Effects of Ultrasonic and Enzymatic Treatment on Cooking and Eating Quality of Sao Hai Rice

Kanokwan Jadwong[1] and Nantawan Therdthai[1,*]

Abstract

Sao Hai rice contains high amylose content which affects textural properties of cooked rice grains. This research aimed to improve cooking quality of high amylose rice, using ultrasonic and enzymatic treatments (protease and α-amylase with concentration at 0, 0.025, 0.05 and 0.1% w/w). Ultrasonic treatment at 40 kHz for 1, 3 and 5 min caused grain cracking, due to the cavitation. For the enzymatic treatment, cooking time and pasting temperature were reduced ($p \leq 0.05$). The protease treated rice had the increased water uptake ratio and the decreased hardness ($p \leq 0.05$). The α-amylase treatment caused decreasing in water uptake ratio, whiteness index, but increasing in adhesiveness ($p \leq 0.05$). The α-amylase treated rice had less hardness than the one from protease treatment. From sensory evaluation, the α-amylase treatment could increase liking scores on softness, adhesiveness, sweetness, overall taste and overall liking of the cooked rice ($p \leq 0.05$).

Keywords: Ultrasonic pretreatment, Enzyme pretreatment: Protease pretreatment, α-amylase, Sao Hai Rice, Cooked Rice

1. Introduction

Rice is the important food of the world, especially in Asia. Most of Asian people produced and consumed rice more than people in other regions. Furthermore, rice is the important crop in Thailand. There are many varieties of rice was developed and produced. Based on amylose content, rice can be classified to 3 types: rice with low, medium and high amylose content. Amylose content is an important factor to cooking and eating quality of the cooked rice. Rice with high amylose has hard texture, compared with rice with low amylose content. Currently, soft cooked rice becomes more popular. Increasing softness of rice containing high amylose will increase value of this rice group. As Soa Hai is one of the high amylose rice, it was selected to study in this project as a prototype softening rice.

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According to previous researches, the proposed methods to soften rice texture included pressurization and enzymatic treatment (Watanabe *et al*., 1991), pressurized cooking (Leelayuthsoontorn and Thipayarat, 2006), ultrasonic treatment of brown rice (Cui *et al*., 2010), pressure-soaking treatment (Tian *et al*., 2014) and ultrasonic combined with enzyme treatment (Zhang *et al*., 2015). With these treatments, physical properties of rice were changed. For example, cell membrane was degraded when high pressure was applied. Surface of rice grains became uneven and cracks after ultrasonic treatment. Springiness and cohesiveness of cooked rice from pressure-soaking treatment was improved.

For enzymatic treatment, Alpha-amylase could hydrolyze amylose in rice, resulting in the reduction of amylose content and hard gel of the cooked rice. Protease could hydrolyze rice protein resulting in the released protein from the rice starch. Water absorption, cooking and eating quality of the treated rice could be improved. Watanabe *et al*. (1991) reported the actinase treatment degraded cell walls and protein-associating membrane. Consequently, it clearly improved quality of aged rice. That was also confirmed by Arai *et al*. (1993). The protease treatment of aged grains was effective, due to two main reasons. Firstly, it liberated starch granules. The second was to remove the granule-associated protein. Then, during rice cooking, the liberated starch without the associated proteins was gelatinized and gave a rise in stickiness.

For ultrasonic treatment, it has been studied to apply in food processing. High-amplitude wave (10 kHz to 1 MHz) tended to generate ultrasonic cavitation (Koo *et al*., 2002). When particles such as rice grains were subjected to sonication in water, some destruction of the surface and grain fragmentation would be expected (Mason and Paniwnyk, 1996). Consequently, water could be penetrated into rice kernel easily, resulting in an increased water absorption ratio during cooking (Cui *et al*., 2010).

As mentioned above, enzymatic treatment and ultrasonic treatment had potential to improve rice quality. Therefore, this study aimed to use alpha-amylase and protease together with ultrasonic treatment to improve texture of cooked Sao Hai rice.

2. Materials and Methods

2.1 Materials

Soa Hai rice (*Oryza sativa* Linn) was bought from Sao Hai, Saraburi Province, Thailand. Rice samples were polished, packed in aluminum foil bag and stored in a refrigerator at 8°C until use.

2.2 Ultrasonic and enzymatic treatment conditions

For ultrasonic treatment**,** rice sample (100 g) was ultrasonically treated in 625 mL water for 0, 1, 3 and 5 min using a sonicator at 40 kHz with 180 W. After the treatment, rice was strained out and spread onto filter cloth for 10 min to allow the surface water to evaporate (Zhang *et al*., 2015). For protease treatment (F: Brenntag ingredients public company limited, Thailand), rice sample (100 g) was treated with 100 ml of protease solution (0.025, 0.05 and 0.1%) in citrate buffer (100 ml) at 55°C and pH = 6 for 20 min. For alpha-amylase treatment (A: Brenntag ingredients public company limited, Thailand), rice sample (100 g) was treated with 100 mL alpha-amylase solution (0.025, 0.05 and 0.1%) in acetate buffer at 65°C and pH = 5.6 for 1 h. After enzymatic treatment, both enzymes were removed by washing the rice grains with water. Then all treated rice samples were dried at ambient temperature for 1 day.

2.3 Determination of rice properties

2.3.1 Determination of moisture contents

Moisture content of rice was determined using an oven method at 105°C (AOAC, 2000). The measurement was conducted in triplicate.

2.3.2 Determination of whiteness

Whiteness of rice was determined using a spectrophotometer (Minolta, CM-3500d, USA). Measurement was based on the Hunter system with L^* (lightness), a^* (redness) and b^* (yellowness). Then the whiteness index (WI) was calculated by Equation.

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

2.3.3 Determination of cooking time

Optimal cooking time of rice was determined using a glass plate-white center method (Zhang *et al*., 2015). Firstly, distilled water (100 mL) was boiled in a 250 mL beaker on a hotplate. Then rice (5 g) was dropped into the boiling water. After 10 min, 10 grains of rice were removed every 1 min and pressed using a glass plate against a black background until uncooked centers in all kernels disappeared. Then the optimal cooking time was recorded for a specified rice sample (Paiva *et al*., 2016).

2.3.4 Determination of water uptake

Rice (1 g) was dropped into boiling water (15 mL) and wait until it reached the optimal cooking time of rice. Then water uptake ratio was calculated as the ratio of the cooked rice weight to the raw rice weight.

$$\text{Water uptake ratio} = \text{Weight}_{\text{cooked rice}}/\text{Weight}_{\text{raw rice}}$$

2.3.5 Determination of microstructure of rice

Microstructure of cross section and surface of rice was determined using a scanning electron microscope (SEM). After treatment, rice samples were immediately dried using a freeze dryer (Lyolab 3000, Thermo Fisher Scientific Heto, Denmark) until their moisture content was less than 5%. After that the rice samples were attached to a SEM stub. The stub and rice sample were coated with a thin film of gold. Microstructure of rice surface was observed at 50X and 300X.

2.3.6 Determination of pasting properties

Pasting properties of rice flour were analyzed using a Rapid Visco Analyser (RVA) (Newport, RVA-4, Australia). Each rice flour suspension (3 g of rice flour was added into 25 mL of distilled water) was equilibrated at 50°C for 1 min, heated at a rate of 12°C/min to 95°C, maintained at that temperature for 2.5 min, cooled to 50°C at a rate of 12°C/min and then kept for 2 min (Zhang *et al*., 2015). After that, the temperature and viscosity profile was recorded.

2.4 Determination of cooked rice quality

Rice samples (100 g) were mixed with water (up to 2 times of the rice weight). Cooking was done using an electric rice cooker (Sharp, KSH-206, Thailand) for the optimal cooking time. Thereafter, the cooked rice was kept warm for 5 min before measuring quality of the cooked rice.

2.4.1 Determination of textural profile of cooked rice

Textural profile of cooked rice was determined using a texture analyser (TA.XT. plus, Stable Micro System, England) with a two-cycle compression. Twenty grains of cooked rice were placed on the objective table and compressed with a 10-cm diameter probe. The pre-test, test and post-test speeds were set at 1.0, 0.5 and 0.5 mm/s, respectively (Horrungsiwat and Therdthai, 2015). The compression distance was 90% of the cooked rice grain. Hardness, adhesiveness, cohesiveness and chewiness of the cooked rice grains were recorded.

2.4.2 Determination of whiteness of cooked rice

Whiteness of cooked rice was determined using a spectrophotometer (Minolta, CM-3500d, USA). Measurement was based on the Hunter system with L*, a* and b*. The whiteness index was calculated using Equation.

2.4.3 Sensory evaluation of cooked rice

Sensory evaluation of cooked rice was carried out using a 9-point hedonic scale. Scores 1 to 9 were from extremely dislike to extremely like in quality, respectively. Untrained panelists (n=50) were used to test the rice samples. Cooked rice samples (10 g) were served at 60°C. Experiment was carried out twice.

2.5 Statistical analysis

Data were analyzed using SPSS statistics 12.0 version. The differences between the mean values of sample were determined by Duncan's multiple-range test (DMRT) at a level of 0.05.

3. Results and Discussion

3.1 Physical properties of Soa Hai rice from ultrasonic treatment

Figure 1 shows images of rice with ultrasonic treatment at 40 kHz with 180 W power for 1, 3 and 5 min, respectively. The surface of rice grains lost natural morphology and became cracked. After cooking, the ultrasonic treated rice was fractured (Figure 2). The degree of fractures was increased when treatment time was increased from 1 to 5 min. It is possible that ultrasonic treatment at 40 kHz affected microstructure of rice by cavitation (Cui *et al*., 2010). Therefore, cooking quality of the rice from ultrasonic treatment was not further determined.

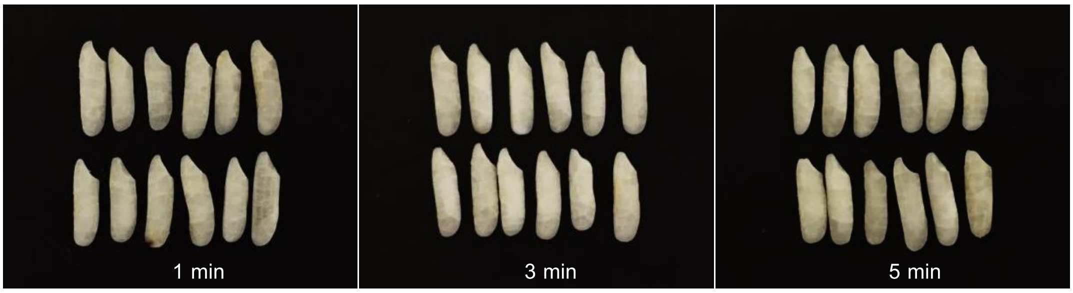

Figure 1 Appearance of rice grain from ultrasonic treatment

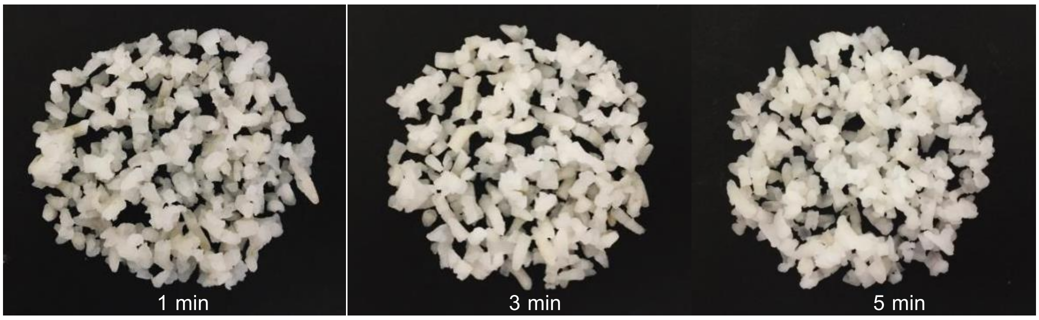

Figure 2 Appearance of cooked rice from ultrasonic treatment

3.2 Properties of Soa Hai rice from enzymatic treatment

Moisture content of untreated rice was about 10.74%w.b. After enzymatic treatment, the moisture content of rice was increased to 12.30–12.54%w.b. (Table 1). With protease treatment, water uptake ratio of rice was improved. In fact, the water uptake ratio was increased from 3.86 to 4.00 with the increased concentration of protease from 0.05%w/w to 0.1%w/w ($p \leq 0.05$). This was because protease hydrolyzed the protein in the starch-protein matrix of rice grain. Thus water absorbability of rice starch was not obstructed by protein (Katekhong, 2013). On the other hand, rice from the alpha-amylase treatment had the decreased water uptake ratio ($p \leq 0.05$). This was because amylose chain was cut into a short chain by alpha-amylase. The short chain of amylose could not form hydrogel (Walter, 1998). Thus the water absorbability of the alpha-amylase treated rice was reduced.

After enzymatic treatment, optimal cooking time was significantly reduced from 20.33 min (untreated rice sample) to 13.00–15.67 min. It is possible that the morphology of rice surface was changed during the enzymatic treatment and influenced on cooking time of rice (Cui *et al*., 2010). This was consistent to the microstructure from SEM. According to SEM, the surface of rice was changed to become rough and porous, compared with the control (Figure 3). This could facilitate water penetration in rice kernels during cooking, resulting in the short cooking time. However, some large cracking was observed in the treated samples, particularly the rice sample from the protease treatment.

Regarding color, after enzymatic treatment, yellowness of rice was significantly decreased ($p \leq 0.05$), particularly with the increased concentration of enzyme from 0.025%w/w to 0.05%w/w. In contrast, whiteness index of rice was significantly increased (Table 2). This was because the protein in starch granules influenced on high yellowness of rice grains. When the protein was hydrolyzed by protease into peptide and amino acid and released from rice grains, the yellowness of rice could be decreased (Pitiphunpong, 2007). Likewise, amylose in rice was hydrolyzed by alpha-amylase and released from the protein-starch matrix, resulting in the increased whiteness index and decreased yellowness.

According to RVA, pasting temperature of the rice flour from enzymatic treatment was lower than that of the untreated rice flour ($p \leq 0.05$). As a result, starch granule was easily swollen; as well as, the peak viscosity significantly increased at low temperature, especially the rice flour from alpha-amylase treatment (Table 3). This was because branch chain of amylose in starch granule was hydrolyzed by alpha-amylase. The internal bond of rice starch became weak. Therefore, swelling and solubility of starch granule could be increased (Sriroth and Piyachomkwan, 2007). Likewise, swelling and peak viscosity of the rice flour from protease treatment were higher than those of the untreated rice flour. This was because protein was hydrolyzed, resulting in the increased opportunity of starch to react with water. Then water absorbability could be increased. In contrast, setback of the treated rice flour was significantly decreased, compared with the untreated rice flour. Therefore retrogradation was hardly occurred. Consequently, texture of cooked rice should not be hard.

Table 1 Moisture content, water uptake, and optimal cooking time of rice from enzymatic treatment

Sample	Moisture content (% wb)	Water uptake ratio	Optimal cooking time (min)
Control	10.74±0.04[d]	3.17±0.04[c]	20.33±0.58[a]
F-0.025	12.39±0.12[bc]	3.85±0.03[b]	13.00±0.00[d]
F-0.05	12.46±0.05[ab]	3.86±0.11[b]	13.33±0.58[cd]
F-0.1	12.30±0.12[c]	4.00±0.07[a]	14.33±0.58[c]
A-0.025	12.43±0.06[abc]	2.91±0.03[d]	14.33±0.58[c]
A-0.05	12.35±0.04[bc]	2.91±0.13[d]	14.33±0.58[c]
A-0.1	12.54±0.06[a]	2.68±0.06[e]	15.67±0.58[b]

Note: Mean values ± standard deviation. means followed by a different superscript letters in column are significantly different ($p \leq 0.05$). Untreated rice (control), Protease (F); 0.025, 0.05 and 0.1% w/w, Alpha-amylase (A); 0.025, 0.05 and 0.1% w/w

Table 2 Yellowness (b*-value) and whiteness index of rice grain from enzymatic treatment

Sample	b*-value	Whiteness index
Control	18.07±0.08[a]	68.23±0.06[f]
F-0.025	15.80±0.18[b]	74.30±0.12[e]
F-0.05	14.51±0.34[c]	75.65±0.25[d]
F-0.1	14.54±0.34[c]	75.90±0.39[cd]
A-0.025	14.35±0.12[c]	76.05±0.08[c]
A-0.05	12.81±0.03[d]	78.27±0.02[b]
A-0.1	12.54±0.06[d]	78.82±0.07[a]

Note: Mean values ± standard deviation. means followed by a different superscript letters in column are significantly different ($p \leq 0.05$). Untreated rice (control), Protease (F); 0.025, 0.05 and 0.1 % w/w, Alpha-amylase (A); 0.025, 0.05 and 0.1 % w/w

Table 3 RVA Viscosity of rice with enzymatic treatment

Sample	Viscosity (RVU)					Pasting temperature (˚c)
	Peak viscosity	Trough	Breakdown	Final viscosity	Setback	
Control	247.17±1.65[a]	188.25±3.18[b]	58.92±1.53[b]	444.71±14.55[a]	256.46±17.73[a]	84.63 ± 0.04[a]
F-0.025	257.59±0.23[a]	200.71±1.12[a]	56.88±1.35[b]	376.54±0.65[b]	175.83±0.47[b]	81.93 ± 0.39[b]
F-0.05	252.25±10.37[a]	192.5±2.47[b]	59.75±7.89[b]	375.2±11.84[b]	182.70±9.37[b]	81.90 ± 0.57[b]
F-0.1	255.29±2.06[a]	189.75±4.95[b]	65.54±2.89[b]	368.91± 2.24[b]	179.17±2.71[b]	81.15 ± 0.64[b]
A-0.025	103.96±1.00[b]	17.59±1.53[c]	86.38±0.53[a]	24.88±2.18[c]	7.29±0.65[c]	81.63 ± 0.04[b]
A-0.05	93.29±2.53[c]	5.46±0.41[d]	87.83±2.12[a]	7.46±0.76[d]	2.00±0.35[c]	79.00 ± 0.07[c]
A-0.1	63.46±2.42[d]	0.00±0.59[d]	63.46±1.82[b]	0.88±0.88[d]	0.88±0.29[c]	79.43 ± 0.60[c]

Note: Mean values ± standard deviation. means followed by a different superscript letters in column are significantly different ($p \leq 0.05$). Untreated rice (control), Protease (F); 0.025, 0.05 and 0.1% w/w, Alpha-amylase (A) ; 0.025, 0.05 and 0.1% w/w

3.3 Quality of Soa Hai cooked rice from enzymatic treatment

According to texture analysis, the cooked rice from enzymatic treatment had significantly lower hardness than the cooked and untreated rice, especially the cooked rice from alpha-amylase treatment (Table 4). This was because molecule of amylose, which was the major component of rice grains were hydrolyzed. The alpha-amylase hydrolyzed amylose molecules at 1,4-glycosidic bond; as a result, the long chain of amylose became short (DP was around 3–9) (Katekhong, 2013). That could decrease the crystallinity of starch granules and obstruct retrogradation of starch (Buranapanichpun, 2001). Moreover, the cooked rice from alpha-amylase treatment had significantly higher adhesiveness than the cooked rice from protease treatment and the cooked and untreated rice. In the case of protease treatment, protein was hydrolyzed, resulting in degradation and removal of proteins in rice grains that would reduce protein's intermolecular between the starch-protein complex formation. This possibly increased the water absorbability of starch (Saleh and Meullenet, 2007). Therefore, starch granules in rice grains could be easily swelled. The hardness of the cooked rice from protease treatment was decreased.

Regarding color of the cooked rice, yellowness of the cooked rice from enzymatic treatment was significantly decreased. In contrast, whiteness index of the cooked rice was significantly increased (Table 5). However, an increase in protease concentration from 0.025%w/w to 0.1%w/w did not affect yellowness. In contrast, the increased concentration of alpha-amylase significantly increased yellowness of the cooked rice. That was because of an increase in glucose (from alpha-amylase hydrolysis) which was possible to enhance maillard reaction. Appearance of the cooked rice with protease and alpha-amylase (A) at 0.025, 0.05 and 0.1% w/w was presented in Figure 4. The cooked rice from protease treatment had some cracking evidence which was consistent to microstructure from SEM (Figure 3 B, C, D). Therefore, only rice from alpha-amylase treatment was selected for further sensory analysis.

From sensory evaluation, liking scores of adhesiveness, softness, sweetness and overall taste were significantly improved when rice was treated by alpha-amylase ($p \leq 0.05$). Moreover, overall liking score was increased to the range of slightly like (6.1–6.6). However, an increase in concentration of alpha-amylase to 0.1% tended to reduce the overall liking score (Table 6). This was too low chewiness and too sweet from too much degree of hydrolysis.

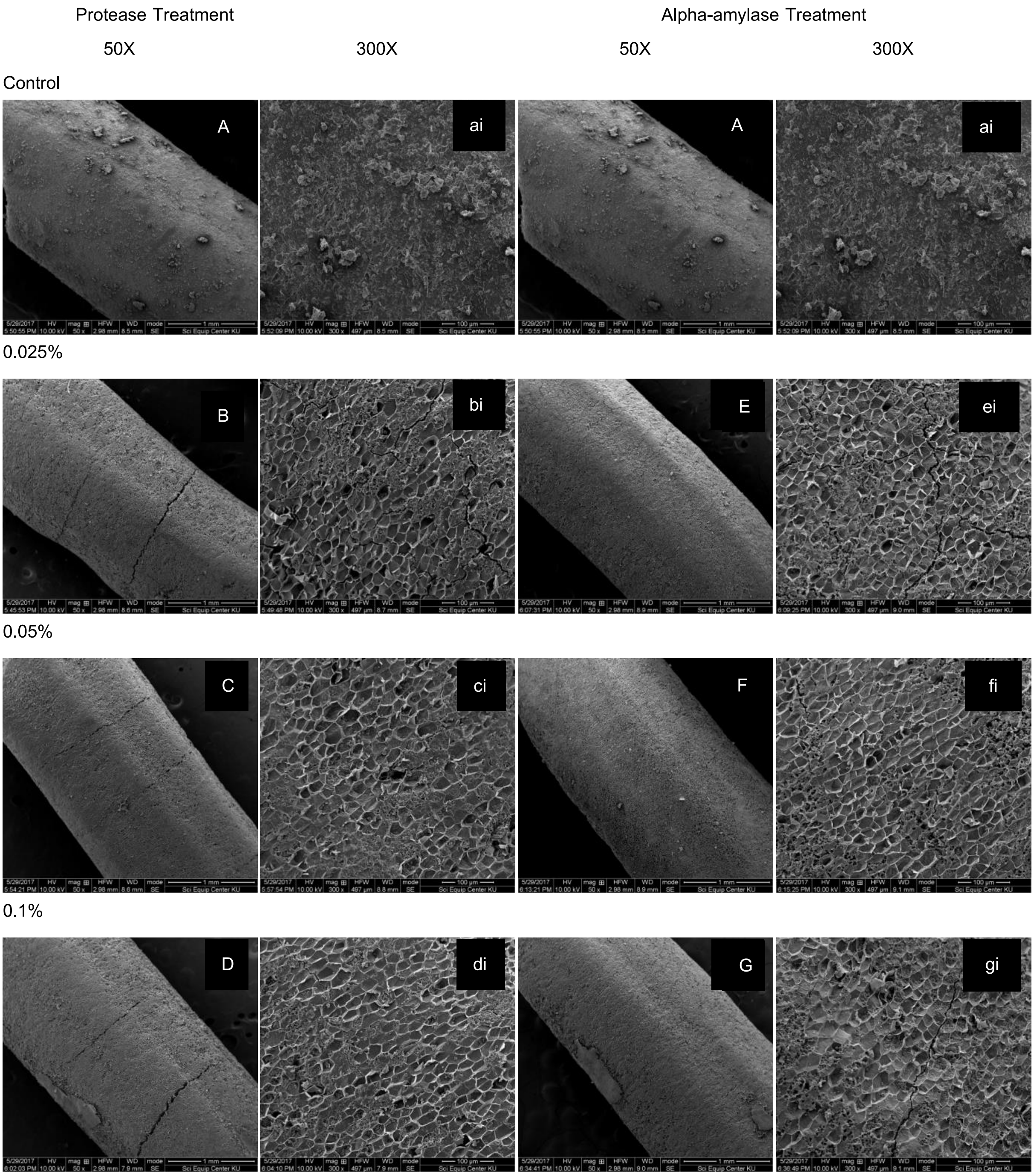

Figure 3 SEM of the surface of rice: Untreated rice (control), Protease; 0.025, 0.05 and 0.1% w/w, Alpha-amylase; 0.025, 0.05 and 0.1 % w/w

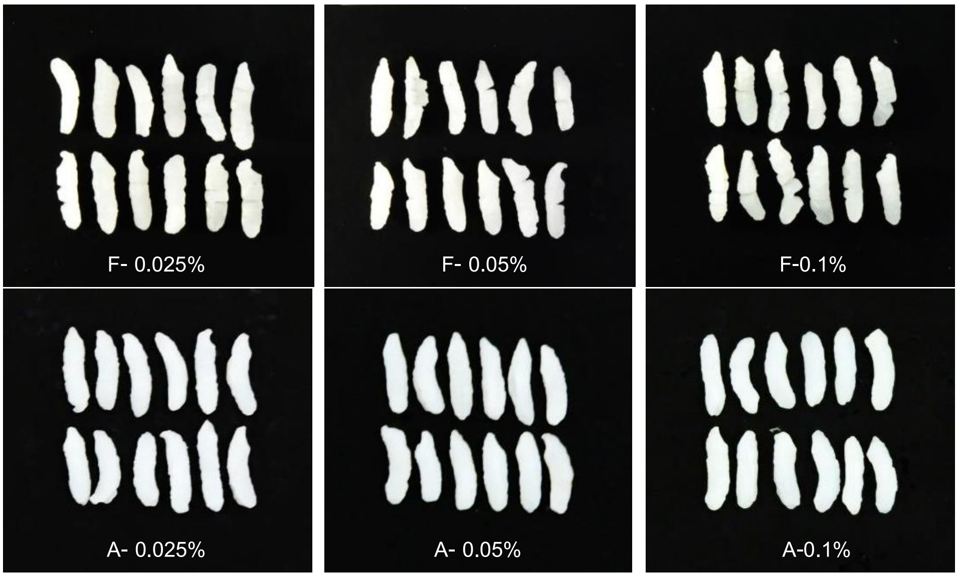

Figure 4 Appearance of cooked rice from Protease treatment (F); 0.025, 0.05 and 0.1 % w/w and cooked rice from Alpha-amylase treatment (A); 0.025, 0.05 and 0.1% w/w

Table 4 Textural properties of cooked rice

Sample	Hardness (N)	Adhesiveness (g.sec)	Cohesiveness	Chewiness
Control	233.15 ± 3.73[a]	-31.64 ± 3.25[a]	0.61 ± 0.01[c]	92.14 ± 4.46[a]
F-0.025	182.62 ± 4.73[b]	-78.18 ± 5.01[b]	0.63 ± 0.01[bc]	84.54 ± 2.52[b]
F-0.05	179.22 ± 3.57[b]	-84.72 ± 5.52[b]	0.63 ± 0.01[ab]	67.01 ± 2.55[c]
F-0.1	172.94 ± 5.22[c]	-108.50 ± 0.84[c]	0.64 ± 0.01[a]	63.77 ± 4.87[c]
A-0.025	156.34 ± 3.45[d]	-401.25 ± 6.75[d]	0.54 ± 0.01[d]	49.40 ± 2.65[d]
A-0.05	133.71 ± 3.79[e]	-424.81 ± 8.34[e]	0.50 ± 0.00[e]	38.09 ± 3.02[e]
A-0.1	117.07 ± 0.98[f]	-545.95 ± 10.25[f]	0.49 ± 0.01[e]	33.50 ± 0.55[f]

Note: Mean values ± standard deviation. means followed by a different superscript letters in column are significantly different ($p \leq 0.05$). Untreated rice (control), Protease (F); 0.025, 0.05 and 0.1% w/w, Alpha-amylase (A); 0.025, 0.05 and 0.1% w/w

Table 5 Yellowness (b*-value) and whiteness index of cooked rice from enzymatic treatment

Sample	b*-value	Whiteness index
Control	12.39 ± 0.14[a]	71.07 ± 0.10[d]
F-0.025	7.30 ± 0.21[e]	74.40 ± 0.05[a]
F-0.05	7.37 ± 0.16[e]	73.99 ± 1.13[ab]
F-0.1	7.69 ± 0.46[e]	73.15 ± 0.83[bc]
A-0.025	8.09 ± 0.12[d]	74.46 ± 0.05[a]
A-0.05	9.12 ± 0.05[c]	73.43 ± 0.47[abc]
A-0.1	10.30 ± 0.02[b]	72.60 ± 0.07[c]

Note: Mean values ± standard deviation. means followed by a different superscript letters in column are significantly different ($p \leq 0.05$). Untreated rice (control), Protease (F); 0.025, 0.05 and 0.1% w/w, Alpha-amylase (A); 0.025, 0.05 and 0.1% w/w

Table 6 Liking scores of cooked rice

Attribute of cooked rice	Control	A-0.025	A-0.05	A-0.1
Whiteness	6.8 ± 1.0^{a}	6.9 ± 1.1^{a}	6.6 ± 1.2^{ab}	6.4 ± 1.3^{b}
Adhesiveness	5.6 ± 1.6^{b}	6.2 ± 1.5^{a}	5.8 ± 1.8^{ab}	6.0 ± 1.5^{ab}
Softness	4.9 ± 1.7^{b}	6.2 ± 1.7^{a}	6.2 ± 1.8^{a}	6.4 ± 1.4^{a}
Sweetness	6.0 ± 1.3^{b}	6.5 ± 1.1^{a}	6.6 ± 1.1^{a}	6.3 ± 1.4^{ab}
Overall taste	6.0 ± 1.5^{b}	6.7 ± 1.3^{a}	6.6 ± 1.5^{a}	6.4 ± 1.5^{a}
Overall liking	5.6 ± 1.5^{b}	6.6 ± 1.3^{a}	6.4 ± 1.7^{a}	6.1 ± 1.6^{ab}

Note: Mean values ± standard deviation. means followed by a different superscript letters in row are significantly different ($p \leq 0.05$). Untreated rice (control), Alpha-amylase (A); 0.025, 0.05 and 0.1% w/w

4. Conclusion

Ultrasonic and enzymatic treatments had significant effect on properties and cooking quality of Soa Hai rice (the high amylose rice). With ultrasonic treatment at 40 kHz, morphological structure of rice surface was changed, resulting in cracking on grain from the cavitation during ultrasonic treatment. With the enzymatic treatment, moisture content, water uptake ratio, optimal cooking time, textural profiles and whiteness index of rice were significantly different from control (untreated rice). In fact, alpha-amylase could hydrolyze starch at junction zone. Therefore, starch molecule could not form hard gel. Hardness of the cooked rice was reduced. Moreover, gelatinization temperature of rice starch was decreased. From sensory analysis, liking score could be significantly improved, particularly liking score on softness, sweetness and adhesiveness. The overall liking score was in the range of slightly like. Therefore, the enzymatic treatment with 0.025–0.05% alpha-amylase would be recommended to improve properties and cooking quality of high amylose rice.

Acknowledgements

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