the main characteristics of the protein are determined by the properties of the endosperm storage proteins. Milled rice contains lower quantities of protein because during the milling some of the protein-rich aleurone cells are removed. Protein content in milled rice is varying from 5.6 to 13.3% (Lásztity, 1996).

A. Classification of rice proteins

Osborne's classification of proteins has been widely used in rice protein chemistry. The protein solubility fractions vary within a wide range depends on the rice variety and the maturity of the grain. The uncertainties of the extraction conditions may also play a role. The distribution of the different solubility fractions in the rice grain is uneven. Also, the distribution of the protein fractions is different in the milling fractions (Lásztity, 1996). Based on Osborne's classification, rice proteins have been classified into four types; albumins, globulin, glutelin, and prolamin, according to their solubility.

1) Albumins

Albumins are proteins soluble in water. Their solubility is not affected by reasonable salt concentrations. In addition, these proteins are coagulated by heat. Rice albumins have a wide range of molecular weight from 10 to 200 kDa (Lásztity, 1996) with major component of 18-20 kDa (Likitwattanasade, 2009). This protein fraction is concentrated in the embryo and aleurone layer. The proportion of albumin is highest in the outer layers of the milled rice and decreases toward the center of the grain. Albumin has the highest lysine content, followed by glutelin, then globulin and prolamin. Globulin is richest in the sulfur amino acids cysteine and methionine, while prolamin is the poorest (Shih, 2004).

2) Globulins

Globulins are proteins insoluble in pure water but soluble in dilute salt solutions and insoluble at high salt concentrations. This proteins show the classic salting in and salting out. (Lásztity, 1996) Rice globulins composes of α-, β-, γ-, and δ- globulins with apparent molecular weights of 25.5, 15, 200 kDa and higher, respectively. Same as rice albumins, this protein fraction is concentrated in the embryo and aleurone layer, and has highest proportion in the outer layers of the milled rice with decreasing toward the center of the grain (Shih, 2004).

3) Glutelins

Glutelins are storage proteins, soluble in dilute acids or bases. Generally, glutelin is largely soluble in acidic (pH below 3.0) or alkali solutions (pH above 10.0). It composes of two major polypeptide subunits classified as α, or acidic, and β, or basic subunits with apparent molecular weights of 30-39 and 19-25 kDa respectively. These two groups of polypeptides are believed to cross-link by disulfide bonding, resulting in glutelin molecules with molecular weight ranging from 64-500 kDa (Shih, 2004). Glutelins is the major fraction in rice, being about 80% of the total protein. Rice glutelins has specific name as oryzenin.

4) Prolamins

Prolamins are the low molecular weight storage proteins which soluble in 70% (v/v) ethyl ethalnol or 50% (v/v) propanol (Lásztity, 1996). Rice prolamin is lack in lysine and sulfur-containing amino acid. It consists of three polypeptide subunits with apparent molecular weights of 10, 13 and 16 kDa (Shih, 2004). The prolamin fraction in rice is quite low (~3-5%) and occur in the highest amount in endosperm (Lásztity, 1996).

B. Forms of rice proteins

In rice storage proteins, up to 95% of endosperm rice protein was observed in the form of discrete particles called protein bodies (PBs). Most of them are concentrated in the peripheral-lateral and peripheral-dorsal cells (Shih, 2004). Three types of PBs have been identified (crystalline, small spherical, and large spherical) (Hamaker, 1994). At least two types of PBs exist in the rice endosperm, PB-I and PB-II. PB-I exhibits a spherical shape while PB-II displays an irregular crystalline morphology. PB-I contains mostly prolamins and glutelins are outstanding component in PB-II (Shih, 2004). Another form of rice endosperm protein is protein matrix. Contrast with PBs, little or no matrix protein has been found in rice endosperm. This discriminates rice from other cereals which a large number of proteins as intergranular matrix are observed. However, connecting protein fibrils between PBs may perhaps could form a network in the rice endosperm and would be difficult to detect even with an electron microscope. A starch granule-associated protein is another form of rice protein which has been identified in kernel. This protein is a starch synthetase and correlates with amylase content (Hamaker, 1994).

2. Protein crosslinking

In recent years, the crosslinking reaction of proteins has made its way into food applications. (Whitehurst and Law, 2002) Modification of protein crosslinking during food processing could alternate functional properties of food product and often without damaging its nutritional quality. Protein crosslinking refers to the formation of covalent bonds between polypeptide chains within a protein (intramolecular crosslinking) or between proteins (intermolecular crosslinking) (Gerrard, 2002). Crosslinking can be introduced to a food matrix by physical, chemical, enzymatic means (Rastall, 2007). Food processing conditions including high temperatures, extremes in pH, particularly alkaline, exposure to oxidizing conditions and uncontrolled enzymatic reaction, can resulting in the formation of protein crosslinking producing substantial changes in the structure of protein. Therefore the functional and nutritional properties of the final product were alternated (Gerrard, 2002).

Crosslinking and aggregation of protein have been mentioned as the important mechanisms for manipulating food products with desirable properties. Protein crosslinking can influence many properties of food, including texture, viscosity, solubility, emulsification properties, gelling properties, foaming properties, and also thermal transition. Crosslinking of proteins could be a tool for providing new types of food or improve traditional food properties. Many traditional foods including yoghurt, cheese, sausage, tofu and surimi, which their textures are derived from a protein gel can be modified by formation of proteins crosslinking. Since crosslinking of protein brings able to formation of gel structures from protein solutions, dispersions, colloidal systems, protein-coated emulsion droplets or protein-coated gas bubbles (Gerrard, 2002).

2.1 Methods for manipulating protein crosslinking during processing

2.1.1 Chemical methods

Many commercial crosslinking agents are available. However, they remain widely used for biochemical and biotechnological applications. Unfortunately, these reagents are not often approved for food use. In practice, they perform the proof of a new commercial crosslinking agent principle studies in which to measure the possible effects of its crosslink introduced into food. If an improvement in functional properties is seen after treatment with the reagent, then further research effort would be merited to find a food approved, cost effective means by which to introduce such crosslinks on a commercial scale (Gerrard, 2002).

2.1.2 Enzymatic methods

The use of enzymes to modify the functional properties of foods is an attracted considerable interest since they are perceived as natural and non-toxic food components (Bonet et al., 2006). Enzymes are specific biological catalysts able to react under mild conditions of temperature and pH, have high specificity, are only required in catalytic quantities, and are less likely to produce toxic products. Thus enzymes are becoming commonplace in many industries for improving the functional properties of food proteins (Gerrard, 2002; Bonet *et al.*, 2006; Rastall, 2007). The benefits of crosslinking enzymes are highly depended on application.

Proteins have several reactive groups for crossliking enzymes, such as glutamine, lysine, tyrosine and cysteine residues. The reactions obtained are depended on the type of enzyme used, the accessibility of the target reactive groups in the biopolymer and the process conditions used (Rastall, 2007).

3. Transglutaminase

Transglutaminase (TGase; protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) catalyses an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues in certain proteins, and leads to covalent crosslinking by the formation of ε-(γ-glutamyl)lysine bond (G-L bond) (Gerrard *et al.*, 2001; Gerrard, 2002; Joye *et al.*, 2009, Motoki and Seguro, 1998; Rastall, 2007). In the absence of amine substrates, TGase catalyses the deamidation of glutamine residues during which water molecules are used as acyl acceptors. TGase can modify proteins by means of amine incorporation, crosslinking and deamidation (Fig. 1 and 2) (Motoki and Seguro, 1998).

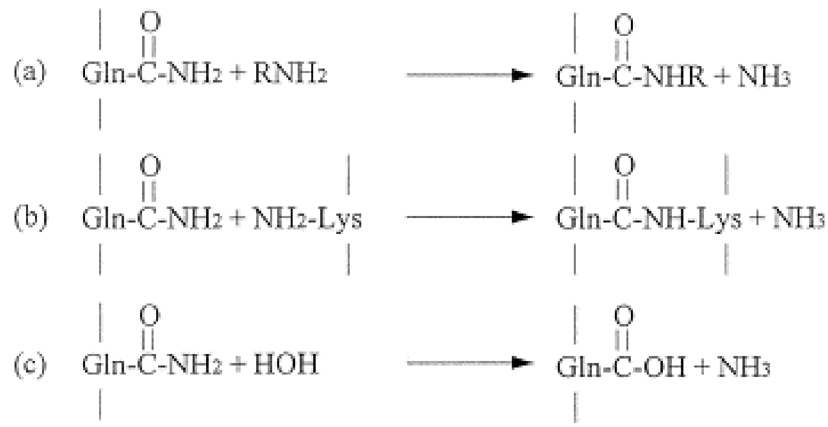


Fig. 1 Reactions catalyzed by TGase; (a) acyl-transfer reaction, (b) crosslinking reaction between glutamine (Gln) and lysine (Lys) residues of proteins or peptides (c) deamidation

Source: Motoki and Seguro (1998)

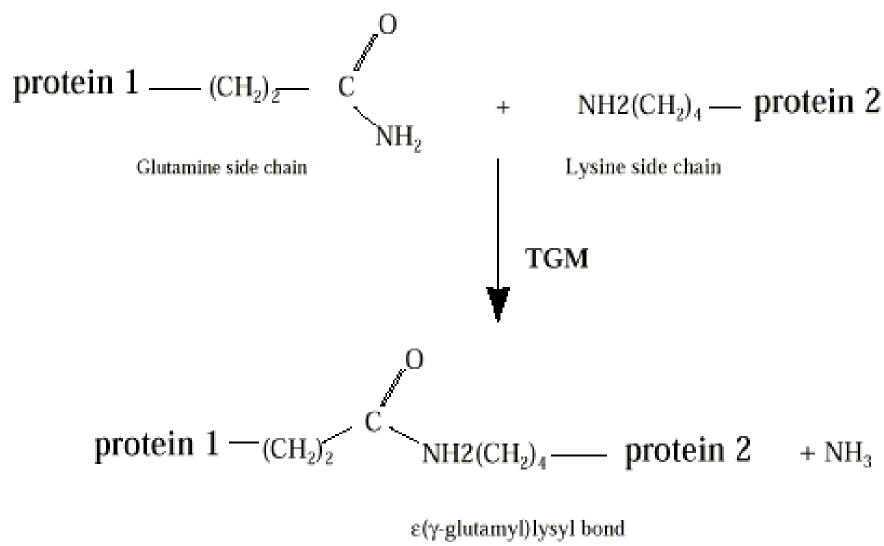


Fig. 2 Crosslinking reaction produced by TGase

Source: Wartiovaara (1999)

TGase are widely distributed enzymes found in various animal tissues and body fluids, fish, birds, invertebrates, amphibians, plants and microbes. They are involved in several biological functions including blood clotting, wound healing, epidermal keratinization and in a number of human disease states. Discovery of microbial TGase (MTGase) from *Streptomyces* and

Streptoverticillium species was made in late 1980s and led to fast development of TGase for various food applications. (Rastall, 2007)

3.1 Characteristics of MTGase

The isoelectric point of MTGase was approximately 8.9. The molecular weight of the enzyme is about 38,000. MTGase comprises of 331 amino acid residues and possibly has a signal peptide of 18 amino acid residues at its amino terminal with a single cysteine residue. It has considered to be a monomeric and simple protein (not a glycoprotein or lipoprotein), although there are two potential glycosylation sites (-Thr-Xxx-Asn-) in the primary structure (Motoki and Seguro, 1998).

Reducing agents, dithiothreitol, 2-mercaptoethanol, and glutathione, would noticeably change the physicochemical properties of MTGase such as thermal stability and sensitivity to heavy metals. Therefore, determination of some properties of the enzyme should be performed in the absence of reducing agents (Motoki and Seguro, 1998).

3.2 Enzymatic properties of MTGase

The pH optimum of MTGase was around 5 to 8. However, the enzyme still expresses some enzymatic activity at pH 4 or 9. Thus, MTGase is considered to be stable over a wide pH range. The optimum temperature for the enzymatic activity was 50°C, and MTGase fully sustained its activity even at 50°C for 10 min. On the other hand, it lost activity within a few minutes on heating to 70°C. MTGase still expressed activity at 10°C, and still retained some activity at temperatures just above the freezing-point (Motoki and Seguro, 1998; Rastall, 2007).

MTGase from a variant of *Streptoverticillium mobaraense* is quite unique from other mammalian enzymes. By definition, TGase require Ca^{2+} for expression of enzymatic activity. However, MTGase is totally independent of Ca^{2+}. This property of MTGase is very useful for modification of functional properties of food proteins since many food proteins, such as milk caseins, soybean globulins and myosins, are susceptible to Ca^{2+} and easily precipitated in the presence of Ca^{2+} and become less sensitive to MTGase. The sensitivity of MTGase toward other cations in the absence of reducing agents has also to be considered. Cysteine residue could be part of the active site of MTGase. Heavy metals such as Cu^{2+}, Zn^{2+}, Pb^{2+} and Li^{+} significantly inhibited MTGase since these heavy metals bind the thiol group of the single cysteine residue (Motoki and Seguro, 1998).

MTGase reacts differently on different substrates. The more non-globular protein/peptides are better substrates than native globular proteins (Whitehurst and Law, 2002). Two or more different proteins can be covalently conjugated to produce new proteins with novel functionalities by the action of MTGase. In addition, MTGase is capable of incorporating amino acids or peptides covalently into proteins. This reaction can improve nutritive values of food or proteins, since covalently incorporated amino acids or peptides behave like amino residues in a protein. Generally, all common amino acids, except lysine, should have their α-carboxyl group either amidated, esterified or decarboxylated to eliminate the negative charge on the α-carboxyl group. On the other hand, ε-amino group of lysine is a primary amine. Consequently, this amino acid acts as a good substrate for MTGase. During reaction, proteins act as acyl donors in such reaction while amino acids, including lysine, act as acyl acceptors (Motoki and Seguro, 1998).

In the case of peptides, both lysine- or glutamine-containing peptides can be substrates for MTGase without modification. Lysine-containing peptides act as the acyl acceptors, while proteins act as the acyl donors. For the glutamine-containing peptides, they act as the acyl donors, while proteins act as the acyl acceptors. In addition, since the substrate specificity of glutamine residue is much higher than primary amines, hydrophobic moieties must be placed on the amino group of the glutamine residue (Motoki and Seguro, 1998).

3.3 Applications of MTGase in food processing

MTGase has been used in many food products such as meat, fish, dairy, soybean and wheat products. The enzyme is possible to produced restructured meat, harden fish protein paste at low temperature, reduce loss during thawing and cooking in frozen fish products, form a heat-resistant firm casein gel, improve the strength of edible soybean film and modify noodles and pasta from wheat products (Motoki and Seguro, 1998). Likewies, MTGase has been successfully used in seafood, surimi, dairy and bakery products (Gerrard, 2002).

4. Pea protein isolate

Legumes are a good supplement for cereal-based foods since both legume and cereal proteins are complementary in essential amino acids. Cereals are deficient in the essential amino acid lysine, while legumes have a high content of this amino acid. Although the most used legume protein is from soybean, pea proteins can also be successfully used in bakery products, obtaining a protein enriched product with better amino acid balance (Marco and Rosell, 2008a).

Presently, using of pea protein isolate in food industry has been increased since it has a suitable proportion of amino acid ratio, with a high level of lysine. The isolated protein from pea contains a higher lysine content in comparison with other cereals. Pea protein isolate provide a good functional properties, including gelling, emulsifying and foaming properties (Nunes *et al.*, 2006). Pea proteins are classified as albumins and globulins. The two major globulins named legumin and vicillin. The proportion of legumin fractions in pea is 20-30% and the vicillin fraction is 20-40% (Makri *et al*., 2006; Marco *et al.*, 2007)

Addition of pea protein isolate in food products purpose to enhance proteins content, homogenize the food matrix, or modify textural property of the products. Moreover, addition of protein which has high level in a specific amino acid promote benefit for athletes or patients that need a specific nutrition. For instance, some commercial pea protein isolate from yellow pea has low calories (3.51 kcal/g), has a high level of lysine, arginine, and branched chain amino acid (leusine, isoleusine, valine), and absence from allergen.

5. Effect of transglutaminase and pea protein isolate addition on properties of wheat and rice products

5.1 Effect of protein crosslinking on extractable protein content

Protein crosslinking causes the change in extractable protein content. Gerrard *et al.* (2001) found that the percentage of extractable gliadin in transglutaminase-treated dough both from bread and croissant was increasing. In the croissant dough, the addition of transglutaminase reduced albumin and globulin fraction. For glutenin fraction, the effect was less pronounced, although the percentage of SDS-insoluble glutenin was drop in the bread dough. The difference in extractable protein in each fraction may due to the different formulation and processing conditions, especially fat content in formula. The extractalbe protein content also affected by pea protein isolate addition.

Marco *et al.* (2007) found that extracted albumin-globulin fraction content from rice dough with pea protein isolate increased. In contrast, a decrease in glutelin and prolamin fractions was observed.

5.2 Effect of protein crosslinking on molecular size of protein

Protein crosslinking promotes the change in molecular size of protein. Gerrard *et al.* (2001) examined the molecular size of each protein fraction extracted from bread and croissant dough with and without transglutaminase using SDS-PAGE analysis. The results were similar for bread and croissant dough. The aggregated protein that was too large to enter into the gel was observed in albumin fraction from the enzyme-treated dough. In addition, the crosslinking of low molecular weight protein and high molecular weight glutenin (HMW-glutenin) was also observed. TGase crosslinking effect on molecular size of protein also exhibited in gliadin (avenalin) and glutelin fractions obtained from oat dough (Huang *et al.*, 2010).

Transglutaminase could also modify protein obtained from rice flour. Marco *et al.* (2007) studied effects of the enzyme on protein extracted from rice flour by SDS-PAGE under both non-reducing and reducing conditions. In both samples in the presence of transglutaminase, a decrease in the intensity or a disappearance of the protein bands was observed. An increase in the protein retained at the top of the stacking and resolving gels was also observed in the presence of TGase. The authors suggested that protein polymer unable to enter the gel increased in molecular weight due to activity of TGase, resulting in polymers of greater size and lower solubility. Under reducing condition, with mercaptoethanol, the result showed a smaller amount of protein retained at the top of stacking and resolving gels. This suggested the relation of disulfide to the occured crosslinking by TGase. Rupture of disulfide bonds between the protein by the reducing agent yielded shorter protein chains that were able to enter the gel. resulting the decreasing the amount of protein that was retained in the gel in the absence of TGase.

5.3 Effect of protein crosslinking on free amino group and thiol group contents

Protein crosslinking affected the free amino group content. Gujral and Rosell (2004) found that addition of transglutaminase enzyme in rice flour dough led to the reduction of free amino group content and a progressive decrease was observed at a concentration of 0.1%. This result indicated the crosslinking between glutamine and lysine residues catalysed by transglutaminase. Otherwise, the addition of the enzyme also promoted the decrease of thiol group, especially at 0.05% concentration, suggested the formation of disulfide bonds was most likely favoured by the proximity of the crosslinked polypeptide chains. The reduce in free amino group with the presence of TGase also reported in oat and wheat doughs. (Huang et al., 2010; Steffolani *et al.* (2010) On the other hand, Marco and Rosell (2008) found that neither transglutaminase nor pea protein isolate did not significantly influence the free amino group content. Thiol group also be influenced by transglutaminase. Marco and Rosell (2008a) found that addition of transglutaminase caused a decrease in thiol group content, particularly at the enzyme level of 0.05%. The result suggested that disulfide bonds attributed to the transglutaminase crosslinking.

5.4 Effect of protein crosslinking on properties of dough

Protein crosslinking causes the modification in rheology of dough. Gujral and Rosell (2004) found that the elastic modulus (G´) of rice flour dough containing transglutaminase enzyme was higher than the viscous modulus (G´´), suggested the solid-elastic like behavior of dough. Addition of the enzyme led to the increase of G´ and G´´ with the enzyme concentration. The G´ remained higher than G´´ at all concentration level. In addition, Marco and Rosell (2008b) found the similar result in rice flour dough treated with transglutaminase including the decreasing of tan δ. Huang *et al.* (2010) stated that cooking stability of oat dough increased when TGase level was 1.5%. Rheological properties of oat dough showed that TGase introduced the increase in G´, G´´ and complex modulus (G*). The effect of TGase was greater in G´´ than G´. Otherwise, the crosslinking induced by TGase reduced tan δ of oat dough treated with the enzyme. These indicated that TGase crosslinking resulting to formation of a network structure. Therefore, viscoelastic behavior and anti-deformation ability of the dough were modified. The highest viscoelastic dough was observed when 1% level of TGase was added.

Pea protein isolate also changed the rheology of rice flour dough. Marco and Rosell (2008b) found that the a decrease in G´ and G´´ of the dough was shown when pea protein isolate was added. The dough had higher G´ than G´´ resulting the viscoelastic solid behavior. Moreover, Marco and Rosell (2008a) found that pea protein isolate produced a significant clearly increase of G´ and G´´ in rice dough. Transglutaminase and pea protein isolate influenced the water absorption of rice flour dough. The increasing in water absorption was shown in both doughs treated with transglutaminase and pea protein isolate. The enzyme and protein isolate modified the dough and brough to a higher water holding capacity dough (Marco and Rosell, 2008b). Huang *et al.* (2010) also observed an increase in cooking stability in oat dough when the TGase level was 1.5%.

Textural property of rice flour dough was modified by both transglutaminase and pea protein isolate addition. Marco and Rosell (2008b) found that transglutaminase-treated dough displayed an increased in hardness and stickiness whereas a decreased in adhesiveness. Pea protein isolate affected hardness, gumminess, springiness and stickiness of dough by decreased the parameter values. However, both enzyme and pea protein isolate addition did not show any effect on cohesiveness of rice flour dough. Protein crosslinking also had an impact on thermal properties of oat dough. Onset temperature and peak temperature of oat dough slightly increased in the samples treated with TGase (Huang *et al.*, 2010).

5.5 Effect of protein crosslinking on quality of bread

For the effect of protein crosslinking on quality of bread, Gujral and Rosell (2004) found that the specific volume of bread made from rice flour dough treated with transglutaminase enzyme was increased, but the value was low at higher concentration. The highest value was observed at 1.0% enzyme concentration. Moreover, they found that the addition of glucose oxidase enzyme could also promote the increase of the specific volume of dough from rice flour. (Gujral and Rosell, 2004a) Otherwise, protein crosslinking by enzymes also affect the crumb hardness. The hardness was decrease when added transglutaminase but was increase at high concentration and the lowest value was observed at a concentration of 1.0%.

5.6 Effect of protein crosslinking on rice sheet and rice gel

Properties of rice sheet and gel were modified regarding to the protein crosslinking. Lin *et al.* (2009) investigated the TGase addition effects on rheological, textural and thermal properties of rice sheet. The rheological property results showed that both G’ and G” values of rice gel. With the increasing level of TGase, the values reached maximum values and then decreased. The authors suggested that this could be related to the limited content of lysine in the cereal protein. The addition of TGase increased the hardness, gumminess and adhesiveness of rice sheet. In contrast, within the dosage level of TGase in this study, a decrease of cohesiveness was observed. Thermal property of rice sheet treated with TGase displayed a slight increase in gelatinization temperature whereas exhibited a decrease in enthalpy.

MATERIALS AND METHODS

1. Materials

1.1. Raw materials

1) Thai rice cultivar Chai nat 1 (amylose 28.5%, protein 6.82%) grown in Ayutthaya province, obtained from the Department of Rice, Ministry of Agriculture and Cooperatives, Thailand
2) Thai rice cultivar Khao Dok Mali 105 (KDML105; amylose 16%, protein 6.27%) grown in Ayutthaya province, obtained from the Department of Rice, Ministry of Agriculture and Cooperatives, Thailand
3) Thai rice cultivar RD6 (waxy rice, amylose 5.5%, protein 5.72%) grown in Chiang Mai, obtained from the Department of Rice, Ministry of Agriculture and Cooperatives, Thailand
4) TGase Activa® STG-M was supplied by Ajinomoto Co., Ltd. (Thailand)
5) Pea protein isolate Pisane® C9

1.2. Instruments and apparatus

1) Rotor mill (Retsch SR300, Germany)
2) 100 mesh test sieve (Retsch, Germany)
3) Refrigerated centrifuge (Kubota, Japan)
4) pH meter (FE-20K, Mettler Toledo, USA)
5) SDS-PAGE apparatus with power supply (Food Protein Bioscience, Kagawa University, Japan)
6) Differential Scanning Calorimeter (Micro DSC VII, Setaram, France)
7) Rapid Visco Analyzer (RVA 3D, Newport Scientific Instruments and Engineering, Australia)
8) Texture Analyzer (Stable Micro Systems TA.XT Plus, UK)
9) Dynamic Mechanical Analyzer (DMA Eplexor, Gabo, Germany)

1.3. Chemical reagents

1) NaCl (Wako, Wako Pure Chemical Industries, Ltd., Japan)
2) NaOH (Wako, Wako Pure Chemical Industries, Ltd., Japan)
3) Ethanol (Wako, Wako Pure Chemical Industries, Ltd., Japan)
4) Hexane (Wako, Wako Pure Chemical Industries, Ltd., Japan)
5) HCl (Wako, Wako Pure Chemical Industries, Ltd., Japan)

6) 2-betamercaptoethanol (Nacalai, Nacalai Tesque, Japan)
7) $(NH_2)_2SO_4$ (Wako, Wako Pure Chemical Industries, Ltd., Japan)
8) Protein marker (board range, Bio-Rad, Japan)
9) Tris(hydroxymethyl)aminomethane (Wako, Wako Pure Chemical Industries, Ltd., Japan)
10) N,N,N',N'-tetramethylethane (TEMED) (Wako, Wako Pure Chemical Industries, Ltd., Japan)
11) Sodium Lauryl Sulfate (SDS) (Nacalai, Nacalai Tesque, Japan)
12) Acrylamide (Nacalai, Nacalai Tesque, Japan)
13) N,N'-methylenebisacrylamide (Nacalai, Nacalai Tesque, Japan)
14) Glycerol (Wako, Wako Pure Chemical Industries, Ltd., Japan)
15) Glycine (Wako, Wako Pure Chemical Industries, Ltd., Japan)
16) Bromphenol Blue (Nacalai, Nacalai Tesque, Japan)
17) Coomassie Brilliant Blue R-250 (Nacalai, Nacalai Tesque, Japan)
18) 2-propanol (Wako, Wako Pure Chemical Industries, Ltd., Japan)
19) Acetic acid (Wako, Wako Pure Chemical Industries, Ltd., Japan)

2. Methods

2.1. Rice flour preparation

Thai rice cultivars; Chai Nat 1 (CN 1; amylose 28.5%, protein 6.8%), Khao Dok Mali 105 (KDML105; amylose 16%, protein 6.3%) were grown in Ayutthaya province. Waxy rice (RD6; amylose 5.5%, protein 5.7%) was grown in Chiangmai province. All samples were obtained as paddy rice from the Department of Rice, Ministry of Agriculture and Cooperatives, Thailand. The paddy rice was dehusked using a McGill sample sheller (Rapsco, Brookshire, TX), and the rice bran removed using a McGill No. 2 mill. Samples were milled to a constant degree of milling (DOM = 90). The DOM was measured using a Satake Milling Meter MM-1B (Satake Engineering Co., Ltd., Tokyo, Japan). Rice grain was then dry milled using Rotor Mill (Retsch SR300, Germany) to decrease the particle size and then sieved through the 100 mesh sieve. Rice flour was kept in plastic bag and stored at 4˚C.

2.2. Rice flour gel preparation

Rice flour suspension was prepared at ratio of rice flour : water 1 : 3 (w/w), with and without TGase (Activa® STG-M; Ajinomoto Co., Ltd., Bangkok, Thailand) and pea protein isolate (PPI) (Pisane® C9; Nutrition Sc Co., Ltd., Nakhonpathom, Thailand. Rice flour was dry mixed with 1% or 2% TGase (w/w of composite flour) and 5% PPI (w/w of composite flour). The suspension was stirred for 30 min using magnetic stirrer. After that, it was preheated in water bath at 52°C for 2 min. 15 g of rice paste was then poured into 2.5 cm diameter can and steamed at 95°C for 15 min. The samples were allowed to be cooled at room temperature and stored in plastic bag. (Gujral and Rosell, 2004; Sasaki *et al.*, 2009).

2.3. Extraction of rice proteins

Rice protein was extracted from rice flour according to the method of Osborne (Ju *et al.*, 2001). Rice flour was defatted with hexane. The defatted flour was subjected to protein extraction. Based on the solubility of protein, four protein fractions (albumin, globulin, glutelin, and prolamin) of rice protein was obtained by sequentially extracting with deionized water for albumin, 5% NaCl for globulin, 0.1M NaOH for glutelin and 70% ethanol for prolamin. Following each extraction, the slurry was centrifuged at 3000g for 30 min. The sediment was used for next fractionation. The supernatant was collected, adjusted pH using 0.1M HCl to the isoelectric pH and centrifugation at 3000g for 30 min. Afterwards, the precipitated proteins were washed twice with distilled water, freeze-dried and stored at -21˚C in plastic bag. The resulting protein powders were used to investigate the reactivity with TGase.

2.4.Analysis of rice proteins treated with TGase

100 µl of rice protein fraction dissolved in buffer solution containing 10 mM Na_2SO_3 and 0.5% SDS (10-20 mg/ml) and was dissolved in 100 µl of Tris-HCl buffer (pH 7.5) and 200 µl pure water. The sample solution was preincubated at 25°C for 5 min before adding of 0.01g/ml TGase. Enzymatic reaction was performed at 25°C for 24 h. Then SDS-sample buffer was added and the sample solution was boiled for 2 min to stop the enzymatic reaction. For the preparation of reducing sample, β-mercaptoethanol (βME) was added into the protein sample as a reducing agent. SDS-PAGE analysis was carried out according to Laemmli's buffer system under reducing and non-reducing conditions, using 10% acrylamide running gel (Laemmli, 1970). 10 µl of sample solution

containing 5-10 mg protein was loaded into the gel and was run at 15mA for about 2.5 h. The gel was stained with Coomassie Brilliant Blue – R250 solution and destained in the solution containing 5% propanol and 7% acetic acid.

2.5.Pasting properties of rice flour

Pasting property of rice flour was measured using Rapid Visco Analyzer (RVA 3D, Newport Scientific Instruments and Engineering, Australia). Rice flour 3 g (±0.01 g) with and without TGase and pea protein isolate, were mixed with 25.0 ml (±0.1 ml) deionized water in aluminum can equipped with plastic paddle. Sample was analyzed using rice profile setting. The test speed was 960 rpm 10 min from the beginning. After that, 160 rpm test speed was used until the end of test. The sample was heated up from 50°C to 95°C and then cooled down to 50°C (AACC, 2000). Pasting temperature, peak viscosity, trough, final viscosity, breakdown and setback were recorded.

2.6.Calorimetric properties of rice flour

Rice suspension was prepared with and without TGase and PPI. 15 g rice flour was mixed with 45 ml deionized water. The suspension was then stirred using magnetic stirrer for 1 hr before analyzed. Differential Scanning Calorimeter (Micro DSC VII, Setaram, France) was used to determine the calorimetric profile of rice flour. 1 mg (±0.02 mg) of rice suspension was weighed in the DSC cell. The reference cell was added with the same weight of deionized water. The test was started at 25°C and hold for 5 min, then heated up to 95°C and hold for 5 min. The sample was then cooled down to 25°C. The heating rate of 1.2 K/min was used throughout the test (Cham and Suwannaporn, 2010). Onset temperature, peak temperature, conclusion temperature and enthalpy were recorded. The amount of rice flour was calculated as % dry basis.

2.7.Textural properties of rice flour gel

Rice flour gel was prepared according to method in section 2.2. The height of the gel was 10 mm. Texture Profile Analysis (TPA) of rice gel was analyzed by Texture Analyzer (Stable Micro Systems TA.XT Plus, UK). The puncture test was conducted using cylindrical probe (6 mm diameter). Calibration of height was performed before the test. Test speed was 1 mm/s. Distance of compression was 5 mm and holding time was 5 s (Vandeputte *et al.*, 2003). The TPA parameters, which were hardness, adhesiveness, springiness, cohesiveness and gumminess, were recorded.

2.8.Viscoelastic properties of rice flour gel

Rice flour gel was prepared according to method in section 2.2. Rice gel was cut into cubic shape with diameter of 20 mm width and 20 mm height. Viscoelastic property of rice flour gel was analyzed using Dynamic Mechanical Analyzer (DMA Eplexor, Gabo, Germany). Linear viscoelastic region (LVR) of static and dynamic force was previously investigated using static dynamic sweep mode. The force range used in the test was 0.5-2.0 N. Frequency sweep test (0.5-10.0 Hz) was then performed using compression mode (Kundu *et al.*, 2010). Geometry of the gel was selected as prism. Static and dynamic load were 1.0 N and 0.5 N respectively. Static and dynamic max strain were 10% and contact force was 0.5 N. Storage modulus (E') and loss modulus (E'') and tan δ were recorded.

2.9.Statistical analysis of data

The experiment was designed using Factorial in CRD. The data was analysed using one-way and two-way ANOVA by SPSS software version 16.0 (SPSS Statistics® 16.0, IBM Corporation, United States) and Duncan's Multiple Range Tests (DMRT), $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

1. Protein patterns of rice protein from three cultivars

Rice protein was extracted into four fractions and protein patterns of rice protein fractions were observed using SDS-PAGE analysis (Fig. 3). The pattern of protein obtained from three cultivars displayed the similar pattern with difference in band intensity. Albumin fraction had bands in a wide range of molecular weights from around 9 to 88 kDa. For globulin fraction, major bands were observed at molecular weight 20-25 kDa and around 50 kDa. Major bands of glutelin fraction had molecular weight at 9 and 28 kDa. For prolamin fraction, the major bands could be observed at molecular weight 9, 21 and 28 kDa, approximately. These results were similar to previous studies (Likitwattanasade and Hongsprabhas, 2010; Oszvald *et al.*, 2008; Renzetti *et al.*, 2012).

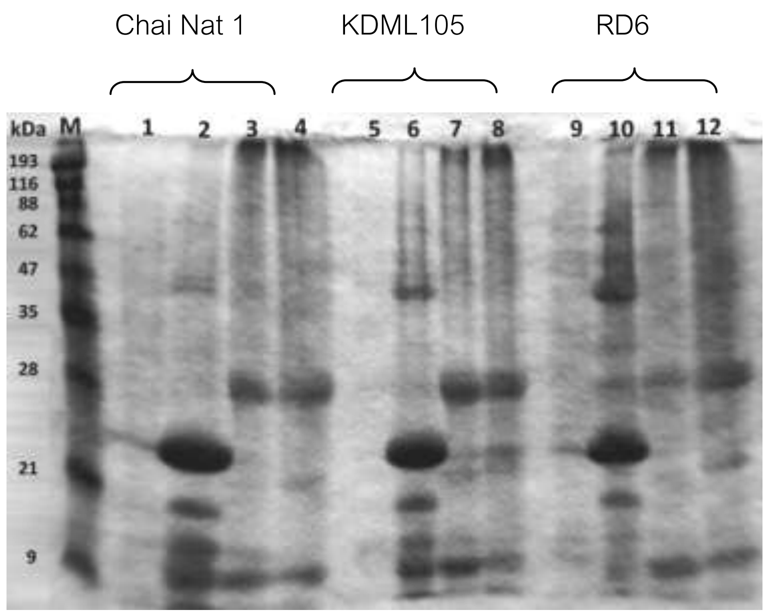


Fig. 3 SDS-PAGE patterns of rice flour protein from three cultivars. (Lane 1, 5, 9: albumin, lane 2, 6, 10: globulin, lane 3, 7, 11: glutelin, lane 4, 8, 12: prolamin)

2. Protein patterns of rice protein fractions treated by TGase

To investigate the effect of TGase on protein fractions, rice proteins were subjected to SDS-PAGE under both reducing and non-reducing conditions. Figures 4a, 4c and 4e showed the SDS-PAGE patterns from three cultivars under reducing condition. In globulin and albumin fractions, protein band shift was observed by the action of TGase in all cultivars. Band intensity of proteins in the range of less than 50 kDa was decreased by the TGase treatment. Furthermore, protein bands at the boundary between stacking and running gels, and/or at the top of stacking gel, were increased clearly in globulin fraction. These suggest the polymerization of proteins through intermolecular crosslinking (Marco *et al.*, 2007). Thus the microbial TGase was found to induce intermolecular crosslinking of proteins of rice albumin and globulin fractions. Renzetti *et al.* (2012) found that no new polypeptides were detected in the TGase-treated flour, and the products of the polymerisation reaction were larger than 230 kDa. High-molecular-weight aggregrated protein by the action of

TGase in globulin and albumin fractions were also observed in buckwheat protein (Renzetti *et al.*, 2008). In contrast, TGase did not induce band shift of proteins in glutelin and prolamin fractions. The cross-linking reaction catalyzed by TGase was depended on the availability and accessibility of glutamine and lysine residues (Renzetti *et al.*, 2012). Since proteins in glutelin and prolamin fractions are generally hydrophobic, so it was reasonable that TGase was difficult to access those proteins. The present study suggested that rice globulin and albumin were susceptible to TGase while glutelin and prolamin were not. For oat dough proteins, Huang *et al.* (2010) found that globulin and gliadin (avenalin) were the desirable substrates for TGase. SDS-PAGE pattern of rice proteins under non-reducing condition are shown in Figures 4b, 4d and 4f. Compared to the gel of reducing condition, protein bands at the boundary between stacking and running gels and/or at the top of stacking gel were dense in all fractions, indicating that S-S crosslinking during the incubation independently of TGase.

However, the degree of S-S crosslinking was higher in the TGase-treated samples. The formation of disulfide bonds due to TGase activity was also reported by Gujral and Rosell (2004), Larre *et al.* (2000), and Marco *et al.* (2007). The formation of disulfide bonds was most likely favoured by the proximity of the cross-linked polypeptide chains. The crosslinking reaction may bring some amino acids closer to each other as the protein molecules become more compact. Thus, the sulfur-containing amino acids may come close to each other leading to the formation of S-S bonds by oxidation. (Gujral and Rosell, 2004). Furthermore, Marco *et al.* (2007) mentioned that the crosslinking reaction may expose the sulfur-containing amino acids, facilitating the formation of these bonds. To sum up, microbial TGase gave two effects to rice proteins: one was direct effect of TGase, that was, the crosslinking reaction between glutamine residues of a protein and lysine residues of another protein, and the other was an indirect effect of the formation of intermolecular disulfide bonds.

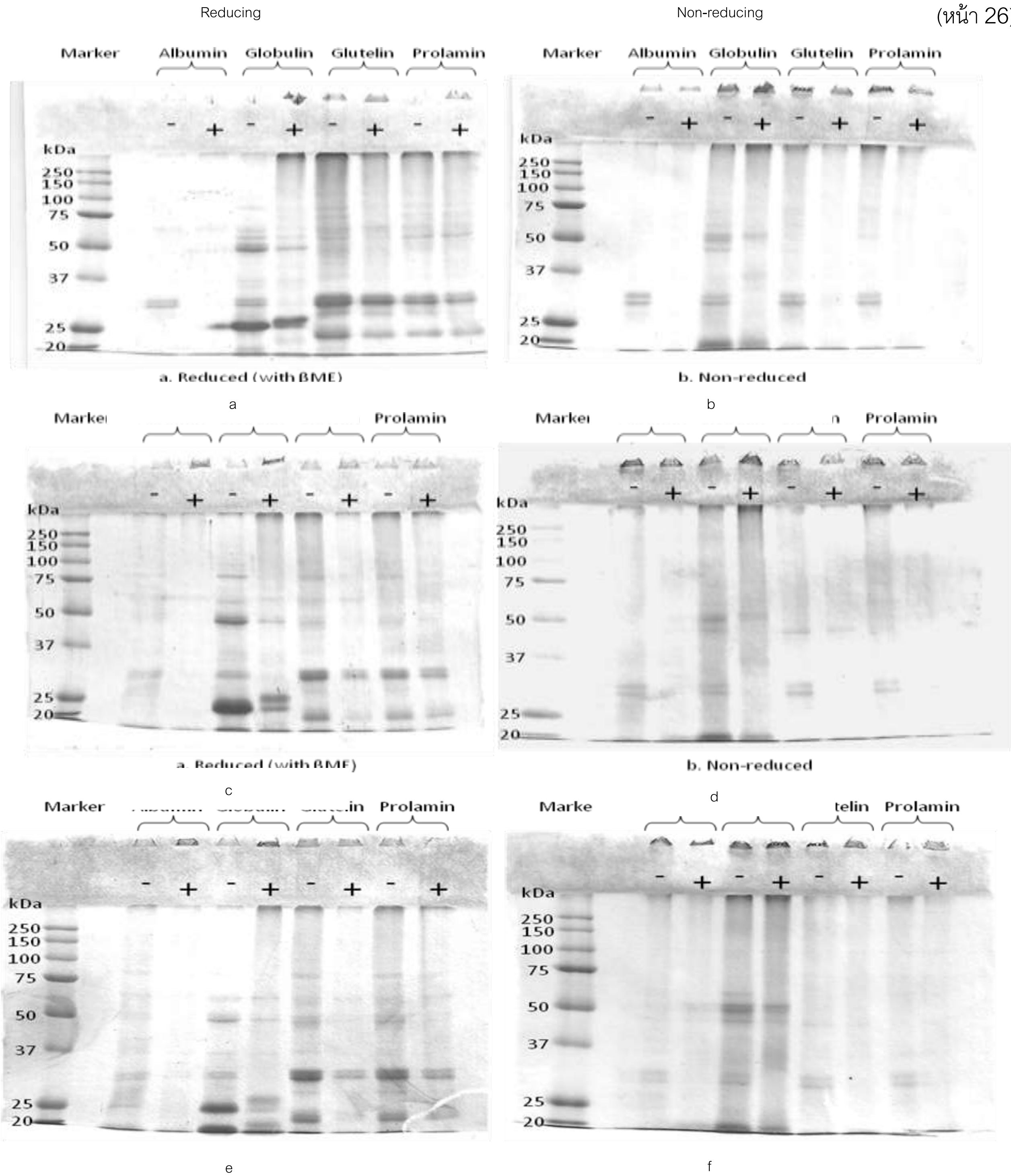


Fig. 4 SDS-PAGE patterns of rice protein fractions from three cultivars with TGase under reducing (a, c, e) and non-reducing condition (b, d, f) of Chai Nat1 (a, b), KDML105 (c, d) and RD6 (e, f); + represent with TGase and – without TGase

3. Effect of transglutaminase and pea protein isolate (PPI) on properties of rice flour gel

3.1 Pasting property of rice flour treated with TGase and PPI

Table 1 shows the RVA profile of rice flour with TGase and PPI treatments. Both TGase and PPI had a significant impact on peak viscosity, through, breakdown, final viscosity and setback in all cultivar. TGase significantly increased (P < 0.05) these parameters while PPI led to a decreasing in the values. The enrichment of PPI by replacing rice flour with the protein resulting in a reduction of starch ratio in the system. PPI, which is a legume protein, has ability to form gels with a great water-holding capacity upon heating. Consequently, the decreasing of RVA profile parameters was observed in the sample with PPI. The effect of TGase in peak time and pasting temperature was negligible. These suggested that TGase crosslinking of rice protein and PPI had minor impact on granule swelling of starch. Nevertheless, the addition of enzyme and PPI seemed to influence the starch granule after gelatinization and pasting stages as observed on final viscosity and setback modification. Rice endosperm protein is almost totally in the form of protein bodies whereas little or no matrix protein has been found. These proteins may form a barrier, which attributed to a continuous protein phase surrounding the starch granule, to expansion of swollen starch granules (Hamaker, 1994).

In our study, PPI showed a synergistic noticeably effect with the presence of TGase only in high amylose rice flour gel. High amylose gel was comparatively firm and compact. As a consequence, the accessibility of TGase to lysine and glutamine residues in this gel was more pronounced. Marco *et al.* (2007) also reported that the effect of the enzyme was greater with the existing of pea protein isolate. Ribotta *et al.* (2012) explored the impact of native and enzymatic modified pea protein isolate on functional properties of protein-cassava starch and protein-corn starch gels. Pasting profile obtained from both protein-starch pastes showed an increase in pasting viscosity, final viscosity and setback with the presence of PPI. The enzyme treatment slightly increased paste viscosity and final viscosity. A decreasing in breakdown was observed in protein-cassava paste while no significant difference was observed in protein-corn paste.

Table 1 Pasting property of rice flour gel (± S. D.)

Cultivar	Treatment	Peak viscosity (RVU)	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)	Peak time (min)	Pasting temperature (RVU)
Chai Nat 1 (High amylose)	Control	2604.0 ± 15.9^{c}	2251.3 ± 20.7^{b}	352.7 ± 23.3^{b}	3761.7 ± 51.5^{c}	1510.3 ± 70.8^{c}	6.5 ± 0.0^{a}	78.9 ± 0.9^{b}
	TG1%	2560.0 ± 62.9^{c}	2065.3 ± 34.1^{c}	494.7 ± 29.7^{a}	3901.0 ± 49.6^{c}	1835.7 ± 23.8^{ab}	6.2 ± 0.0^{bc}	76.3 ± 1.0^{c}
	TG2%	2735.0 ± 52.2^{b}	2269.0 ± 108.1^{b}	466.0 ± 70.8^{a}	4090.0 ± 78.0^{b}	1821.0 ± 178.1^{ab}	6.4 ± 0.1^{b}	76.2 ± 1.0^{c}
	PPI	2148.0 ± 30.6^{e}	1760.3 ± 18.7^{e}	387.7 ± 37.1^{b}	3543.3 ± 115.2^{d}	1783.0 ± 129.1^{ab}	6.1 ± 0.1^{c}	81.6 ± 1.4^{a}
	TG1% + PPI	2243.7 ± 11.4^{d}	1888.0 ± 26.1^{d}	355.7 ± 35.4^{b}	3494.0 ± 104.8^{d}	1606.0 ± 130.6^{bc}	6.2 ± 0.1^{bc}	79.9 ± 0.0^{ab}
	TG2%+ PPI	2961.7 ± 17.1^{a}	2421.3 ± 54.9^{a}	540.3 ± 42.8^{a}	4345.0 ± 77.1^{a}	1923.7 ± 131.8^{a}	6.2 ± 0.1^{bc}	79.4 ± 0.9^{b}
KDML105 (low amylose)	Control	3419.7 ± 8.3^{b}	2666.3 ± 31.8^{b}	753.3 ± 38.2^{c}	4257.3 ± 14.6^{b}	1591.0 ± 17.4^{b}	6.6 ± 0.0^{a}	76.3 ± 0.4^{a}
	TG1%	3346.3 ± 13.3^{c}	2549.7 ± 28.3^{c}	796.7 ± 15.0^{c}	4146.7 ± 59.5^{c}	1597.0 ± 31.2^{b}	6.6 ± 0.0^{a}	73.6 ± 1.3^{bc}
	TG2%	3584.0 ± 42.6^{a}	2778.7 ± 122.0^{a}	805.3 ± 98.6^{c}	4587.7 ± 106.1^{a}	1809.0 ± 23.1^{a}	6.5 ± 0.1^{a}	72.1 ± 0.0^{c}
	PPI	3029.3 ± 11.4^{e}	1998.3 ± 49.4^{e}	1031.0 ± 40.2^{a}	3420.0 ± 35.3^{e}	1421.7 ± 14.5^{c}	6.2 ± 0.1^{b}	74.4 ± 0.1^{ab}
	TG1% + PPI	3078.0 ± 13.1^{d}	2172.3 ± 64.3^{d}	905.7 ± 61.5^{b}	3565.0 ± 69.3^{d}	1392.7 ± 20.3^{c}	6.3 ± 0.1^{b}	75.2 ± 2.4^{ab}
	TG2%+ PPI	2981.0 ± 21.1^{f}	2153.7 ± 12.9^{d}	827.3 ± 14.2^{bc}	3563.7 ± 13.4^{d}	1410.0 ± 1.7^{c}	6.3 ± 0.0^{b}	74.9 ± 1.2^{ab}
RD6 (waxy)	Control	3123.3 ± 28.9^{c}	1700.3 ± 21.8^{d}	1423.0 ± 19.3^{a}	1965.7 ± 16.0^{d}	265.3 ± 10.1^{d}	4.2 ± 0.0^{b}	64.6 ± 0.5^{a}
	TG1%	3189.3 ± 32.6^{b}	1824.0 ± 5.6^{b}	1365.3 ± 34.7^{b}	2141.0 ± 7.6^{b}	317.0 ± 12.2^{b}	4.2 ± 0.1^{ab}	64.8 ± 0.0^{a}
	TG2%	3311.3 ± 10.6^{a}	1896.7 ± 12.2^{a}	1414.7 ± 22.8^{a}	2278.7 ± 32.1^{a}	382.0 ± 20.0^{a}	4.2 ± 0.0^{a}	66.2 ± 2.0^{a}
	PPI	2947.3 ± 15.0^{e}	1641.3 ± 18.7^{e}	1306.0 ± 4.0^{c}	1882.0 ± 22.5^{e}	240.7 ± 9.0^{e}	4.2 ± 0.0^{b}	65.4 ± 0.5^{a}
	TG1% + PPI	3005.3 ± 19.5^{d}	1765.0 ± 29.8^{c}	1240.3 ± 41.4^{b}	2012.7 ± 32.3^{c}	247.7 ± 2.5^{d}	4.3 ± 0.0^{a}	65.7 ± 1.4^{a}
	TG2%+ PPI	2957.7 ± 18.61^{e}	1695.0 ± 6.3^{d}	1262.7 ± 15.4^{cb}	1989.3 ± 2.3^{cd}	294.3 ± 4.0^{c}	4.2 ± 0.0^{ab}	64.6 ± 1.2^{a}

$^{a\text{-}c}$ Means in the same column followed by the same lowercase superscript letter are not different using Duncan's test at $P > 0.05$.

3.2 Thermal property of rice flour treated with TGase and PPI

Thermal property of rice flour was measured by DSC. A single endothermic peak for rice flour was obtained approximately between 67 and 74ºC (data not shown). The mean of observed value was exhibited in Table 2. The addition of TGase and PPI had small effect on thermal properties of rice flour gels especially waxy rice. Protein bodies crosslinking may have minor influenced on starch granule swelling comparing with protein matrix. Difference of calorimetric profile of rice flour paste was affected by the difference of amylose/amylopectin ratio in each cultivar rather than protein crosslinking because protein crosslinking may still not formed unless the flour gel have been set. High amylose rice flour gel was more susceptible to TGase accounted for its packed amylose gel which enhance the accessibility of glutamine and lysine residues to the enzyme. The modification of thermal properties due to difference of amylose proportion was also reported in previous studies. Varavinit *et al.* (2003) stated that amylose content provided a highly positive correlation with onset, peak and conclusion temperatures of Thai rice isolated starch. The authors found that these parameters were raised with the increasing amount of amylose. The increasing of gelatinization temperatures induced by TGase was observed in rice sheet (Lin *et al.*, 2009). TGase treatment was found to be related with changes of water holding capacity and surface hydrophobicity of the gel pastes. Nahid *et al.* (2010) reported that TGase treatment could improve the solubility of legumes protein isolate. The protein treated with TGase has a lower level of free amino groups. As a result, a decrease in the surface hydrophobicity and an increase in electrostatic repulsion of the protein molecules were observed. The authors suggested that these could enhance protein-protein interaction and surface adsorption of protein.

Table 2 Calorimetric profile of rice flour gel (± S. D.)

Cultivar	Treatment	Onset Temp. (°C)	Peak Temp. (°C)	Conclusion Temp.(°C)	Enthalpy (J/g)
Chai Nat 1 (High amylose)	Control	69.2 ± 0.1^{c}	73.9 ± 0.2^{b}	79.3 ± 0.4^{c}	5.8 ± 0.1^{a}
	TG1%	69.3 ± 0.1^{bc}	73.9 ± 0.1^{b}	79.3 ± 0.1^{c}	5.4 ± 0.1^{bc}
	TG2%	69.3 ± 0.1^{bc}	74.0 ± 0.0^{b}	79.4 ± 0.1^{c}	5.3 ± 0.1^{c}
	PPI	69.4 ± 0.1^{b}	74.3 ± 0.0^{a}	79.9 ± 0.9^{b}	5.5 ± 0.1^{b}
	TG1%+PPI	69.6 ± 0.1^{a}	74.4 ± 0.1^{a}	80.4 ± 0.3^{a}	4.8 ± 0.1^{d}
	TG2%+PPI	69.6 ± 0.1^{a}	74.4 ± 0.0^{a}	80.3 ± 0.1^{a}	4.7 ± 0.1^{d}
KDML105 (low amylose)	Control	60.8 ± 0.2^{b}	67.7 ± 0.1^{c}	73.9 ± 0.2^{ab}	5.3 ± 0.1^{a}
	TG1%	60.9 ± 0.2^{b}	67.8 ± 0.2^{c}	73.7 ± 0.1^{ab}	5.3 ± 0.1^{a}
	TG2%	61.8 ± 0.8^{a}	67.8 ± 0.0^{c}	72.5 ± 2.5^{b}	5.2 ± 0.2^{a}
	PPI	61.3 ± 0.1^{ab}	68.1 ± 0.1^{b}	74.7 ± 0.2^{a}	5.3 ± 0.3^{a}
	TG1%+PPI	61.4 ± 0.1^{ab}	68.2 ± 0.1^{a}	75.2 ± 0.0^{a}	5.2 ± 0.1^{a}
	TG2%+PPI	61.4 ± 0.4^{ab}	68.1 ± 0.0^{ab}	75.3 ± 0.4^{a}	5.0 ± 0.2^{a}
RD6 (waxy)	Control	63.3 ± 1.0^{a}	67.8 ± 0.1^{a}	74.6 ± 0.0^{a}	5.9 ± 0.4^{a}
	TG1%	59.1 ± 0.1^{b}	67.8 ± 0.1^{a}	74.6 ± 0.4^{a}	5.6 ± 0.3^{a}
	TG2%	59.1 ± 1.2^{b}	67.8 ± 0.2^{a}	74.5 ± 0.2^{a}	5.4 ± 0.9^{a}
	PPI	64.0 ± 1.4^{a}	68.4 ± 0.8^{a}	74.9 ± 0.2^{a}	4.9 ± 0.8^{a}
	TG1%+PPI	59.7 ± 0.3^{b}	68.2 ± 0.3^{a}	74.7 ± 0.2^{a}	5.4 ± 0.2^{a}
	TG2%+PPI	59.8 ± 0.4^{b}	68.2 ± 0.1^{a}	74.7 ± 0.2^{a}	5.3 ± 0.2^{a}

$^{a\text{-}c}$ Means in the same column followed by the same lowercase superscript letter are not different using Duncan's test at $P > 0.05$.

3.3 Textural property of rice flour gels treated with TGase and PPI

TPA profile of rice flour gels, with and without TGase and PPI, were shown in table 3. Hardness and gumminess increased with the increase in TGase concentration, particularly at 2% level, in all cultivars ($P < 0.05$). Both TGase and PPI promoted significant impact on hardness, but with a relatively lower in value compared to sample with TGase alone. This could be related to the formation of PPI gel. PPI is a legume protein and has ability to form gel. The gel network could be formed into fine-stranded and coarse networks depending on the gel formation conditions (Makri *et al.*, 2006). When the mixture of polysaccharides and the protein isolate are both presented, a two component gel is formed. The structure of the network formed depends on the experimental conditions (mixing procedure, time, temperature, etc.) but mainly on the nature and the ratio of the two components present. If no complex can be formed, two different networks coexist and the gel formed could belong to one of the following categories: phase-seperated network gel, interpenetrating network gel or filled gel. Depending on their ratio, the two components could form either a filled gel or a phase-seperated network gel (Makri *et al.*, 2006). The PPI gel occurred in the system, which has different properties such as water holding capacity compared to rice gel alone or the one with TGase, reflected the relatively difference in hardness. For the others texture profile parameters, adhesiveness, springiness and cohesiveness showed minor modification by addition of TGase and PPI.

The present results suggested that TGase promoted alteration of rice protein and resulting in changes in textural property of rice flour gel. The gel treated with TGase had stronger structure which requires higher force and energy to disintegrate, resulting in a greater of hardness and gumminess. The improvement of food texture according to the action of TGase was reported in previous study and similar result was found by Lin *et al.* (2009). The authors stated that the addition of MTGase increased hardness and gumminess in rice gel sheet. Meanwhile, cohesiveness was no affected by the enzyme (0.01-0.3 U/mg) and adhesiveness was hardly affected resulting in an increase in the value when 0.3 U/mg of MTGase was added.

Table 3 Texture Profile Analysis (TPA) profile of rice flour gel (±S.D.)

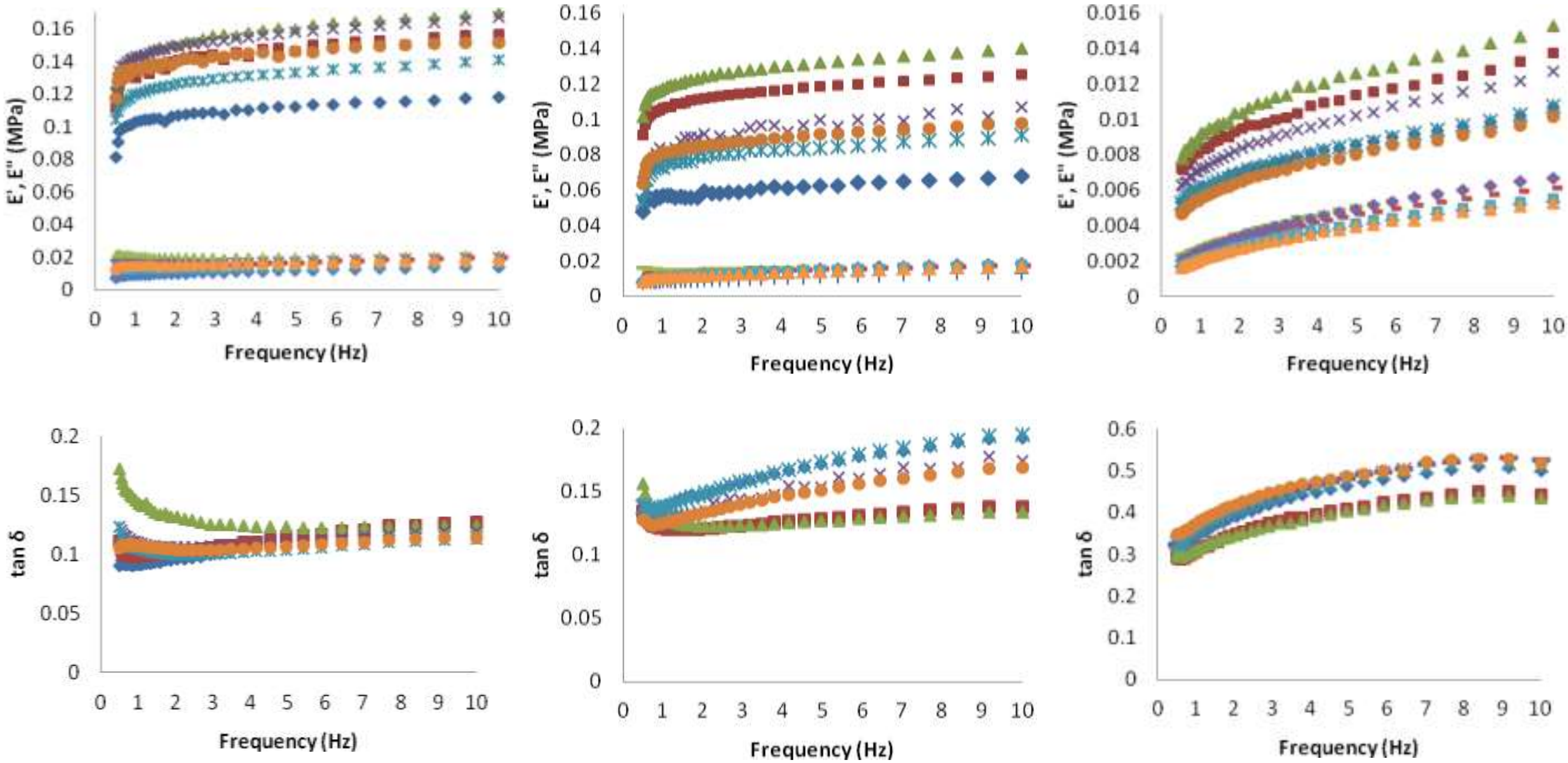
Cultivar	Treatment	Hardness (g)	Adhesiveness (g·sec)	Springiness (g)	Cohesiveness	Gumminess (g)
Chai Nat 1	Control	267.8 ± 40.5[c]	-134.5 ± 45.4	0.6 ± 0.1[b]	0.7 ± 0.1[a]	181.1 ± 33.0[bc]
	TG1%	308.7 ± 21.6[ab]	-82.3 ± 71.1	0.6 ± 0.1[b]	0.7 ± 0.1[a]	219.7 ± 20.7[a]
	TG2%	325.6 ± 24.4[a]	-126.1 ± 61.8	0.6 ± 0.1[b]	0.7 ± 0.1[a]	225.1 ± 20.3[a]
	PPI	281.6 ± 32.2[bc]	-154.7 ± 10.7	0.6 ± 0.0[b]	0.6 ± 0.1[a]	171.7 ± 42.0[c]
	TG1%+PPI	280.8 ± 31.8[bc]	-82.5 ± 64.1	0.8 ± 0.1[a]	0.7 ± 0.0[a]	196.4 ± 12.1[abc]
	TG2%+PPI	316.4 ± 34.6[ab]	-122.7 ± 44.7	0.7 ± 0.1[a]	0.7 ± 0.1[a]	215.6 ± 32.7[ab]
KDML105	Control	49.9 ± 7.6[c]	-33.6 ± 14.6[a]	0.7 ± 0.1[a]	0.7 ± 0.1[a]	33.9 ± 1.8[c]
	TG1%	79.5 ± 7.9[a]	-47.2 ± 16.5[a]	0.8 ± 0.0[a]	0.7 ± 0.0[a]	51.2 ± 3.7[a]
	TG2%	72.8 ± 11.2[ab]	-44.8 ± 22.9[a]	0.8 ± 0.1[a]	0.7 ± 0.1[a]	46.9 ± 6.5[ab]
	PPI	50.8 ± 7.9[c]	-24.4 ± 13.0[a]	0.7 ± 0.1[a]	0.7 ± 0.1[a]	34.3 ± 4.1[c]
	TG1%+PPI	62.9 ± 7.4[b]	-37.5 ± 20.8[a]	0.7 ± 0.1[a]	0.7 ± 0.1[a]	43.3 ± 6.5[b]
	TG2%+PPI	68.4 ± 6.0[b]	-37.7 ± 18.0[a]	0.7 ± 0.1[a]	0.7 ± 0.1[a]	45.3 ±4.0[b]
RD6	Control	12.7 ± 2.9[c]	-4.5 ± 1.9[a]	0.6 ± 0.1[a]	0.6 ± 0.1[ab]	7.7 ± 1.2[b]
	TG1%	16.7 ± 1.8[ab]	-8.1 ± 1.3[bc]	0.6 ± 0.1[a]	0.6 ± 0.1[ab]	9.7 ± 0.6[a]
	TG2%	18.5 ± 2.2[a]	-11.2 ± 2.0[d]	0.7 ± 0.1[a]	0.5 ± 0.1[b]	9.8 ± 0.9[a]
	PPI	12.9 ± 1.7[c]	-6.5 ± 2.8[ab]	0.6 ± 0.1[a]	0.7 ± 0.1[a]	8.6 ± 1.1[ab]
	TG1%+PPI	14.4 ± 2.1[bc]	-9.3 ± 1.4[cd]	0.6 ± 0.2[a]	0.7 ± 0.1[a]	9.3 ± 0.9[a]
	TG2%+PPI	16.2 ± 1.5[ab]	-10.9 ± 1.4[d]	0.6 ± 0.1[a]	0.6 ± 0.1[ab]	9.7 ± 1.1[a]

[a-c] Means in the same column followed by the same lowercase superscript letter are not different using Duncan’s test at $P > 0.05$.

3.4 Viscoelastic property of rice flour gels treated with TGase and PPI

Higher value of storage modulus (E´) than loss modulus (E´´) throughout the range of frequency was observed in gels obtained from all cultivars for every treatment (Fig. 6). The higher of E´ than E´´ indicated the elastic-like behavior of the gels (Ribotta *et al.*, 2012). Although, E´´ was not significantly different when frequency increased, E´ increased slightly with an increase in frequency in Chai Nat 1 and KDML 105 whereas the RD6 gels showed a slowly raise of E´ with frequency. These results suggested that both high-amylose and low-amylose gels were strong gels, which has a more withstand structure to the load at various frequencies. Waxy (RD6) rice flour gel was relatively weak but with strong structure that was not stable under increasing in frequency.

TGase and PPI modified E´ and E´´ resulted in the shift of the values of all cultivar. 2% level of TGase showed the highest shift of E' compared to control. The modification of viscoelastic property by the action of TGase was investigated in the previous studies. Marco and Rosell (2008) found that addition of TGase increased both G´ and G´´ in rice flour treated with the enzyme and pea protein isolate. For E´´, the effect of TGase was more obviously effect than PPI. Lower tan δ was particularly observed in KDML105 and RD6 after TGase addition whereas the tendency of value observed in Chai Nat 1 remained the same as the control (Fig. 5). Gujral and Rosell (2004) suggested the similar results and also indicated the decrease in tan δ with the increasing level of TGase. Likewise, the effect of TGase was observed in oat dough by Huang *et al.* (2010). The authors implied that TGase induced a raise of G´ and G´´ and a decrease in tan δ with increasing level of the enzyme. The efficient impact of TGase and pea protein isolate was also reported in gels (Lin *et al.*, 2009; Ribotta *et al.*, 2012). This result suggested that TGase led to the more structured and solid-like gel (Ribotta et al, 2012). Viscoelastic property of product is greatly associating with the continuous protein phase (Gujral and Rosell, 2004). Rice flour dough observed by scanning electron microscopy (SEM) showed a disrupted-like structure where starch granules were hold together by the proteins. When TGase (1% w/w) was added, an uniform distribution of the starch granules through a more compact rice flour dough structure was observed (Marco and Rosell, 2008). Consequently, alteration of rice protein due to TGase crosslinking and the appearance of PPI resulting in changes in viscoelastic behaviour of rice flour gel. The improvement of products by TGase and legume protein was also reported in previous studied. Marco and Rosell (2008) observed a more compact rice flour dough structure when 1% w/w of TGase was added using scanning electron microscope. In addition, the authors also found that rice flour with pea proteins seemed to be integrated in a compact structure.



a b c

Fig. 5 Storage modulus (E′), loss modulus (E′′) and tan δ of (a) Chai Nat 1, (b) KDML105, (c) RD6 rice flour gels (◆- Ctrl, ■- with 1% TGase, ▲- with 2% TGase, ×- with PPI, ✳- with 1% TGase + PPI, ●- with 2% TGase + PPI)

CONCLUSION AND RECOMMENDATION

SDS-PAGE results suggested the polymerization of proteins by the action of TGase was mainly in globulin and albumin fractions. Results indicated the occurrence of S-S crosslinking during the incubation. The addition of TGase could modify properties of rice flour gel. Physicochemical properties of rice flour gel were modified by the action of the enzyme, depending on the existing of PPI and difference in cultivar. RVA profile displayed the minor effect of TGase crosslinking and PPI on granule swelling of starch. Nevertheless, the addition of enzyme and PPI seemed to influence the starch granule after gelatinization and pasting stages as observed on final viscosity and setback modification. Moreover, PPI showed a synergistic noticeably effect with the presence of TGase only in high amylose rice flour gel. For calorimetric property of rice flour gels, the addition of TGase and PPI had small effect on this property especially waxy rice. High amylose rice flour gel seemed to be more susceptible to TGase accounted for its packed amylose gel which enhance the accessibility of glutamine and lysine residues to the enzyme. Both TGase and PPI altered textural property of rice flour gel promoting significant impact on hardness, but with a relatively lower in value compared to sample with TGase alone. Conversely, adhesiveness, springiness and cohesiveness showed minor modification by addition of TGase and PPI. The effect of TGase was more obviously effect than PPI on viscoelastic property of rice flour gel providing a clearly increase in storage modulus and whereas decrease in tan δ. From these results, introducing of rice protein crosslinking using enzymes could improve properties of rice flour. Consequently, protein crosslinking enzyme provided better utilization of rice flour. However, application data in foods should be more investigate. Other studied such as spectroscopic analysis would lead us to understand more about the formation of the crosslinking.

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