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Removal of Soluble Proteins during Fermentation Process for Improving Textural Properties of Traditional Thai Rice Noodles, Kanom-jeen

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Abstract

Traditional Thai fermented rice noodles called *Kanom-jeen* are characterized by a unique flavor and pleasing texture, and widely consumed as a staple food in Thailand and throughout the Indochina region. This study aimed to determine the effect of the fermentation process on rice noodle protein content and composition, and the relationship to Kanom-jeen texture. The protein content of rice flour decreased during the fermentation process. SDS-PAGE showed that the protein bands at 20-33 kDa, representing protein body-II (PB-II) disappeared during rice fermentation, while the bands around 13 kDa representing protein body-I (PB-I) remained. Microstructural analysis of noodles demonstrated that unfermented Kanom-jeen was composed of cluster-like structures of PB-II proteins, while fermented Kanom-jeen contained only uniformly spherical protein bodies of PB-I in starch gel. Both results give the product a stronger texture. Therefore, the fermentation process is necessary during Kanom-jeen preparation to obtain the desired specific texture.

Discipline: Food

Additional key words: enzymatic modification; protein composition, microscopy

Introduction

Kanom-jeen is a traditional fermented rice noodle, popularly consumed as a staple food in Thailand. Similar rice noodles are also prevalent in other food cultures in the Indochina region, such as *Bun* (Vietnam), *Nom Banvuchock* (Cambodia), *Khao Bun* (Laos) and *Mohinga* (Myanmar). In Thailand, two types of Kanom-jeen (non-fermented and fermented) are commercially available. Consumers prefer fermented Kanom-jeen, however, due to its unique flavor and textural characteristics (Oupathumpanont et al. 2008, Pisitkul & Rengpipat 2014).

The traditional preparation of Kanom-jeen involves soaking high amylose rice for 4-5 h in water; fermentation of the soaked rice grains for 3 days with aeration; wet-milling; followed by the fermentation of ground flour in saline solution for 3 days; filtration; kneading and pre-gelatinization; re-kneading to form a viscous slurry; and then extruding the viscous slurry into noodles and cooking in boiling water (Chunta et al. 2014).

The fermentation process was considered a key step in achieving the desirable attributes of the Kanom-jeen product (Li et al. 2001, Keatkrai & Jirapakkul 2010, Zhou et al. 2010). Besides taste and aroma, the specific texture of Kanom-jeen is thought to derive from the fermentation process. An analysis of chemical components revealed that the total protein content of the fermented rice flour

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was always lower than that of unfermented one, and that even starch properties such as amylose content did not change (Lu et al. 2008 a, b).

These findings postulate the hypothesis that during the fermentation process, changes occur in the chemical composition, particularly in proteins, resulting in the specific texture of Kanom-jeen.

Rice endosperm of milled or broken rice used for Kanom-jeen production contains storage proteins. These storage proteins are localized as protein bodies. The type I protein body (PB-I) has a spherical shape, is rich in prolamin, and can be difficult to digest, while the type II protein body (PB-II) is irregularly shaped and consists mainly of glutelin, an easily digestible protein (Tanaka & Masumura 1988, Kubota et al. 2010). The role of these PBs in rice gel or noodle production is still not fully understood.

The purpose of this study was to determine the effect of the fermentation process on protein composition localized as protein bodies, and the subsequent textural changes specific to Kanom-jeen.

Materials and methods

1. Rice materials

Broken rice and fermented rice flour produced following the traditional process were purchased from a local Kanom-jeen flour producer in Udon Thani Province, Thailand. Naturally fermented rice flour was provided as wet rice cake. The producer also uses a broken rice mixture of high (around 30%) and medium (> 20%) amylose rice varieties to maintain product quality. Therefore, the rice material used in this study contains two varieties of rice.

2. Rice flour preparation

Wet-milled rice flour was prepared by soaking broken rice in water for 2 hrs., then draining and wet-milling the rice using a Vita-Mix blender (Bangkok, Thailand), and finally drying it overnight at 45°C in a drying oven (Binder, Germany). The dried flour was then re-ground in a mortar to obtain a fine powder.

The fermented wet rice cake was broken into small pieces, then dried and re-ground as described above to obtain fermented rice flour. The wet-milled and fermented rice flours had a moisture content of 11.5% and 12.3%, respectively. Both were kept in a refrigerator until use.

3. Kanom-jeen preparation

The wet-milled or fermented rice flour (100 g) was thoroughly mixed with water (75 mL) to yield dough, and then formed into two balls 6 cm in diameter. The balls of dough were pre-gelatinized in boiling water for 2.5 min, where partial gelatinization of starch at around 31.4% was achieved (about 1 cm below the ball surface). The balls of dough were then re-kneaded with a small amount of boiling water to obtain a smooth and creamy texture. The dough was then extruded into noodles in boiling water for 1 min., then immediately cooled with water and drained for further analysis.

4. Protein and starch analysis

Total protein content was determined according to AACC method 46-10.01, and the protein composition of the rice flour samples was investigated using SDS-PAGE. In brief, total proteins were extracted from the wet-milled and fermented rice flours (100 mg) with 700 μL of extraction buffer (0.25M Tris-HCl, pH 6.8, 4% SDS, 8M urea, 20% glycerol, 5% 2-mercaptoethanol) at 100°C for 3 min. The extracts were immediately cooled to room temperature and then centrifuged. The protein composition of the supernatants (5 μL) was separated in 5-20% gradient SDS-PAGE and visualized using Quick-CBB PLUS (Wako, Japan).

Amylose content was determined by using the Amylose/Amylopectin Assay Kit (K-AMYL, Megazyme, Ireland).

Differential Scanning Calorimetry (DSC) thermograms of the flours were obtained with a SII Exstar 6200 DSC system (SII, Japan). The wet-milled or fermented rice flour (10 mg) was weighted in silver pan (70μL, ϕ 5 mm), with water (50 mg) being added with a syringe. The sealed pan was used for a DSC analysis heating range of 40-120 °C at 0.5°C/min. with the pan with water (60 mg) as the reference. Two gelatinization peaks representing two varieties of rice were observed in the thermograms.

5. Textural analysis

The hardness and elongation properties of cooked Kanom-jeen noodles were measured with a texture analyzer (TA-XT2i, Stable Micro Systems, UK), equipped with a 50-mm cylinder probe and a spaghetti noodle probe. The hardness of Kanom-jeen noodles was recorded as the force (N) required to compress the sample at 70% strain, while elongation was measured as the distance (mm) before breakage.

6. Protein microstructural analysis

Kanom-jeen noodles were sliced at thickness of 10 μm using a rotary microtome (RV-240, Yamato Koki, Japan) equipped with freezing sample dye (MC-802A, Yamato Koki, Japan). The thin noodle sections were placed on MAS-GP Type A coated glass slides (Matsunami Glass, Japan), and fixed with 10% glutaraldehyde. The noodle sections were stained with Coomassie Brilliant Blue R-250 (CBB), and then mounted and covered with Softmount coverslips (Wako, Japan). All samples were examined under a light microscope (BX-3500TL, Wraymer, Japan) to determine localization of the protein image.

7. Statistical analysis

All measurements were performed in duplicate. Analysis of variance (ANOVA) was conducted for protein content and texture, and the mean separations were determined by Duncan's multiple range test ($p < 0.05$) using SPSS version 12.0.

Results and discussion

1. Protein content and components

Table 1 lists the protein content of fermented and unfermented noodles. The protein content was 8.67% in unfermented noodles and 4.83% in fermented noodles. The decrease is thought to be caused by fermentation that changed the protein molecules into less complex forms, and leach out with water due to microorganism activity.

Figure 1 shows the protein composition analyses of fermented and wet-milled rice flour by using SDS-PAGE. The results revealed that the protein composition was changed during the fermentation process, where the protein band with molecular mass around 20-33 kDa disappeared. The 22-23 kDa protein bands should be glutelin (Xia et al. 2012), and the 33 kDa protein band might be globulin (Kato et al. 2000, Ito et al. 2005). Both glutelin and globulin consisting of 60-65% rice endosperm protein are known as PB-II, which is easily digested during the fermentation stage (Furukawa et al. 2000, Yamagata et al. 1982).

After fermentation, the 13 and 55 kDa protein bands still remained. The 13 kDa protein band was reported as PB-I (Furukawa et al. 2000, Kumagai et al. 2006, Xia et al. 2012), while the 55 kDa band could be granule-bound starch synthase (Krishnan & Chen, 2013). Thus, the fermentation process strongly impacted the quantity of PB-II, but not PB-I. This suggested that the prolamin portion (PB-I) may interact with other rice components and remain through both fermentation and cooking processes. Fermented rice products that contain less digestible proteins may be suitable for patients with chronic kidney disease who need to control protein intake (Kopple 2001).

The amylose content and gelatinization temperature in the DSC thermograms for wet-milled and fermented rice flours were 17.61% and 17.78%; 65.7°C, 74.7°C and 65.5°C, 75,1°C, respectively, and had no significant difference (Table 1). The amylose content is known to

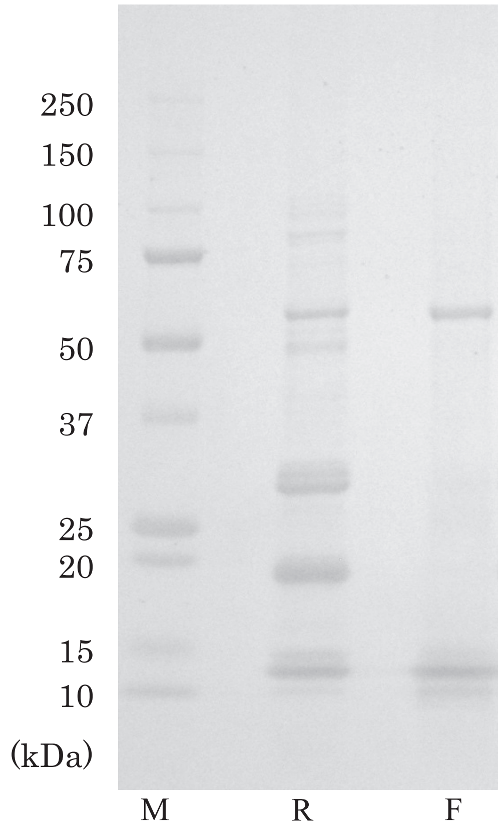

Fig. 1. Protein composition of wet-milled rice flour and fermented rice flour
M: molecular weight marker; R: wet-milled rice flour; F: fermented rice flour

Table 1. Summary of the textural analysis of rice noodles prepared with and without fermentation

Sample	Amylose content (%)	Protein content (%)	Hardness (N)	Elongation (mm)
Unfermented noodles	17.61 ± 0.69^a	8.67 ± 0.05^a	7.33 ± 0.78^a	11.57 ± 2.77^a
Fermented noodles	17.78 ± 0.47^a	4.83 ± 0.36^b	8.29 ± 0.89^b	16.29 ± 3.70^b

Means are significantly different ($p < 0.05$).

be the key parameter for pasting properties, and thus rice noodle characteristics. The DSC thermograms also showed no significant difference, including gelatinization temperature and enthalpy on a carbohydrate basis. Because the fermentation process did not affect the gelatinization properties of starch, resulting in no structural changes of starch, the unique texture of Kanom-jeen might be introduced by changes in protein content and composition.

2. Textural analysis

The fermentation process provided specific textural properties for Kanom-jeen by inducing better gel properties. Our results indicated that Kanom-jeen noodles prepared from fermented rice flour demonstrated higher hardness and elongation than that from unfermented flour (Table 1). This might be related to the change in protein composition, especially in the content of PB-II, which has been reported to influence the textural properties of rice gel (Li et al. 2001, Lu et al. 2003). However, how the protein composition in rice affects the textural properties of Kanom-jeen noodles still requires confirmation.

3. Microstructure of noodles

CBB staining clearly showed the localization of protein composition in both fermented and unfermented Kanom-jeen (Fig. 2). For unfermented Kanom-jeen, a cluster-like PB-II protein formed a network in the gel, resulting in a less uniform starch gel structure (A and B in Fig. 2). Conversely, fermented Kanom-jeen showed no cluster-like protein with a uniform scattering of small protein PB-I bodies, producing a stronger noodle with higher hardness and elongation.

Our findings thus suggested that the rice protein composition influenced the textural properties of Kanom-jeen prepared with or without fermentation, and that the removal of PB-II during the fermentation process conferred the unique texture of Kanom-jeen.

The traditional method of fermentation to prepare Kanom-jeen was abandoned a long time ago. However, this ancient technique can be utilized as a new technology to produce food with enhanced gel properties and added nutritional benefits.

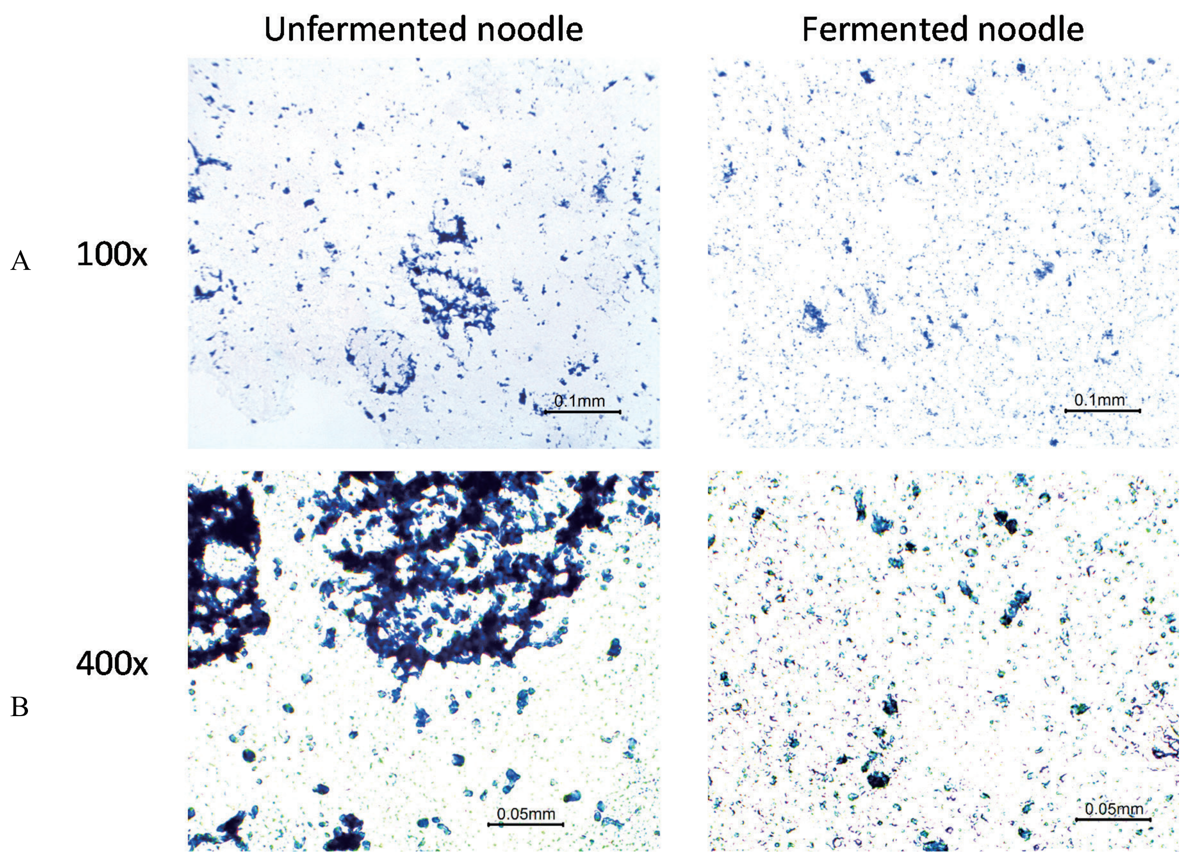

Fig. 2. Protein localization of unfermented and fermented Kanom-jeen using CBB staining as examined under a light microscope at 100x (A) and 400x (B)

Conclusions

Our study provided evidence suggesting that the PB-II protein content significantly affected the textural properties of fermented Kanom-jeen. Selective PB-II protein removal during the fermentation process produced a uniform gel microstructure of Kanom-jeen noodles consisting of only spherical PB-I proteins with a superior texture. Cluster-like PB-II protein structures were commonly found in gels of non-fermented Kanom-jeen noodles, resulting in a less uniform microstructure. These structural differences indicated that the traditional fermentation process can be considered viable to remove PB-II proteins from rice endosperm in order to provide a stronger gel.

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