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Proteolysis and Lipid Oxidation during 'Mu Som' Fermentation as Affected by Different Ratios of White and Black Glutinous Rice

Prassanee Tubbiyam[1] Manat Chaijan[2] Siriporn Riebroy Kim[1]* Amporn Jamphon[1] Taweesak Techakriangkrai[1] and Tipakorn Muangteuk[1]

[1] Major in Food and Nutrition, Department of Home Economics, Faculty of Agriculture, Kasetsart University

[2] Food Technology and Innovation Center of Excellence, Department of Agro-Industry, School of Agricultural Technology, Walailak University

[1] 50 Chatuchak, Bangkok 10900 Thailand

[2] 222 Thaiburi, Nakhon Si Thammarat 80161 Thailand

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Abstract

Changes in muscle protein proteolysis and lipid oxidation of 'Mu Som' added with different white and black glutinous rice ratios (w:w) at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100) during 72 h fermentation at 30°C were investigated. During 36 h fermentation, B100 exhibited a higher rate of fermentation than others as indicated by lowered pH and lactic acid production ($p<0.05$). Due to higher acid production rate, the proteolysis of muscle proteins as evidenced by the increase in trichloroacetic acid (TCA)-soluble peptides and smaller molecular weight of proteins appeared as electrophoresis were more pronounced. These might contributed to higher released water and expressible water as well as pale color. Higher black glutinous tice, higher released water and expressible water were observed. The lipid oxidation in all samples was observed during fermentation. All samples had no difference in likeness scores of all attributes ($p>0.05$). From the results, the ratio of white and black glutinous rice might contribute to proteolysis which effected on released water of Mu Som.

Keywords : Mu Som; Black Glutinous Rice; Proteolysis; Lipid Oxidation

**** Corresponding Author. Tel.: +662 579 5514 ext.128, E-mail Address: siriporn.r@ku.th***

1. Introduction

Proteolysis and lipolysis in fermented meat has been widely studied. These are influenced by many factors such as product formulation, starter culture and processing condition. Generally, the fermentation of meat could be achieved by lactic acid bacteria to pH lowering and other secondary metabolites against undesirable microorganisms such as spoilages and pathogens. For meat fermentation, the use of sugars (dextrose, glucose, sucrose/ lactose, corn syrup, and starches) by lactic acid bacteria (LAB) to assure rapid acidification has become common practice [1]. In Thailand, cooked rice and cooked glutinous rice are usually used as ingredients for pork fermentation. These are substrate for lactic acid production through the glycolysis [2]. The decrease in pH leads to changes in its characteristics and has the impact on proteolysis and lipid oxidation of resulting products [3]. Proteolysis and lipolysis originated from endogenous muscular enzymes, such as cathepsins, which are active at the pH range found in fermented sausage or from microbial enzymes were reported [4]. L.T. Yin et al. [5] concluded that the decrease in pH not only gives the product a unique lactic acid flavor, but also increases the firmness of texture and mouth feeling due to the acid denaturation and degradation of muscle proteins. Changes in lipid during pork fermentation have been studied. J.M. Aro Aro et al. [6] reported that proteolysis of sarcoplasmic and myofibrillar proteins were observed in sausage during fermentation.W. Visessanguan et al. [7] reported that lipid oxidation occurred during Thai pork sausage (Nham) fermentation. However, changes in lipid oxidation during fermentation of Mu Som have not been reported.

Mu Som is one of fermented pork, which is produced from sliced pork meat (or belly or tendon) mixed with salt, cooked rice and chopped garlic. In general, cooked rice or glutinous rice and other adjuncts can be added as a carbon source for lactic acid fermentation. The mixture is generally fermented in sealed packages until pH decrease to lower than 4.6 and sourness. Fermented Mu Som (pH < 4.6) is usually consumed by deep frying [8]. The amount of carbon source used is very important for fermentation rate. The fermented meat product is slightly sour and salty and is of a relatively firm and tender texture. Recently, P. Tubbiyam et al. [9] reported that Mu Som produced from cooked glutinous rice showed a greater acceptability than those produced from cooked rice. The proteolysis and lipid oxidation occurred during Mu Som fermentation. These phenomena usually influence on the perception of fermented Mu Som. Nowadays, consumers pay a lot of attention to the relationship between food and health. Regarding pigmented rice in Thailand, black glutinous rice has been paid more attention due to its composition such as anthocyanin, gamma oryzanol, vitamins and iron [10]. The applications of black glutinous

rice are both colored pigment as food and pharmaceutical ingredients [11]. Black glutinous rice is excellent source of phenolic compounds, which can act as antioxidant. Due to the increasing demand for Mu Som in the market, a larger amount of Mu Som has been produced continuously. Nevertheless, no information on the use the black glutinous rice for Mu Som production has been reported. Therefore, the objectives of this study were aimed to monitor changes in proteolysis and lipid oxidation of Mu Som produced from different ratio of cooked white and black glutinous rice.

2. Research Methodology

2.1 Preparation of Cooked Glutinous Rice

White and black glutinous rice, Raitip®, were purchased from supermarket. One kilogram of glutinous rice was washed with tap water then strained and soaked with water at ratio of glutinous rice: water was 1:2 (w:w) at ambient temperature for 8 h. After drainage, the soaked glutinous rice was added with water at ratio of 1:1 (w:w) then transferred to the rice cooker (Sharp, Thai City Electric Co. Ltd., Thailand). Both cooked white and black glutinous rice were allowed to stand at room temperature before use (30 min).

2.2 Preparation of Mu Som

Mu Som was prepared as described by P. Tubbiyam et al. [9]. Stripped pork (1cm × 6 cm × 1 cm) (100 g) was added with salt (2 g), chopped garlic (5 g). The different ratio of white and black glutinous rice was added as follows: 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W20B75) and 0:100 (100B). After the ingredients was thoroughly mixed, then stuffed into plastic bags (approximately 100 g each) and sealed tightly before incubation at 30ºC for 72 h. Samples were taken every 12 h for microbiological, chemical and physical analyses.

2.3 Microbiological Analysis

Total viable count (TVC) and lactic acid bacteria were determined using plate count agar (PCA) and Man Rogosa Sharpe (MRS), respectively [12]. TVC and LAB counts were reported as log colony-forming unit (CFU)/g sample.

2.4 Determination of pH and Total Acidity (TA)

The pH of the sample was determined according to the method of S. Benjakul et al. [13]. The TA was determined by the method of [12]. The TA was calculated as lactic acid and expressed as percent TA (w/w).

2.5 Determination of Trichloroacetic acid (TCA)-soluble Peptides

TCA-soluble peptides were determined according to the method described by D.H.Greene and J.K. Babbit [14]. Sample (3 g) was homogenized with

27 ml of 5% TCA (w/v). The homogenate was kept in ice for 1 h and centrifuged at 12,000×*g* for 5 min. The soluble peptides in the supernatant were measured by the method of Q.H.Lowry et al. [15] and expressed as mmole tyrosine/g sample.

2.6 Sodium Dodecyl Sulfate –Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Sample (3 g) was homogenized with 27 ml of solubilizing agent (2% SDS, 8 M urea and 2% β-mercaptoethanol). The homogenate was heated at 85ºC for 1 h, followed by centrifugation at 10,000×g for 15 min at room temperature. The protein concentration of supernatant was determined by the Lowry method [15]. SDS-PAGE was performed using 4% stacking gel and 10% running gel according to the method of U.K. Laemmli [16] with a vertical gel electrophoresis unit (Mini-Protein II; Bio-Rad Laboratories, Richmond, California, USA).

2.7 Determination of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined according to the method of J.A. Buege and S.D. Aust [17]. TBARS value was calculated from the standard curve of malonaldehyde (MDA) and expressed as mg MDA/kg sample.

2.8 Determination of Released Water, Expressible Water and Water Holding Capacity

The percentage of water released from sample was determined according to the method of Y. Nakao et al. [18]. The sample in package was weighed (A) and then removed from the casing. The water released on its surface was wiped with filter paper (Whatman No. 4) and the sample was then reweighed (B). The empty casing was weighed (C). The percentage of released water was calculated according to the following equation:

$$\text{Released water}\,(\%) = 100 \times \{(A\text{-}B) - C\}/(A - C)$$

Expressible water of samples was measured according to the method of T. Funami et al. [19] as modified by W. Visessanguan et al. [20]. The expressible water was determined as the weight loss after the compression of sample. The expressible water content was calculated as the ratio of the apparent expressible water to the total moisture content of the Mu-Som according to the following equations:

Expressible water (%) =
[100 × Apparent expressible water content] /Total moisture content
Where Apparent expressible water content $= 100 \times (W_{before} - W_{after})$,

W_{before} = weight before compression;

W_{after} = weight after compression.

Water holding capacity of samples was determined according to the method of R. Ofstad et al. [21] with a slight modification. Sample (15 g) was wrapped with double layers cheesecloth and transferred onto centrifugation tube. Sample was centrifuged at 324×*g* (4°C) for 15 min and it was reweighted. The water holding capacity was calculated and expressed as % by weight of sample before centrifugation.

2.9 Determination of Color

The color of sample was measured in the L*, a*, b* mode of CIE (Angle 101, illuminant D65) using a HunterLab (ColorFlex, Hunter Associates Laboratory, Reston, VA, USA).

2.10 Sensory Evaluation

Fermented Mu Som samples (pH < 4.6) were evaluated for acceptance by an untrained 50-member panel. The panelists were staffs and students in the Department of Home Economics, of age ranging from 22 to 45 years, Faculty of Agriculture, Kasetsart University. Panelists had sensorial acquaintance with lactic acid fermented pork. A nine-point hedonic scale, in which a score of 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation [22]. Samples were prepared by deep-frying for 5 min in palm oil at 140ºC using an electric deep fryer. Individual samples of each sample were placed on dish (diameter 3.0 cm) and the samples were covered with aluminium foil. Samples were randomly selected and coded with three-digit random numbers and presented to the panelists at room temperature. During evaluation, the panelists were situated in private booths. Room temperature water was given to rinse the mouth between samples. The panelists evaluated each sample for appearance, color, texture, taste, flavor, and overall liking.

2.11 Statistical Analysis

Analysis of variance (ANOVA) was performed and comparisons between means were analyzed by Duncan's multiple range test [23].

3. Results and Discussion

3.1 Changes in TVC and LAB Counts during Mu Som Fermentation

Changes in TVC and LAB counts during Mu Som fermentation are shown in **Fig. 1A** and **Fig. 1B**, respectively. TVC and LAB counts were greatly increased during the first 24 h of fermentation. LAB counts of Mu Som added with 100% black glutinous rice (B100) was higher than other samples. As fermentation time proceeded, both TVC and LAB were about 9 log CFU/g. Additionally, LAB was mainly observed during Mu Som fermentation. LAB were also suggested as the mainly role in fermented meat [20]. In general, antimicrobial activity of plant phenolic compounds against pathogens has been studied. A. Cisowska

et al. [24] reported that Gram-positive bacteria usually are more susceptible to the phenolic acid and anthocyanin actions than Gram-negative bacteria. From the results, the present of anthocyanin in Mu-Som formula would rather to give an opportunity for LAB growth. From the results, higher LAB count (First 24 h fermentation) might be resulted from different compositions between white and black glutinous rice.

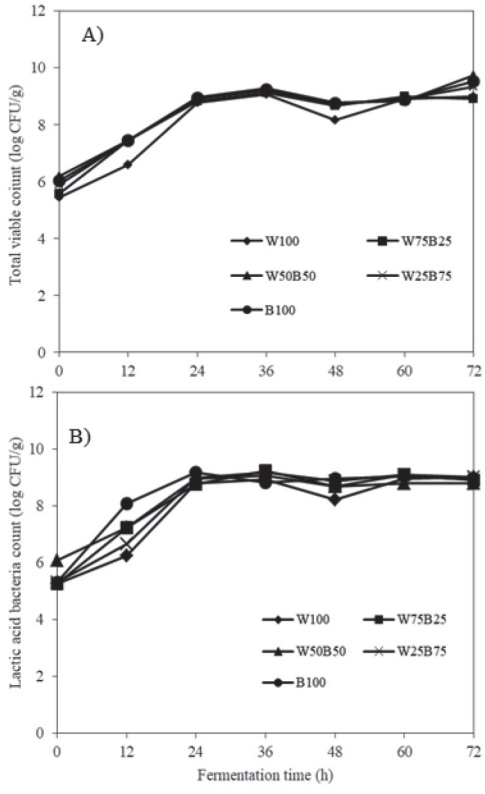

Fig. 1 Changes in total viable count (A) and lactic acid bacteria (B) during fermentation of Mu-Som added with white and black glutinous ratio at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100)

3.2 Changes in pH and Total Acidity (TA)

Changes in pH and TA of Mu Som added with different ratios of white and black glutinous rice during fermentation are shown in **Fig. 2A** and **Fig. 2B**, respectively. The initial pH of both samples was about 6. The increase in TA was generally accompanied by the decrease in pH value throughout the fermentation. The pH value of Mu Som must be lower than 4.6 as determination by Thai Community Product Standard (TCPS) no.876/2557 [8]. The pH of W50B50, W25B75, and B100 decreased to 4.6 within 60 h, while those of W100 and W75B25 were slower (72h). During fermentation, LAB convert glucose to lactic acid, which is mainly responsible for pH decrease. The decrease in pH observed during fermentation might be due to lactic acid produced by LAB (**Fig. 1B**). Although cooked white glutinous rice had higher total sugar content than that in cooked black glutinous rice (data not shown), B100 exhibited higher rate of pH decrease and TA increase during 36 h of fermentation. These might be due to the effect of other compounds in black glutinous rice such as antimicrobials from anthocyanin. The present of some antimicrobials can promote the growth of LAB which is lactic acid producer. Generally, lactic acid was the major organic acid in nham [20]. The low-salt fermented meat and fish products always contain a carbohydrate source, and rich

starch is assumed to be a substrate for fermentation [25]. Saisith et al. [25] reported that the addition of cooked rice to Som-fug (fermented fish mince) caused a gradual decrease in pH. Paludan-Müller et al. [26] reported that garlic added to Som-fug served as an important carbohydrate source (Fructans) for the fermentation and showed antimicrobial activity. Generally, the combination of low pH and organic acids (Mainly lactic acid) is the main preservation factor in fermented fish products. From the results,

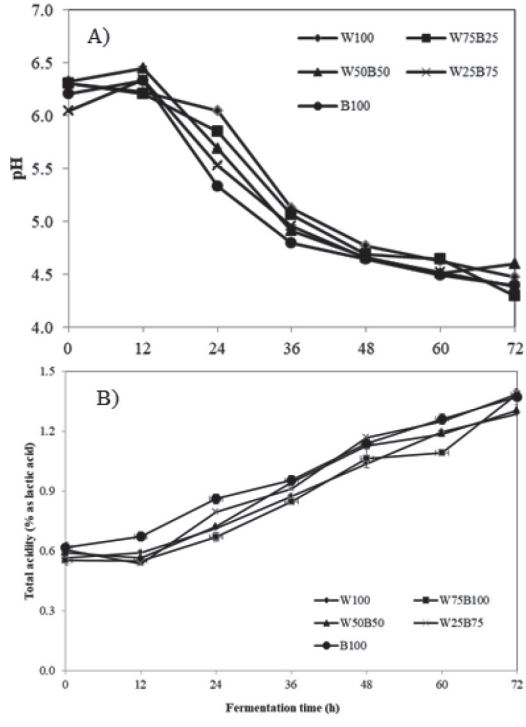

Fig. 2 Changes in pH (A) and total acidity (B) during fermentation of Mu-Som added with white and black glutinous ratio at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100)

the pH decreasing rate of samples added with higher 50% of black glutinous rice was about 0.02 unit/h, which was similar to Som-fug fermentation [25]. Additionally, the rapid growth of LAB causing pH to decrease by lactic acid production is essential to prevent to prevent spoilage and pathogen.

3.3 Changes in Proteolysis during Mu Som Fermentation

No difference in TCA-soluble peptides in all samples at initial fermentation ($p>0.05$). After 24 h of fermentation, the increase in TCA-soluble peptides was observed in all samples ($p<0.05$) (**Fig. 3**). The greater TCA-soluble peptides indicated the higher hydrolysis of muscle proteins during fermentation [20], [27]. Catheptic enzymes were active under the acidic condition, leading to the hydrolysis of muscle proteins [26]. After 24 h of fermentation, higher TCA-soluble peptides in Mu Som added with higher portion of black glutinous rice was observed ($p< 0.05$). Additionally, the trend of peptides in all samples was similarity. Degradation of myofibrillar and sarcoplasmic proteins resulted in an increase in peptides, which may be related to the development of flavor and aroma of fermented meat [6], [20]. W. Vissessanguan et al. [20] suggested that low pH values have been reported to stimulate hydrolysis of myofibrillar proteins. The development of flavor was

due to the several low molecular weight compounds, including peptides, amino acids, aldehydes, organic acids, and amines [28], [29].

Electrophoretic pattern of muscle proteins in Mu Som added with different ratios of white and black glutinous rice during fermentation is shown in **Fig. 4**. When the fermentation time increased, myosin heavy chain (MHC) was markedly degraded as observed by the much lowered MHC band intensity with the appearance of protein bands with lower molecular weight. For B100, MHC slightly disappeared after 60 h of fermentation. This might be resulted from pH lowering (**Fig. 2A**) W. Visessanguan et al. [20] and S. Riebroy et al. [27] reported that MHC was the most susceptible to proteolysis during meat and fish fermentation. Both indigenous muscle and microbial proteases contributed to the degradation of muscle proteins [6]. From the results, it was suggested that the more rapid acidification by higher black glutinous rice portion contribute to pH of protease activity.

3.4 Lipid oxidation during Mu Som fermentation

TBARS was used as indices to assess the level of lipid oxidation in meat fermentation [7]. **Fig. 5** shows change in TBARS value of Mu Som

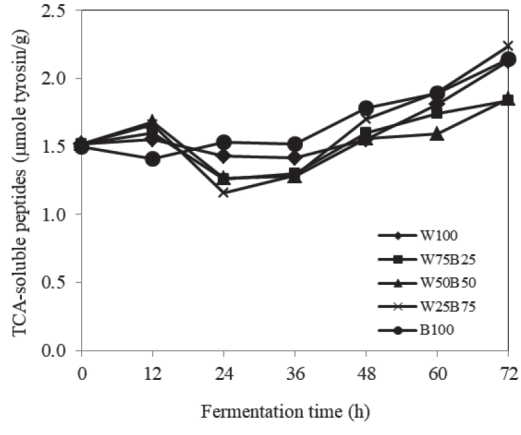

Fig. 3 Changes in trichloroacetic acid (TCA) soluble peptides during fermentation of Mu-Som added with white and black glutinous ratio at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100)

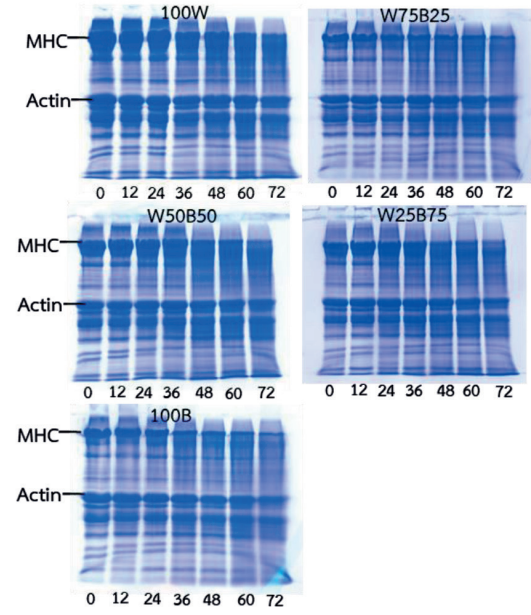

Fig. 4 SDS-PAGE pattern of muscle proteins in Mu Som added with white and black glutinous ratio at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100). MHC: Myosin heavy chain

added with different ratios of white and black glutinous rice during 72 h fermentation. At initial of fermentation, TBARS values were 0.49 – 0.53 mg MDA/kg. During preparation, shredding, mincing and mixing of the meat increased the surface area and exposed it to oxygen and oxidation catalysts [30]. This result indicated that oxidation of lipid occurred during processing of Mu Som such as slicing and mixing. After 36 h fermentation, TBARS of all samples tended to be decreased. The highest TBARS value was found in B100 after 24 h fermentation ($p<0.05$).

The antioxidant activity of anthocyanin in black glutinous rice has been reported [31], [32], but the stability of anthocyanin might be limited by pH, temperature, oxygen, and light. Furthermore, free radicals can be produced from iron which was found in black glutinous rice but it was traceable in white glutinous rice [33]. Additionally, the LAB isolated from fermented pork (Nham) produce hydrogen peroxide [34]. W. Visessanguan et al. [7] discussed that lipid oxidation in Nham might be resulted from hem pigment, myoglobin, which is strong prooxidants when they are activated by hydrogen peroxide. Lipid oxidation is responsible for a reduction in nutritional quality as well as changes in flavor [7]. Decrease in TBARS of samples during 24 to 36 h might be resulted from volatile compounds development such as esters and alcohols [35]. From the result, sample added with more black glutinous rice had more lipid oxidation during Mu Som fermentation.

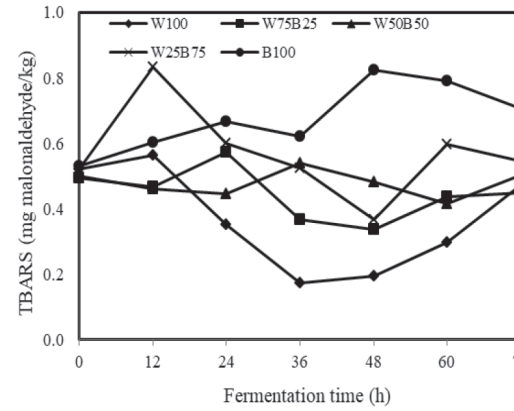

Fig. 5 Changes in TBARS of Mu Som added with white and black glutinous ratio at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100) during fermentation 72 h at 30°C

3.5 Changes in physical properties

Fig. 6 shows released water (A), expressible water (B) and water holding capacity (C) of Mu Som added with different ratios of white and black glutinous rice during fermentation. The increases in released water and expressible water contents of Mu Som samples were observed as the fermentation proceeded. During 72 h of fermentation, the increases in both parameters were observed in all samples ($p<0.05$), while their WHC decreased sharply throughout fermentation. W. Visessanguan et al. [20] explained that the increasing amount of released and expressible water were

probably responsible for the increased weight loss. The amount of released water or expressible water from the meat is an indicator of its water holding capacity [19]. These phenomena might be resulted from pH changes affecting on muscle protein degradation and denaturation, leading to a reduction in their ability to bind water. W. Visessanguan et al. [20] reported that degradation of protein and an increase in the concentration of peptides and amino acids results in an increase in the intracellular osmotic pressure. The results indicated that the ability and retaining water trends of Mu Som were similar though they were added with different ratios of white and black glutinous rice.

L*, a* and b* values of Mu Som added with different white and black glutinous rice ratios are shown in **Fig.7**. As the color of glutinous rice, higher L* in W100 was observed throughout the fermentation ($p<0.05$). No difference in L* value of samples added with black glutinous rice during 24 to 60 of fermentation ($p>0.05$). The difference in a* value of all samples was noticeable throughout the fermentation. Higher black glutinous rice portion showed higher a* value, however, this value decreased obviously during first 12 h fermentation. The lowering pH of samples caused the decreased a*. At the beginning of fermentation, the addition of black glutinous rice to Mu-Som showed higher b* value than that added with white glutinous rice ($p<0.05$). During 12–72 h, all samples had similarly trend of change in b* value. However, the trend of change in b*value was observed throughout fermentation.

Generally, the color of fermented pork could be grayish-pink, depending on the part of meat and amount of blood remaining in the flesh. The characteristic color of meat is a function of the meat pigments and light-scattering properties of the fibers. In general, the pigments responsible for the color of muscle food are myoglobin and hemoglobin. [36]. The growth of LAB influence on the change in color of fermented pork and it can be associated with acid production, protein denaturation, and stability of nitrosomyoglobin [37]. However, nitrite/ nitrate compounds were not used as ingredients/additives in this study. The color of samples was pigments in ingredients such as myoglobin, anthocyanin, and some chemical reactions (non-enzymatic reaction). In addition, sarcoplasmic proteins in muscle, involving myoglobin, are among the most labile proteins in connection with pH and temperature damage [37]. Higher a* value indicated a higher redness. From the results, fermented (at 48 h), Mu Som added with black glutinous rice was more reddish than that of those added with white glutinous rice. This was probably due to the retained pigments though the higher released water. Black glutinous

rice contained two major anthocyanins which are cyaniding-6-*O*-glucoside and peonidin-3-*O*-glucoside [38]. When pH lowering, the flavylium cation (red color) is the predominant compound [39]. Therefore, the acidification contributes to color of anthocyanin in black glutinous

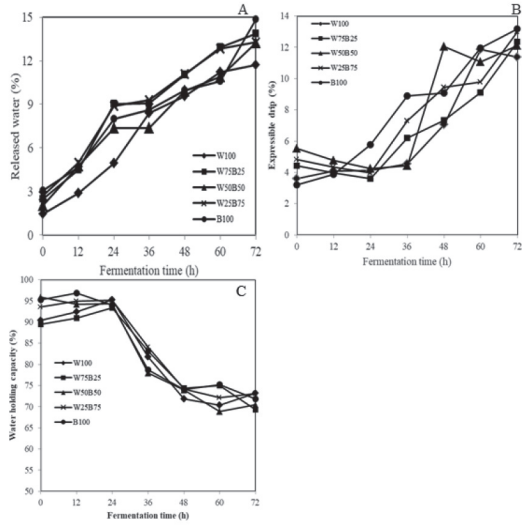

Fig. 6 Changes in released water content (A), expressible water (B) and water holding capacity (C) of Mu Som added with white and black glutinous ratio at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100)

rice during Mu-Som fermentation.

3.6 Sensory evaluation

Table 1 shows likeness scores (9-point hedonic scale) of fried fermented Mu Som (pH < 4.6) added with different ratios of white and black glutinous rice are shown in **Table 1**. No difference in likeness scores in all attributes among samples (p>0.05). P. Tubbiyam et al. [9]

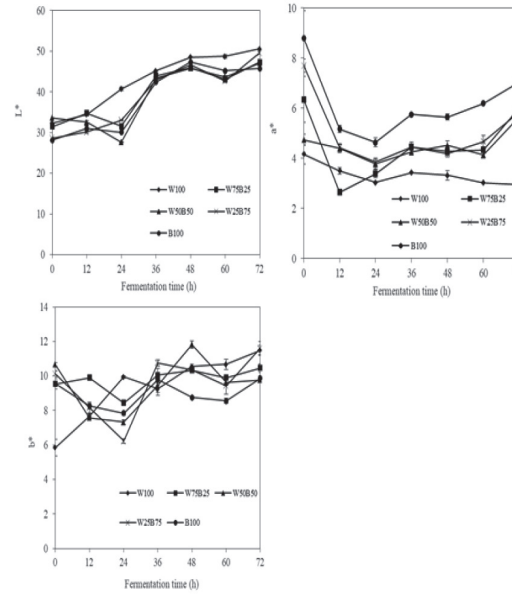

Fig. 7 Changes in L*, a* and b* of Mu Som added with white and black glutinous ratio at 100:0 (W100), 75:25 (W75B25), 50:50 (W50B50), 25:75 (W25B75) and 0:100 (B100)

reported that Mu Som added with glutinous rice had a higher likeness scores, particularly texture likeness score. The firmness and tenderness of fried fermented Mu Som are important for acceptability. These might be related with released water and water holding capacity (**Fig. 6**). According to the pH lowering, the denaturation and degradation of muscle proteins occurred during fermentation and these affected on functional properties, i.e. water holding capacity. Additionally, flavor of fermented Mu Som is due to products from fermentation of carbohydrates, lipolysis and lipid oxidation, proteolysis

and salt. However, different ratios of white and black glutinous rice did not effect on acceptability of fermented Mu Som. According to fermentation, the addition of black glutinous rice to Mu-Som contributed to shorten fermentation time and some characteristics such as lipid oxidation and color. Panelists gave information for all samples evaluated that they could accept all samples due to those were similar even color was different. This could be confirmed that black glutinous rice can be used as an alternative ingredient for

Table 1 Likeness scores of cooked Mu Som (pH <4.6) added with different ratios of white and black glutinous rice

Attributes	Samples*				
	100W	**75W25B**	**50W50B**	**25W75B**	**B100**
Appearance^{ns***}	7.86±0.93**	7.74±0.85	7.78±0.95	7.58±0.97	7.72±0.99
Color^{ns}	7.68±0.92	7.68±0.96	7.64±0.96	7.46±0.86	7.40±0.97
Odor^{ns}	7.76±0.94	7.68±0.96	7.54±0.99	7.52±0.99	7.42±0.99
Taste^{ns}	7.48±0.99	7.78±0.89	7.66±0.92	7.50±0.89	7.52±0.99
Texture^{ns}	7.36±0.90	7.44±0.93	7.32±0.94	7.36±0.96	7.32±0.98
Flavor^{ns}	7.53±0.97	7.97±0.85	7.56±0.88	7.44±0.91	7.44±0.97
Overall liking^{ns}	7.60±0.90	7.70±0.79	7.68±0.96	7.44±0.97	7.60±0.99

*Mu-Som added with white and black glutinous ratio at 100:0 (W100); 75:25 (W75B25); 50:50 (W50B50); 25:75 (W25B75) and 0:100 (B100).

**Mean±SD from fifty evaluations.

***ns: no significant difference (p>0.05)

Mu-Som production.

4. Conclusion

Mu Som with higher ratio of black glutinous rice showed the faster rate of fermentation as shown in LAB growth and pH lowering. This contributed to proteolysis of muscle proteins, resulting in released water and water holding capacity. Lipid oxidation of Mu Som could not be retarded when black glutinous rice ratio increased. However, the likeness scores of all samples were not different.

5. Acknowledgement

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