CHAPTER 7

EFFECTS OF ACCELERATED AGING OF MILLED RICE ON CHANGES IN STARCH GRANULE MORPHOLOGY, THERMAL AND PROTEIN PROPERTIES AND STORAGE STABILITY OF FRESHLY HARVESTED RICE CV. KDML 105

7.1 Introduction

Aging affects various rice physico-chemical properties which are related to changes in cooking and pasting characteristics of stored rice. These changes had been reported to associate with change in protein property (Hamaker and Griffin, 1990; Martin and Fitzgerald, 2002; Fitzgerald, *et al*., 2003; Zhou *et al*., 2003). Although amount of protein in rice does not change considerably during storage, their chemical properties can be substantially altered. This protein influenced many rice attributes, especially paste viscosity (Fitzgerald, *et al*., 2003; Zhou *et al*., 2003) and texture of cooked rice (Chrastil, 1994; Martin and Fitzgerald, 2002). It had been reported that the main storage protein in rice was oryzenin (Sugimoto *et al*., 1986; Chrastil and Zarins, 1992) and its molecular weight increased significantly during storage (Chrastil, 1990b). This increase was correlated with an increase in disulfide bonding between cysteine, the protein sub unit which contain sulfhydryl group (Chrastil and Zarins, 1992). The formation of disulfide bond of the protein sub unit decreased its solubility and could affect pasting property as studied via RVA (Zhou *et al*., 2003). Change in rice protein property that occurs during natural aging may be enhanced by high temperature. Derycke *et al*. (2005a) had demonstrated that proteins impacted on pasting and cooking properties of non-parboiled and parboiled rice. This confirms the effect of high temperature during parboiling process on rice protein property. High temperature had also been reported to influence thermal property (Altay and Gunasekaran, 2006; Jaisut *et al*., 2008) and morphology of rice starch granule (Jaisut *et al*., 2008). However, it was also reported that morphology of starch granule was not affected when low moisture content starches was heated (Anderson and Guraya, 2006; Pukkahuta *et al*., 2007).

In this present study, accelerated aging of freshly harvested milled rice was achieved by high temperature treatment, and thus the rice starch granule and its component would likely be affected. The current experiment was, therefore, conducted to investigate the basis of changes caused by accelerated aging treatments and to determine stability of the treated rice. Investigations were mainly focused on changes in starch granule morphology, thermal and protein properties. Storage stability of the accelerated aging rice in terms of color, textural and pasting properties, 2-acetyl-1-pyrroline and *n*-hexanal quantities was also verified and monitored over a 6-month storage period.

7.2 Materials and Methods

7.2.1 Rice Samples and Preparations

KDML 105 rice sample was obtained from the same lot that was prepared for the experiment conducted in Chapter 6. Preparation of rice samples and accelerated aging treatments were also done as mentioned in Chapter 6. Immediately after aging treatments, all samples including fresh rice (control) were placed into zip-locked plastic bags and kept at -20^{o}C for further analyses. For storage stability investigation, the rice samples were packed and stored as described in section 7.2.5.

7.2.2 Investigation of Morphological and Structural Changes of Rice Starch Granule using Scanning Electron Microscopy

Microscopic study was done to examine change in the structure or morphology of starch granules and gelatinization of the starch that might occur during accelerated aging treatment. Cross section of middle part of the fresh and accelerated aging milled rice kernels including their corresponding flour samples were viewed on a scanning electron microscope (SEM). The samples were mounted on metal stubs using double-sided adhesive tape and coated with gold palladium (~6 nm thickness) using a SPI-MODULE™ Sputter Coater (SPI Supplies® Division of Structure Probe, Inc., Japan). Samples were then observed using a JEOL JSM-5910 LV scanning electron microscope (JEOL Technics LTD., Japan) at an accelerating voltage of 15 kV. The SEM images were captured by automatic image capturing software (SEM Control User Interface Version 5.08, JEOL Technics LTD., Japan) and the magnification was 5,000 times.

7.2.3 Investigation of Thermal Properties of KDML 105 Rice Flour

Thermal properties of freshly harvested and accelerated aging rice flour samples were analyzed using a Differential Scanning Calorimeter (DSC, TA Q100, TA Instruments, Newcastle, DE). The DSC was calibrated with indium and sapphire for temperature and heat capacity values prior to the analysis. Rice flour sample (approximately 4 mg) was accurately weighed into aluminum DSC pan and distilled water was added using a micro syringe to obtain a dry matter to water ratio of 1:2 (w/w). The pan was hermetically sealed and subjected to a presoak period for 45 min for sample equilibration at room temperature, and then transferred to the DSC cell and equilibrated to 35°C. Thermal scan was performed from 35 to 110°C at a heating rate of 2°C/min and the DSC thermogram derived was analyzed as illustrated in Figure 7.1. Determination for thermal transitions in terms of onset (T_o: the

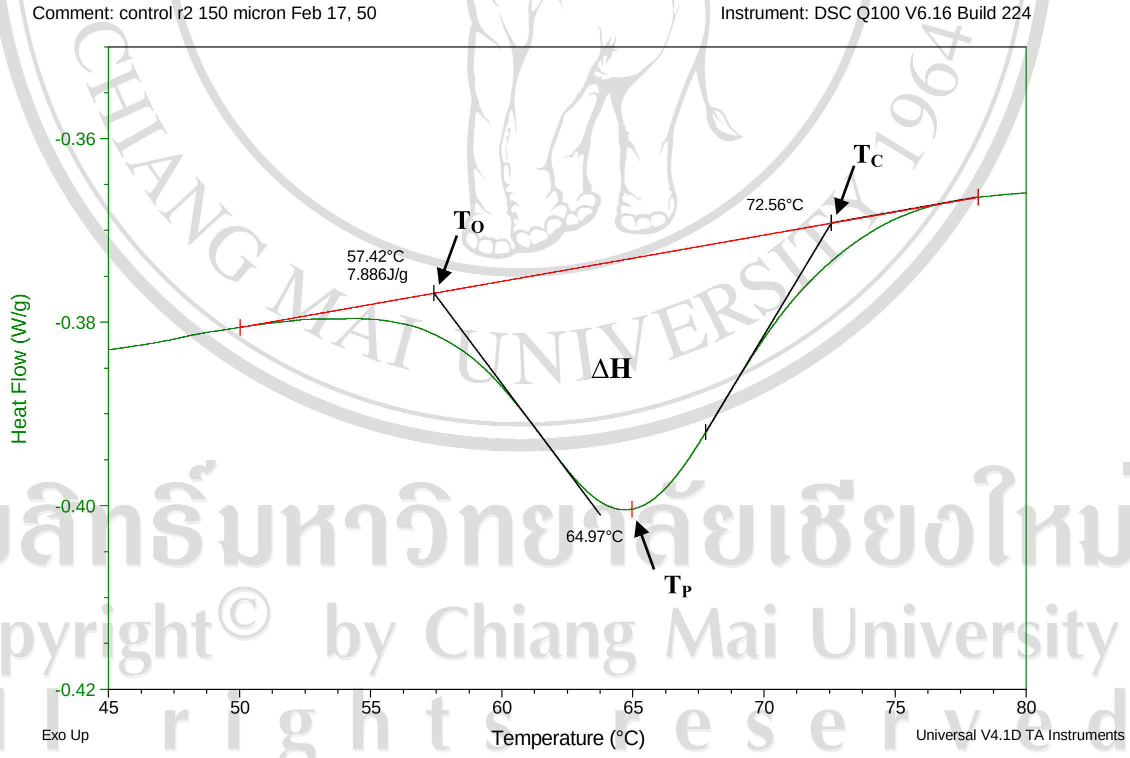


Figure 7.1 The DSC thermogram illustrating phrase transition; onset (T_O), peak (T_P), and conclusion (T_C) temperatures of gelatinization and the gelatinization enthalpy (ΔH) of KDML105 rice flour.

temperature at which the tangential line from the lower temperature side of the peak intersects with the baseline), peak (T_P: the temperature at the tip of the peak), and conclusion (T_C: the temperature at which the tangential line from the high temperature side of the peak intersects with the baseline) temperatures of gelatinization was done and the gelatinization enthalpy (ΔH) in Joules per gram of flour sample (on the basis of 11% MC wb) was calculated from the area of the peak endotherm employing the Universal Analysis 2000 software (version 4.1D, TA Instruments, Newcastle, DE).

An empty pan of similar weight to sample pan was used as reference pan for all samples. In all runs, the space surrounding the sample cells and the outer surfaces of the pans was flushed with dry nitrogen gas at a rate of 50 ml/min in order to prevent condensation on the outside of the cells during the run. Two measurements were performed for each rice sample.

7.2.4 Investigation of Changes in Protein Properties

A Rapid Visco Analyser (RVA) (model 4, Newport Scientific, Warriewood, NSW, Australia) was employed to examine the increases in disulphide bonding of protein sub unit of milled rice samples after accelerated aging treatment. The measurement was performed as described by Hamaker and Griffin (1990). Rice samples were ground to pass through a 0.5 mm screen (Cyclotec 1093 sample mill, Tecator, Hogenas, Sweden) and the resulting flour (3 g on the basis of 12% MC) was poured into a RVA canister and the weight was made up to 28 g with distilled water or solutions containing 10 mM dithiothreitol (DTT, Fluka 43819) or 40 U mg^{-1} proteinase (bovine pancreatic chymotrypsin, Sigma no. C-4129) (as used by Hamaker and Griffin (1990) with modification in the concentration of DTT and proteinase). Samples containing proteinase-treated flour and their corresponding controls were incubated at room temperature at pH 7.2 for 2 hr prior to the RVA measurement. The measurement was performed using temperature profile as follows: initial holding at 50°C 1 min, heating to 95°C in 3.8 min, holding at 95°C for 2.5 min, cooling to 50°C in 3.8 min, and final holding at 50°C 1.4 min. For sample with DTT, the temperature profile described above was altered by extending the initial 1 min holding at 50°C to 5 min holding at 50°C. In the RVA run, the paddle speed was set at 960 rpm in the first 10 sec for homogenizing the sample and then adjusted to 160 rpm. Differences in the RVA parameters between samples (pasting temperature, peak viscosity, trough (viscosity at 95°C after holding), final viscosity (viscosity at 50^{o}C), breakdown (peak viscosity minus trough) and setback (final viscosity minus peak viscosity) were recorded and analyzed.

7.2.5 Investigation of Storage Stability

The effects of the selected accelerated aging treatments were studied in combination with different packaging materials for the storage periods of 6 months. Rice samples (380 g. each) were packed in three packaging materials, namely sealed polyethylene bag, nylon laminated bag and aluminum laminated bag. The bags were closed by hot seaming and the latter two materials were processed under vacuum application. The samples were stored in ambient condition. Storage stability of the rice samples were evaluated in terms of changes in physico-chemical properties such as color, pasting properties and textural properties and aroma quality in terms of amount of 2-acetyl-1-pyrroline and relative amount of *n*-hexanal. Evaluation was done at 2-month interval for the 6-month storage period. Preparation of rice samples, accelerated aging treatment and determination of color, pasting properties, textural properties, amount of 2-acetyl-1-pyrroline and relative amount of *n*-hexanal were done using the same methods described in Chapter 4.

7.2.6 Statistical Analysis

Data regarding thermal and protein properties, pasting and textural properties, color parameters and the quantities of 2-acetyl-1-pyrroline and *n*-hexanal of KDML 105 rice samples were analyzed using analysis of variance (ANOVA) to determine the treatment effects. Duncan's multiple range test ($P<0.05$) was done to compare treatment means.

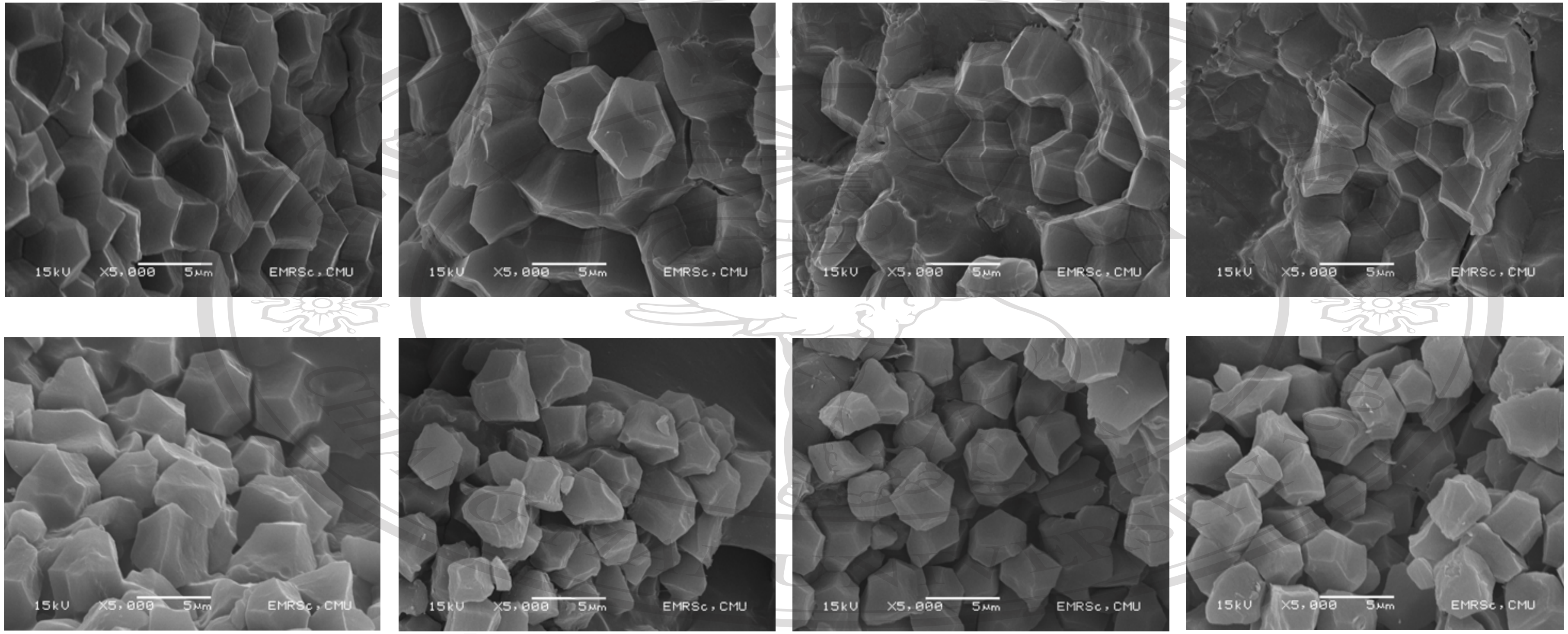
7.3 Results and Discussion

7.3.1 Investigation of Morphological and Structural Changes of Rice Starch Granule using Scanning Electron Microscopy

The SEM micrographs of the cross section area of fresh and accelerated aging milled rice kernels and their starch granules from flour samples are presented in Figure 7.2. The cross section areas of the kernel samples were scanned thoroughly to observe gelatinization of starch or some damage of the rice starch granules that would be caused by the high temperature of aging treatments. This was done since gelatinization of starch molecule is associated with the disruption of starch granular structure. Observation of starch granules in this way would be better and be more advantageous for the investigation of heat effect on starch of this present work than observation via birefringence of the starch granule from flour samples. This is because some portion of starch granule would be damaged by grinding action in the preparation of flour and may give artifact or misleading results.

The starch granules observed from the kernel and also from flour samples by SEM at the same magnification (5000X) revealed that their morphology and structure were not disrupted by the accelerated aging treatments. Fresh and accelerated aging starch granules did not show any significant differences in their polygonal granular morphologies. The micrographs indicated that surface of the starch granules did not lose their flatness and smoothness which suggested that the gelatinization of starch granule did not likely take place in the rice kernel samples although the temperature and exposure duration ranges used in this accelerated aging treatment were comparable to the conditions required for completion of the gelatinization of starch granule in processing of parboiled rice (Bhattacharya, 1985; Islam *et al*., 2004). In the production of such parboiled rice, high grain moisture content (>30% wet basis) has to be achieved by soaking method before going to steaming step so that the starch granule would have enough moisture to complete its gelatinization.

For the accelerated aging technique used in this current study, gelatinization of starch and disruption of starch granule was prevented by limiting rice grain moisture content (approximately 13.3%wb) during heat aging. Comparable research results have been reported on starches treated with heat-moisture treatment using



Fresh rice (control)

Accelerated aging at 100°C – 100 min

Accelerated aging at 110°C – 45 min

Accelerated aging at 120°C – 25 min

Figure 7.2 Scanning electron microscopy of freshly harvested KDML105 rice starch granule after accelerated aging with different conditions as indicated under the micrographs.

microwave and autoclave as heat source. Anderson and Guraya (2006) reported that granule morphology of waxy and non-waxy rice starches having 20% moisture content (wb) was not affected by microwave heat-moisture treatment as observed under SEM. Pukkahuta *et al*. (2007) also found a similar result if potato starch of 20% moisture content (wb) was treated with heat-moisture treatment using an autoclave. Thus, exposure of the low moisture content milled rice to high temperature condition of this present study could enhance aging process of rice without disruption of starch granular structure and the practice could produce milled rice of having physical appearance and functional characteristics identical to naturally aged milled rice.

7.3.2 Investigation of Thermal Properties of KDML 105 Rice Flour Samples using Differential Scanning Calorimeter

The thermal transition characteristics of freshly harvested and accelerated aging milled rice samples were studied from their flour samples using a DSC device. The scanning temperature range was 35 to 110°C with a ramp rate of 2°C/min which was the range covering the gelatinization region of normal rice starch and flour. This temperature range and heating rate could complete the gelatinization process of the rice starch granule as demonstrated by the thermogram of ungelatinized flour sample and the rescanning thermogram of the same flour (gelatinized) in Figure 7.3. The DSC thermograms of KDML 105 fresh rice flour and flour samples derived from different accelerated aging treatments are also shown in Figure 7.4 and the values of their thermal transitions in terms of onset (T_O), peak (T_P) and conclusion (T_C) temperatures of gelatinization, gelatinization temperature range ($\Delta T = T_C - T_O$) as well as gelatinization enthalpy (ΔH) are summarized in Table 7.1.

All the thermal property parameters, T_O, T_P, T_C, ΔT and ΔH of the accelerated aging rice flour samples significantly differed from those of fresh rice, though statistical significant difference for T_C was P=0.0601. The results indicated that T_O, T_P and T_C of the accelerated aging rice samples shifted to higher temperature. High gelatinization transition temperatures are an indication of aging. Zhou *et al*. (2003) reported that gelatinization of naturally-aged rice shifted to higher temperature as determined by DSC. Thus, the increases in gelatinization temperatures of the flour

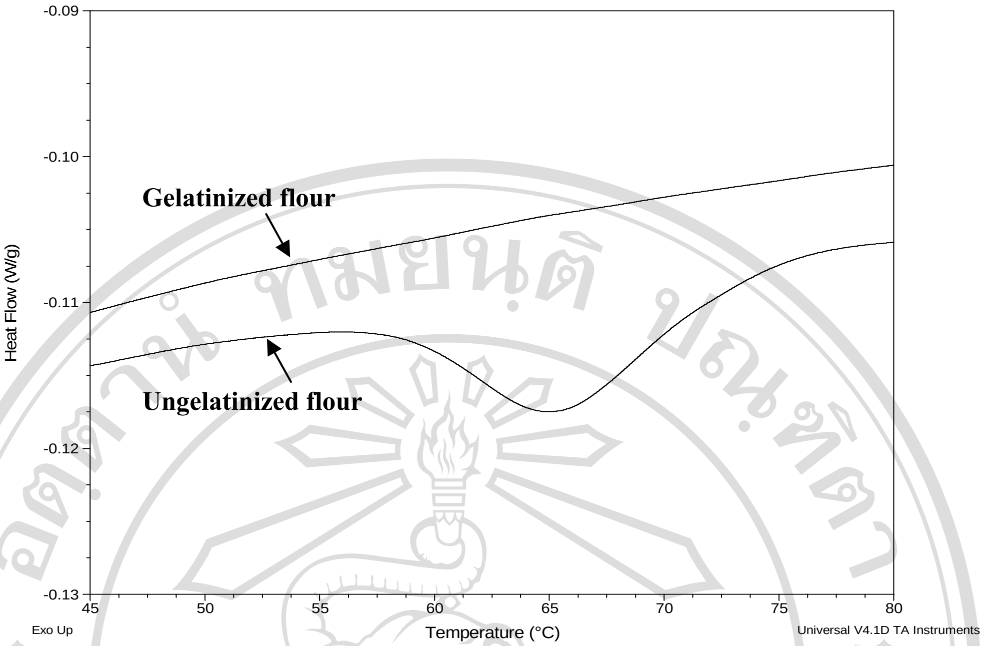


Figure 7.3 DSC thermogram of ungelatinized flour sample and the rescanning thermogram of the same flour.

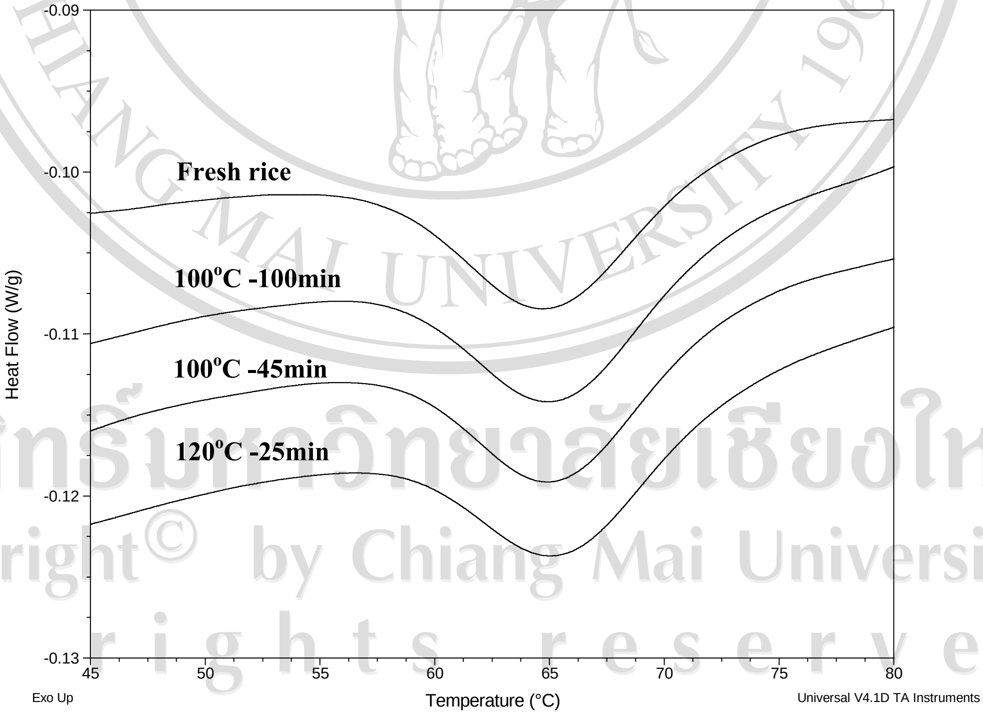


Figure 7.4 DSC thermograms of flour from freshly harvested and after accelerated aging with different conditions of rice cv. KDML105. Their aging conditions are indicated above the thermograms.

Table 7.1 Thermal transition characteristics in terms of onset (T_O), peak (T_P), and conclusion (T_C) temperatures and gelatinization enthalpy (ΔH) of flour samples (in Joules per gram of 11% MC wb) of KDML 105 freshly harvested rice as affected by accelerated aging treatments.

Accelerated aging treatments (Temperature –time)	Transition temperature (°C)				ΔH (J g^{-1})
	T_O	T_P	T_C	T_C- T_O	
Fresh rice	57.52±0.135[b]	65.02±0.061[c]	72.34±0.111	14.83±0.061[a]	7.79±0.128[a]
100°C–100 min	58.13±0.053[a]	65.31±0.031[b]	72.47±0.113	14.35±0.075[b]	7.20±0.108[b]
110°C–45 min	58.08±0.083[a]	65.33±0.094[ab]	72.57±0.090	14.49±0.106[b]	7.18±0.020[b]
120°C–25 min	58.20±0.208[a]	65.42±0.031[a]	72.60±0.101	14.41±0.269[b]	7.07±0.103[b]

Means (± SD) followed by the same letters in a column are significantly different by DMRT (*P*<0.05) samples implied that aging had already taken place after milled rice had received accelerated aging treatments. Increases in the transition temperatures of these accelerated aging rice samples may be attributed to less solubility of their starch granules caused by the aging treatments. The accelerated aging conditions of this study could enhance rate of oxidation process of rice constituents including oxidation of starch granule-bound protein. This protein could become more restricted to hydration and swelling of the starch granules which retarded starch gelatinization and hence the temperature of gelatinization shifted to higher position when determined by DSC thermograms. The increase in T_O, T_P and T_C of this work are in line with results studied by Altay and Gunasekaran (2006) who conducted an experiment to investigate effects of drying temperature (20 and 100°C), water content of starch (30, 50, 70 and 90% w/w), and heating rate (1, 3, 5, 7, 10, and 15°C/min) on gelatinization temperatures of corn and waxy corn starches. They reported that high drying temperature increased transition temperature of gelatinization of the starch samples. Increases in T_O, T_P and T_C and decrease in ΔH of KDML 105 brown rice with increasing drying temperatures (130 and 150°C), moisture content (28.2 and 33.3% dry basis) and tempering time (30, 60 and 120 min) of fluidized bed drying process have been reported by Jaisut *et al.* (2008). Increases in thermal transition of both studies were attributed to the decrease in starch solubility.

In the study of Jaisut *et al.* (2008), high temperature from fluidized bed drying and relatively high grain moisture caused the rice starch to be partially gelatinized. For this present study, a slight decrease in ΔH of flour from the accelerated aging rice samples was found. This may probably be attributed to the order structure of starch molecules inside the granules of some portion of the milled rice sample partially disrupted or gelatinized during the aging treatments. Dissociation of amylose-lipid complexes could also be accounted for this slight decrease in ΔH. The amylose-lipid complexes could be formed in dry heat treated rice (Jaisut *et al.*, 2008) or in low MC parboiled rice (Derycke *et al.*, 2005b) and could be detected as a second endotherm on DSC thermogram. However, the second endothermic peak was not detected on the DSC thermograms of the current study as shown in Figure 7.5. Thus, the ΔH was determined only from the first endothermic peak. This limitation was due to the type of aluminum pan used to contain flour sample in the DSC test, and could also be

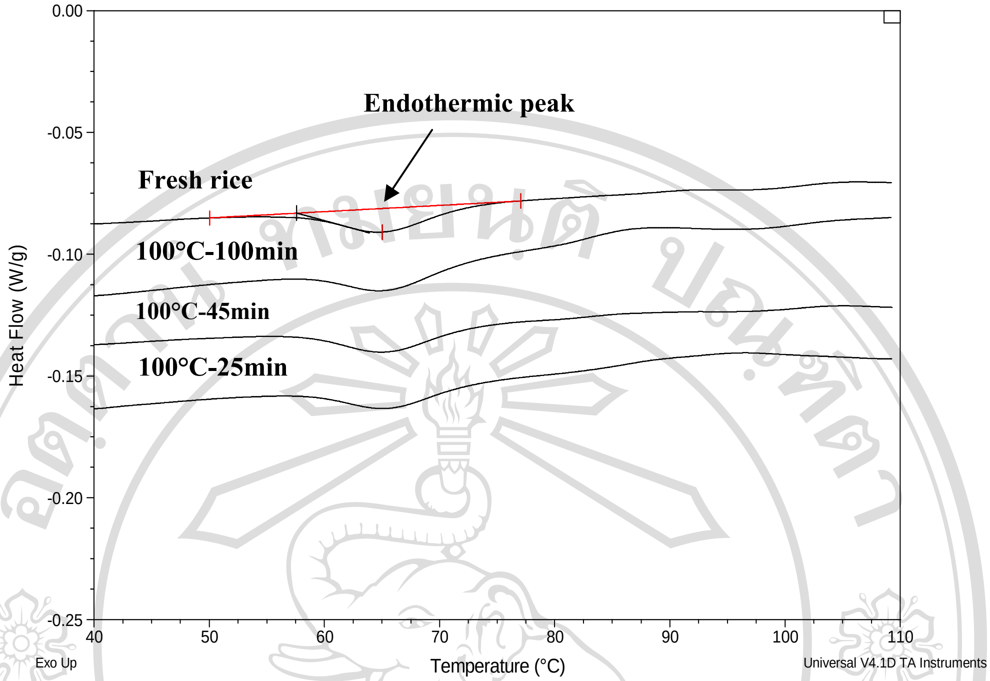


Figure 7.5 DSC thermograms of KDML105 flour sample showing single endothermic peak. Their aging conditions are indicated above the thermograms.

attributed to the scanning temperature range (35-110°C) which was lower than the temperature range (40 to 150°C) reported to find the second endotherm in heated KDML 105 brown rice (Jaisut *et al*., 2008). All the accelerated aging rice flour samples showed narrow temperature range between T_O and T_C, indicating that homogeneity of aged rice was derived from this accelerated aging technique. Based on the results, it can be concluded that accelerated aging conditions of this study is effective in altering the freshly harvested rice to be rice of having aged character as regarded to their thermal properties changes.

7.3.3 Investigation of Changes in Protein Properties

Change in chemical structure related to aged rice property has been focused on the change occurred in rice storage protein, particularly cross-linking between cysteine, the sub unit of glutelins or oryzenin (Chrastil, 1990b, Chrastil and Zarins, 1992 and 1994). To investigate change at molecular structure level of starch granular protein, pasting curve obtained from RVA had been employed (Martin and Fitzgerald, 2002; Fitzgerald, *et al*., 2003; Zhou *et al*., 2003). Results from this current work indicated that accelerated aging treatment changed the freshly harvested rice to become aged as illustrated by their RVA viscograms in Figure 7.6.

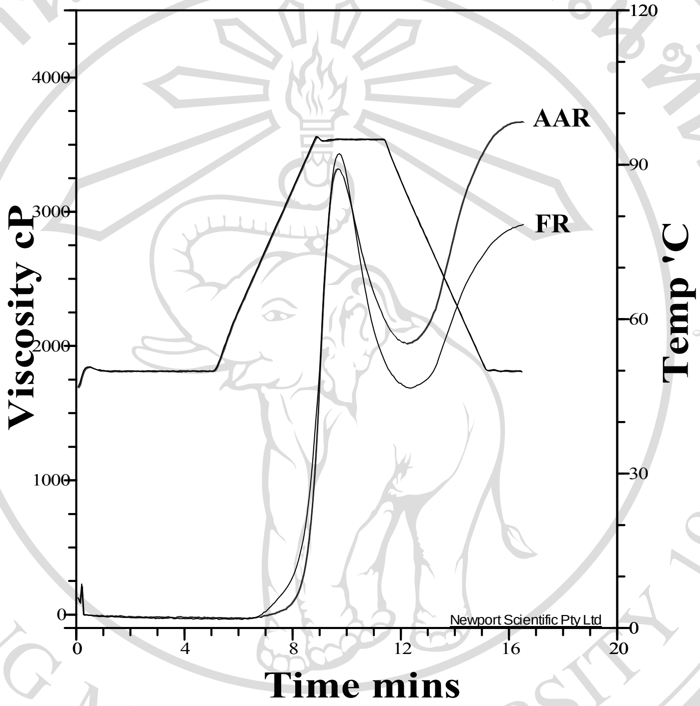


Figure 7.6 RVA viscograms of flour from freshly harvested rice cv. KDML 105 (FR) and of flour from the rice after accelerated aging by exposure to temperature of 100°C for 100 min (AAR).

Accelerated aging rice flour samples performed their RVA pasting curves different from the corresponding fresh rice flour. This was possibly attributed to the increase in cross-linking between the protein sub units caused by the heat aging treatments. During accelerated aging, the disulfide cross-links may rapidly be generated between both intra and inter-links of the protein molecules and that the protein network become a barrier to water penetration, hydration and swelling of the starch granules as seen through the change in their RVA performances in the Figure 7.6. McDonough *et al*. (2008) suggested that the tight association of protein matrix not only restricted swelling but could limit starch leaching from the granule as well, and consequently result in a decrease in peak viscosity and breakdown values. Results of this present study are in line with their report.

To verify whether changes in RVA viscosity of the accelerated aging rice samples were due to change in rice protein property caused by cross-linking between sulfhydryl groups of cysteine units, the protein structure of the starch granule samples in this study was altered by using dithiothreitol (DTT). This was to cleave disulfide bridges of the protein sub unit. The results showed the effect of the reducing agent on reversing RVA viscosity of the rice flour samples and proved that the disulfide bonding of rice storage protein was concerned. The RVA pasting curves of the accelerated aging samples operated with 10 mM DTT solution behaved almost similar to the typical viscogram of fresh rice as illustrated in Figure 7.7.

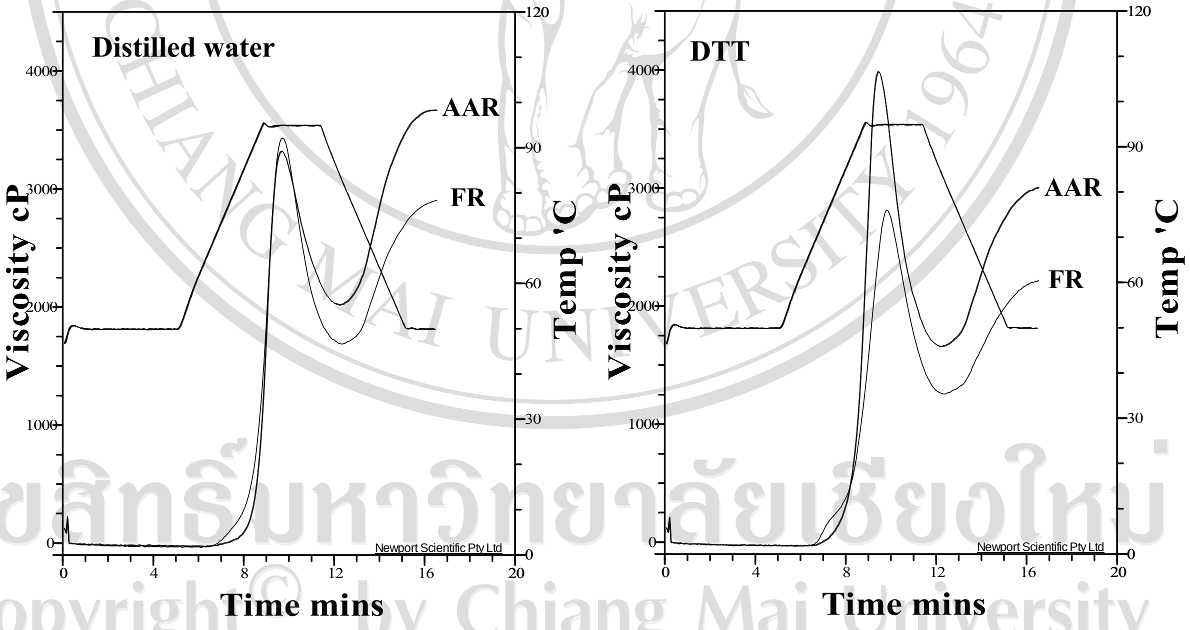


Figure 7.7 RVA viscograms of flour from freshly harvested rice cv. KDML105 (FR) and the flour after accelerated aging with temperature of 100°C for 100 min (AAR) when operated with distilled water and with solution containing dithiothreitol (10 mM DTT).

From Figure 7.7, different responses to DTT between fresh and accelerated aging samples were observed. All the RVA parameter values measured from freshly harvested and accelerated aging rice samples are summarized in Table 7.2. In the presence of DTT, pasting temperature, trough and final viscosity of the accelerated aging samples decreased markedly while peak viscosity increased. These significant changes resulted in a greater increase in breakdown and decrease in setback value. The different responses to DTT between freshly harvested and accelerated aging rice samples could be explained by the following assumption.

In the presence of DTT, the decrease in RVA peak viscosity of fresh rice sample was probably attributed to DTT cleave disulfide bond of the starch granule bound protein while other constituents of the granule have not yet been more organized to form intra and inter linkages of their molecules. Therefore, decrease in disulfide bridge by DTT resulted in increase in fragility of the starch granule and that the granule was broken easily in shearing stress during heating in the RVA measurement. In the case of accelerated aging samples, not only disulfide linkage was developed, but cross-linking between other molecular constituents of the starch granule also occurred. Since DTT has a specific effect to reduce only the disulfide bridge, so the granule was still rigid by some other cross-links or other organizations and was able to swell further, resulting in the increase in peak viscosity. The effect of DTT on pasting behavior of fresh and accelerated aging rice samples in this study was also confirmed by conducting a test with 8-month naturally-aged rice to prove that naturally-aged rice responded to DTT in the same way as accelerated aged sample did. The results provided evidence to confirm that DTT had the same effect on both aged samples as demonstrated by the RVA viscograms of the naturally-aged rice in Figure 7.8.

Table 7.2 RVA viscosity parameters of freshly harvested and accelerated aging milled rice flour operated with distilled water and dithiothreitol (10mM DTT) solution.

Accelerated aging treatments (Temperature –time)	RVA viscosity parameters (cP)						
	Pasting temp. (°C)	Peak viscosity	Trough	Final viscosity	Breakdown	Setback	Peak time
<u>Flour with water</u>							
Fresh rice	84.9±0.43^{b}	3322±69^{c}	1769±16^{b}	2960±80^{c}	1553±55^{c}	-363±39^{c}	9.8±0.04^{b}
100°C–100 min	87.5±0.52^{a}	3005±15^{d}	2074±18^{a}	3715±25^{a}	931±4^{f}	710±16^{a}	9.8±0.04^{b}
110°C–45 min	87.7±0.08^{a}	3052±32^{d}	2032±25^{a}	3609±35^{b}	1020±15^{e}	557±40^{b}	9.7±0.00^{b}
120°C–25 min	87.4±0.47^{a}	3058±8^{d}	2034±33^{a}	3579±14^{b}	1024±26^{e}	521±8^{b}	9.8±0.04^{b}
<u>Flour with DTT</u>							
Fresh rice	71.7±0.43^{d}	2723±24^{e}	1423±28^{d}	2355±36^{d}	1300±48^{d}	-367±57^{c}	10.0±0.04^{a}
100°C–100 min	82.2±0.10^{c}	3789±87^{a}	1660±32^{c}	2912±32^{c}	2128±67^{a}	-877±81^{e}	9.5±0.04^{d}
110°C–45 min	81.9±0.51^{c}	3647±32^{b}	1715±54^{bc}	2920±55^{c}	1932±40^{b}	-728±42^{d}	9.6±0.04^{c}
120°C–25 min	81.8±0.48^{c}	3695±42^{b}	1707±33^{c}	2888±33^{c}	1988±16^{b}	-807±33^{c}	9.6±0.04^{c}

Means (± SD) followed by the same letters in a column are not significantly different by DMRT (*P*<0.05)

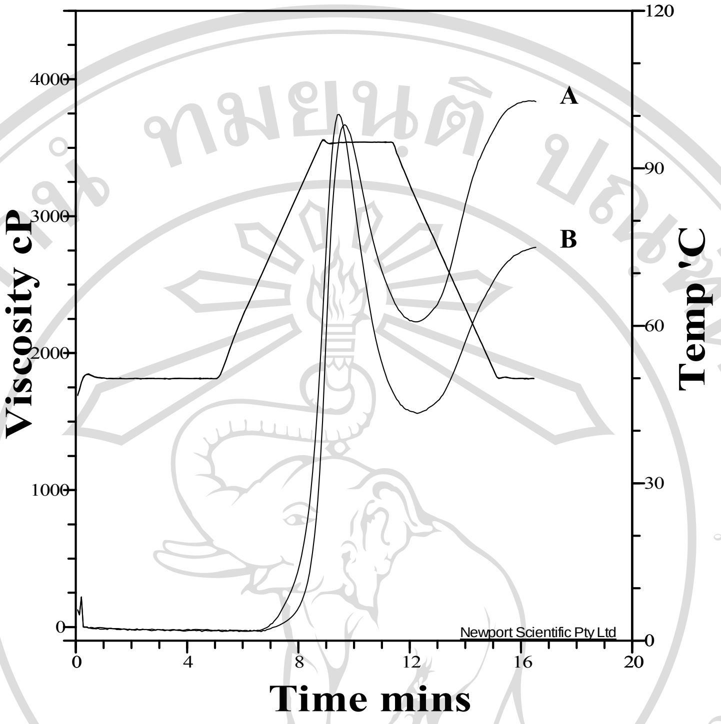


Figure 7.8 RVA viscograms of flour from 8-month naturally-aged KDML 105 rice sample performing with distilled water (A) and (B) with solution containing dithiothreitol (10 mM DTT).

To prove this assumption, solution containing proteinase enzyme (40U mg^{-1}) and solution containing a mixture of the enzyme and 10 mM DTT were used instead of water. The RVA viscosities of freshly harvested and accelerated aging samples changed in a similar manner after given with the enzyme and the mixture solutions as demonstrated in Figure 7.9. These changes confirmed the above assumption in which linkages other than disulfide have been involved and the changes indicated the starch-bound protein were concerned in the rigidity of the rice starch granule and this rigidity was increased by the accelerated aging treatment.

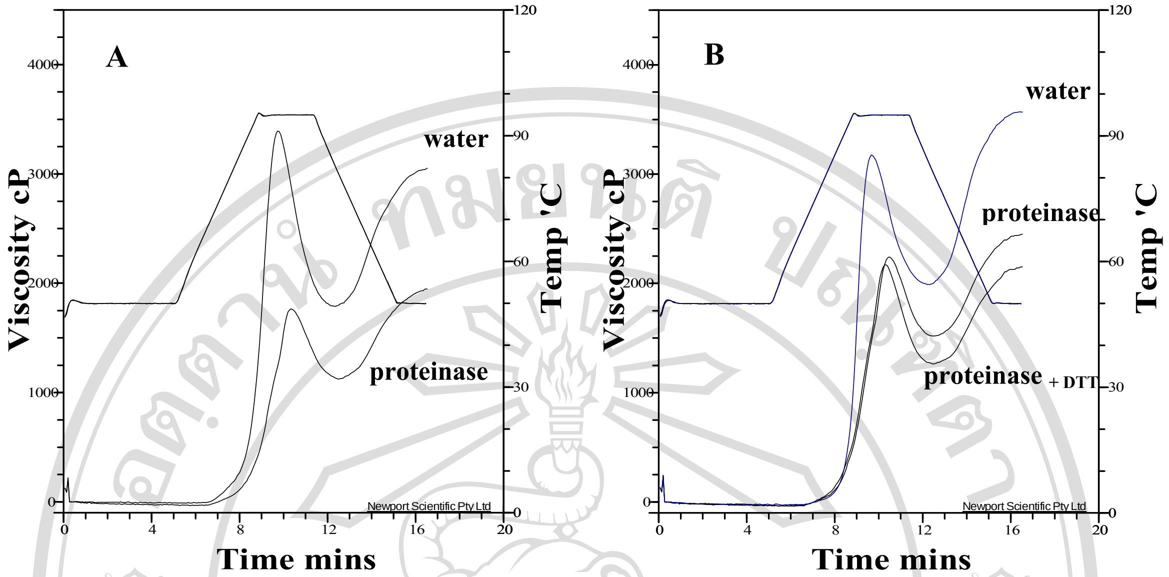


Figure 7.9 **RVA viscograms of flour from freshly harvested rice cv. KDML 105 (A) and of flour from the rice after accelerated aging with temperature of 100°C for 100 min (B) when operated with distilled water and with solution containing proteinase enzyme (40 U) and solution containing mixture of proteinase enzyme (40 U) and dithiothreitol (10 mM DTT).**

Result from this present study was in agreement with result of an experiment conducted earlier by Martin and Fitzgerald (2002) who determined the effect of protein on pasting properties of flour from various rice varieties having different amylose content and differed in aged levels. In their study, addition of DTT to stored rice flour resulted in increases in peak viscosity and amount of breakdown while decreased final viscosity. An opposite result was observed in that work in which DTT lowered viscosity curve of fresh rice flour. However, RVA viscosity of both stored and fresh rice was decreased in the presence of protease enzyme. They concluded that protein exerts its influence on viscosity through the increase in network linked by disulfide bonds and through binding water during dispersed and viscous phase of the gelatinized starch.

7.3.4 Investigations of Storage Stability

The effects of accelerated aging treatments on stability of the rice products as measured by the changes in amount of 2-acetyl-1-pyrroline and relative amount of *n*-hexana are shown in Figures 7.10 and 7.11, respectively. During storage, the amount of 2-acetyl-1-pyrroline of all rice samples decreased from their respective initial values of 5.04, 3.33, 3.78 and 3.94 ppm for unaged fresh rice and rice aged with 100°C for 100 min, 110°C for 45 min and 120°C for 25 min, respectively. The decrease was slow in the first 2 months after given accelerated aging treatments. These decreases occurred constantly with the accelerated aging samples showing higher rate of reduction. Packaging materials contributed in protecting loss of this prime aromatic volatile. Comparing among packaging types, aluminum laminated bag significantly showed its highest efficiency. At the end of 6-month storage period, almost half of 2-acetyl-1-pyrroline contents in accelerated aging rice volatilized out. The unaged sample packed in aluminum laminated bag lost its 2-acetyl-1-pyrroline content by 25% while rice aged with 100°C for 100 min, 110°C for 45 min and 120°C for 25 min lost 42.8, 38.9 and 50.4%, respectively. The decrease in 2-acetyl-1-pyrroline content of unaged (ordinary) and accelerated aging milled rice suggested that aroma of the rice could not be stabilized. Reduction of 2-acetyl-1-pyrroline during storage of milled rice in this study is similar to the results obtained by previous researches (Widjaja *et al*., 1996a; Yoshihashi *et al*., 2005).

Relative amount of *n*-hexanal generated during storage of milled rice in the different packaging types was monitored over a period of 6 months. In general, accelerated aging samples showed slightly higher amount of *n*-hexanal than unaged rice along the storage time as indicated by the area ratios of *n*-hexanal/DMP in Figure 7.11. During milling process, lipid bodies of rice bran were disrupted and their residuals were retained on the surface of milled rice kernel. These oil residuals were readily oxidized by lipid hydrolysis enzyme or by auto-oxidation process which resulted in the development of off-odor compounds including *n*-hexanal on stored milled rice (Lam and Proctor, 2003). In the present study, slightly increase in *n*-hexanal of the accelerated-aged rice might partly attribute to higher rate of auto-oxidation of the rice lipid breakdown residuals. This occurrence would be a consequent effect of accelerated aging when high temperature was used. Regarding to the effects of packaging types,

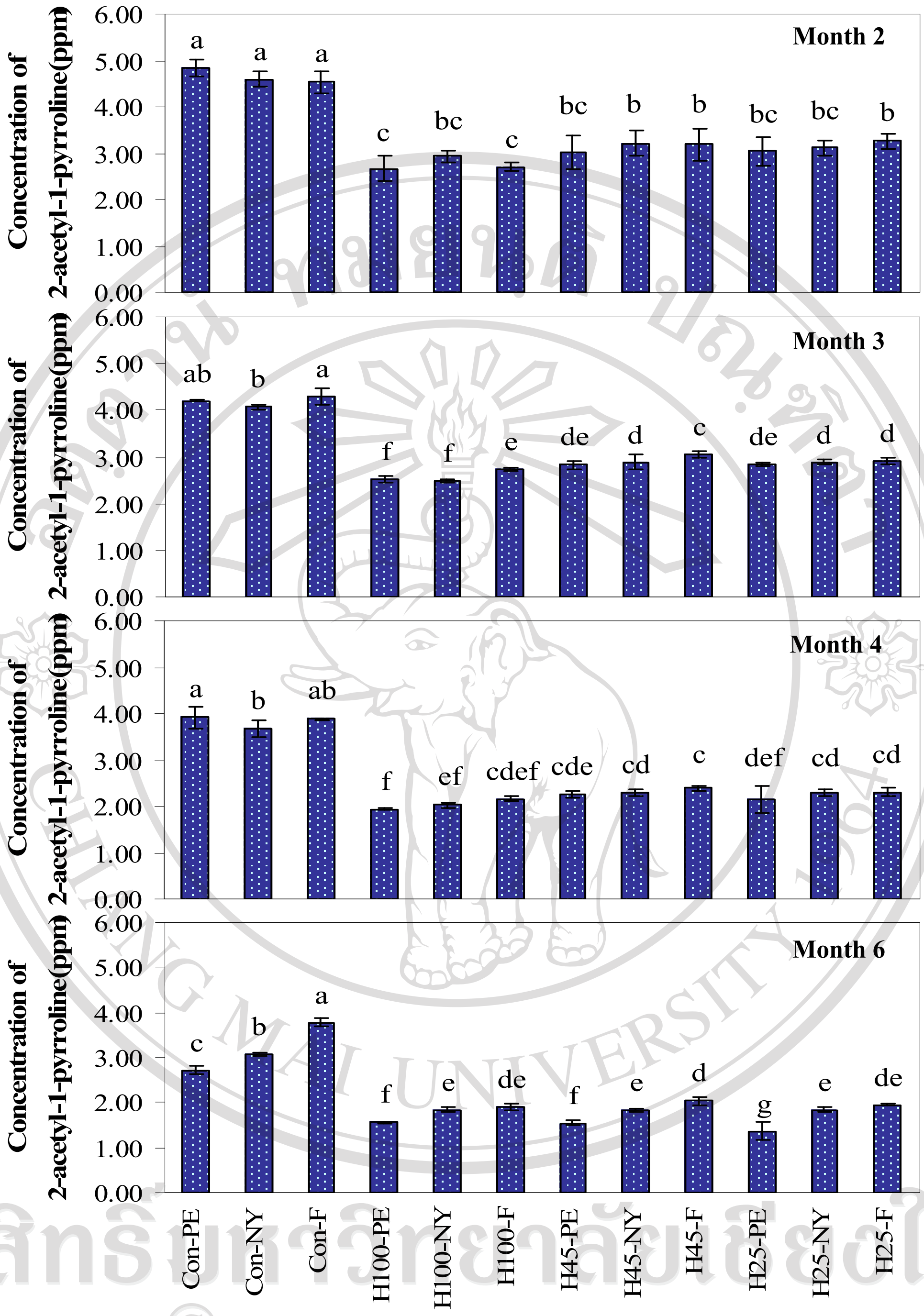


Figure 7.10 **Change in 2-acetyl-1-pyrroline concentration of KDML105 milled rice after given different accelerated aging treatments and stored in different packaging types in ambient condition for a period of 6 months. The initial values (at month 0) were 5.04, 3.33, 3.78, and 3.94 ppm in Con, H100, H45, and H25, respectively. Con; fresh rice: H100; heat 100°C–100 min: H45; heat 110°C–45 min: H25; heat 120°C–25 min: PE; polyethylene bag: NY; nylon laminated bag: F; aluminum foil laminated bag. Vertical bars (±SD) with the same letters are not significantly different by DMRT (*P*<0.05).**

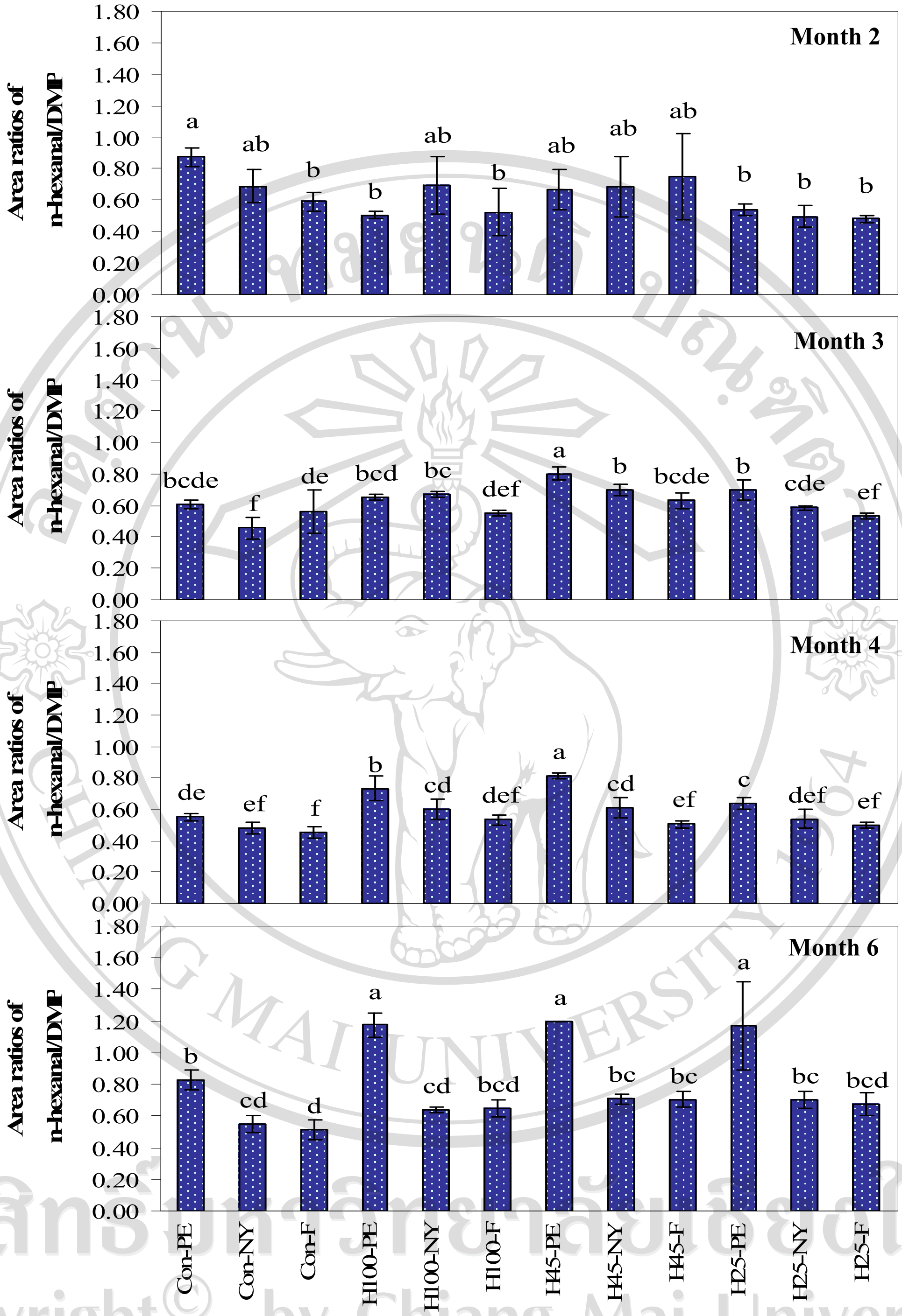


Figure 7.11 Change in area ratios of *n*-hexanal/DMP of KDML105 milled rice after given different accelerated aging treatments and stored in different packaging types in ambient condition for a period of 6 months. The initial values (at month 0) were 0.60, 0.47, 0.41, and 0.37 in Con, H100, H45, and H25, respectively. Con; fresh rice: H100; heat 100°C–100 min: H45; heat 110°C–45 min: H25; heat 120°C–25 min: PE; polyethylene bag: NY; nylon laminated bag: F; aluminum foil laminated bag. Vertical bars (±SD) with the same letters are not significantly different by DMRT (P<0.05).

polyethylene bag showed its less effectiveness in preventing *n*-hexanal development. Investigation along the 6-month storage period, nylon and aluminum laminated bags consistently showed lower *n*-hexanal as compared to that of the polyethylene bag. However, *n*-hexanal generated during storage of the milled rice samples in this study was in the range of 0.48 to 1.18. The values were in the normal range as respected to values obtained in the ordinary stored rice samples discussed earlier in Chapters 5 and 6. These values are acceptable by rice consumers as regarded to the fact that rice are naturally stored for a period of time before milling and trading in market.

The effects of accelerated aging treatments on stability of RVA viscosity, cooked rice hardness and adhesiveness and grain yellowness (*b**value) are shown in Figures 7.12, 7.13, 7.14, 7.15 and 7.16, respectively. RVA pasting curves of the aged

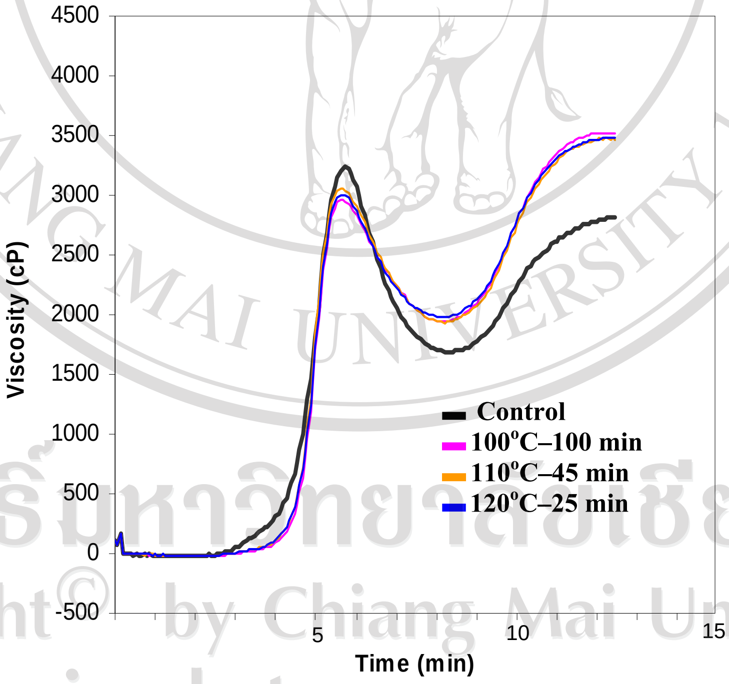


Figure 7.12 RVA pasting curves of KDML105 rice flour just after given different accelerated aging treatments.

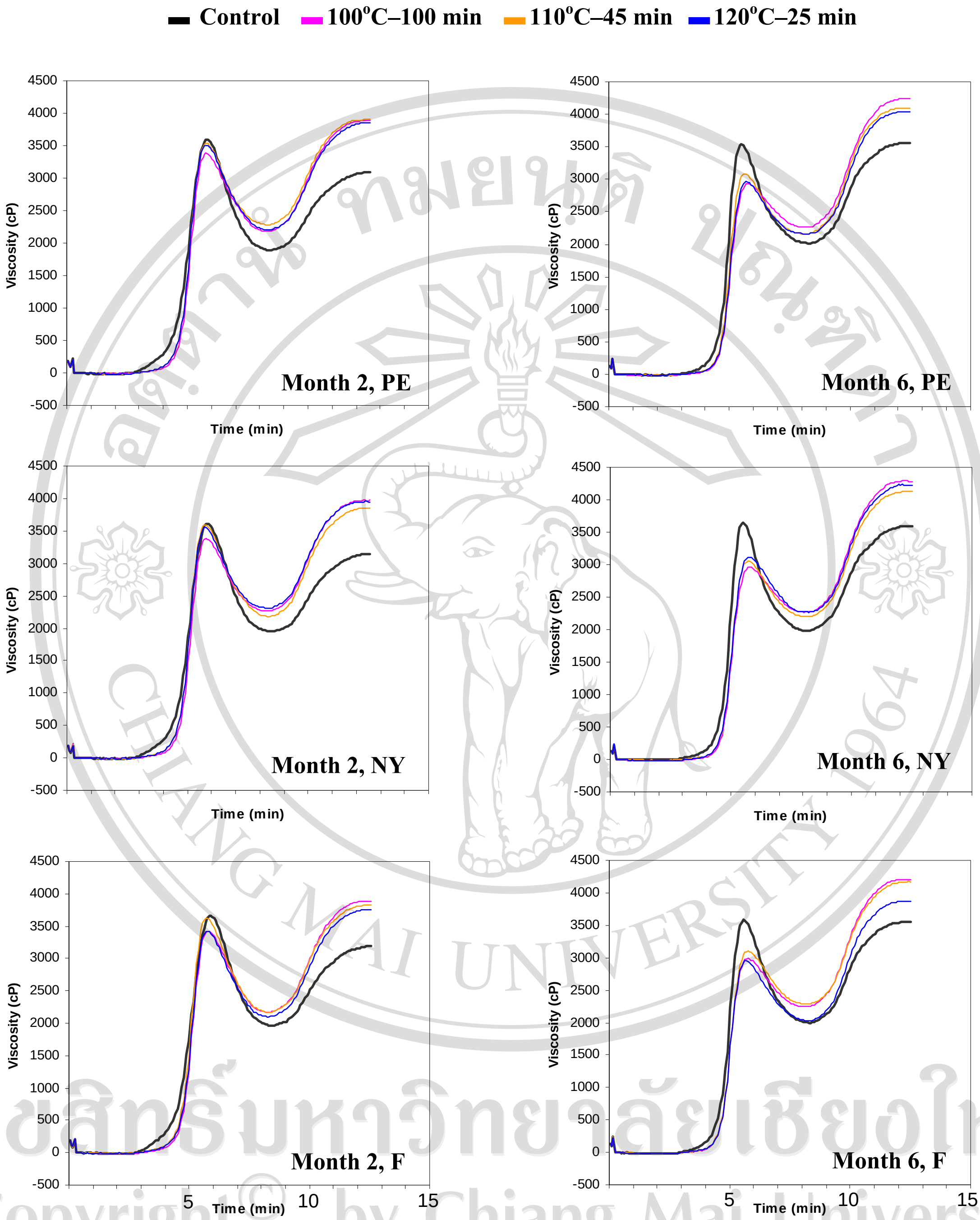


Figure 7.13 **Change in RVA pasting curves of flour from KDML105 milled rice after given different accelerated aging treatments and stored in different packaging types in ambient condition for a period of 6 months. PE; polyethylene bag: NY; nylon laminated bag: F; aluminum foil laminated bag.**

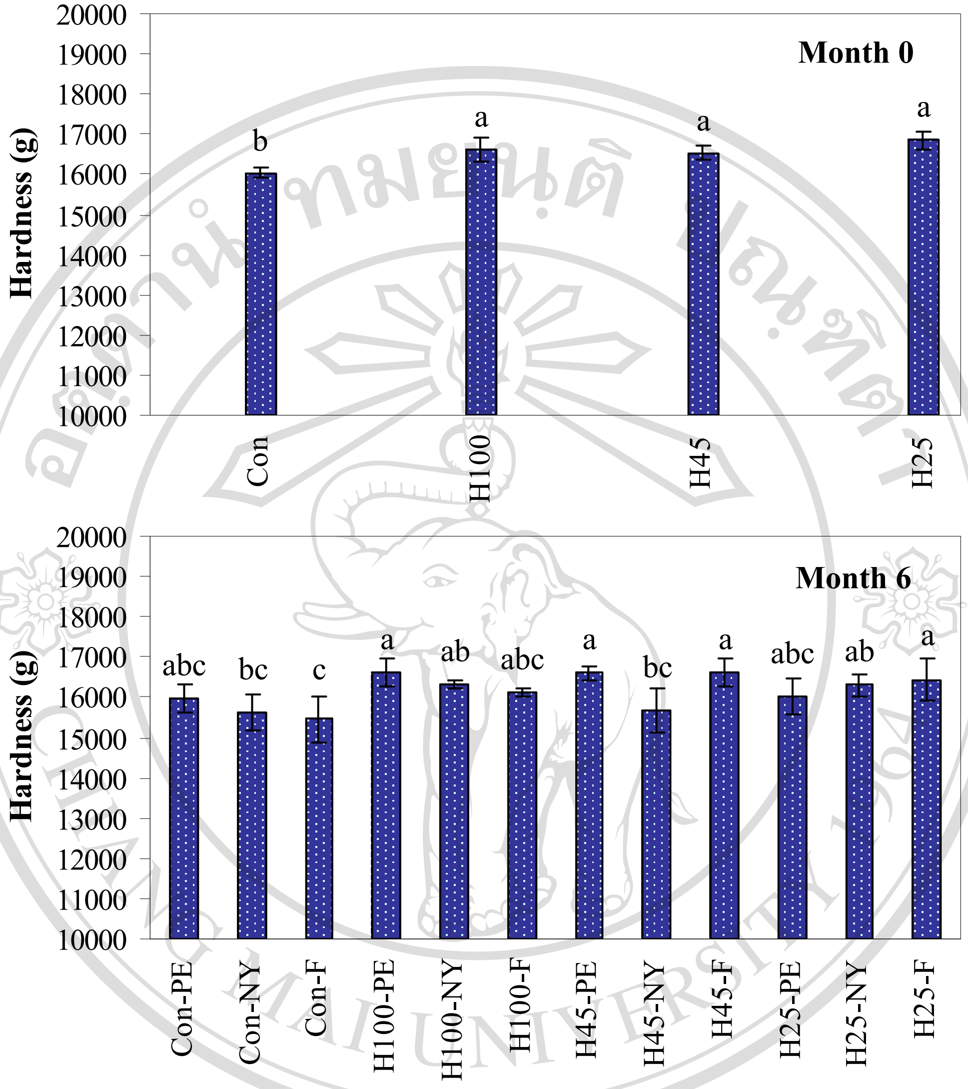


Figure 7.14 **Change in hardness of cooked rice from KDML105 milled rice after given different accelerated aging treatments and stored in different packaging types in ambient condition for a period of 6 months. Con; fresh rice: H100; heat 100°C–100 min: H45; heat 110°C–45 min: H25; heat 120°C–25 min: PE; polyethylene bag: NY; nylon laminated bag: F; aluminum foil laminated bag. Vertical bars (±SD) with the same letters are not significantly different by DMRT (*P*<0.05).**

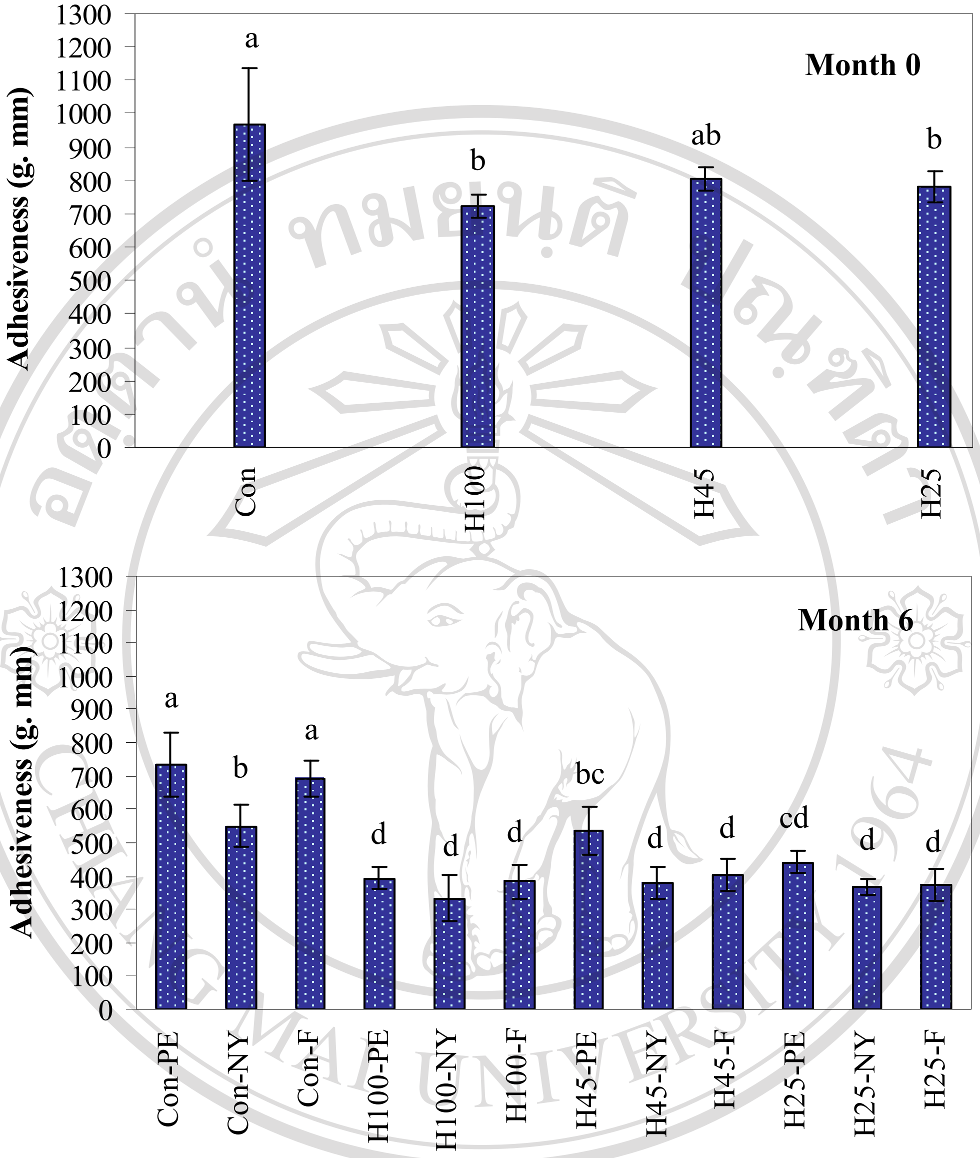


Figure 7.15 **Change in adhesiveness of cooked rice from KDML105 milled rice after given different accelerated aging treatments and stored in different packaging types in ambient condition for a period of 6 months. Con; fresh rice: H100; heat 100°C–100 min: H45; heat 110°C–45 min: H25; heat 120°C–25 min: PE; polyethylene bag: NY; nylon laminated bag: F; aluminum foil laminated bag. Vertical bars (±SD) with the same letters are not significantly different by DMRT (*P*<0.05).**

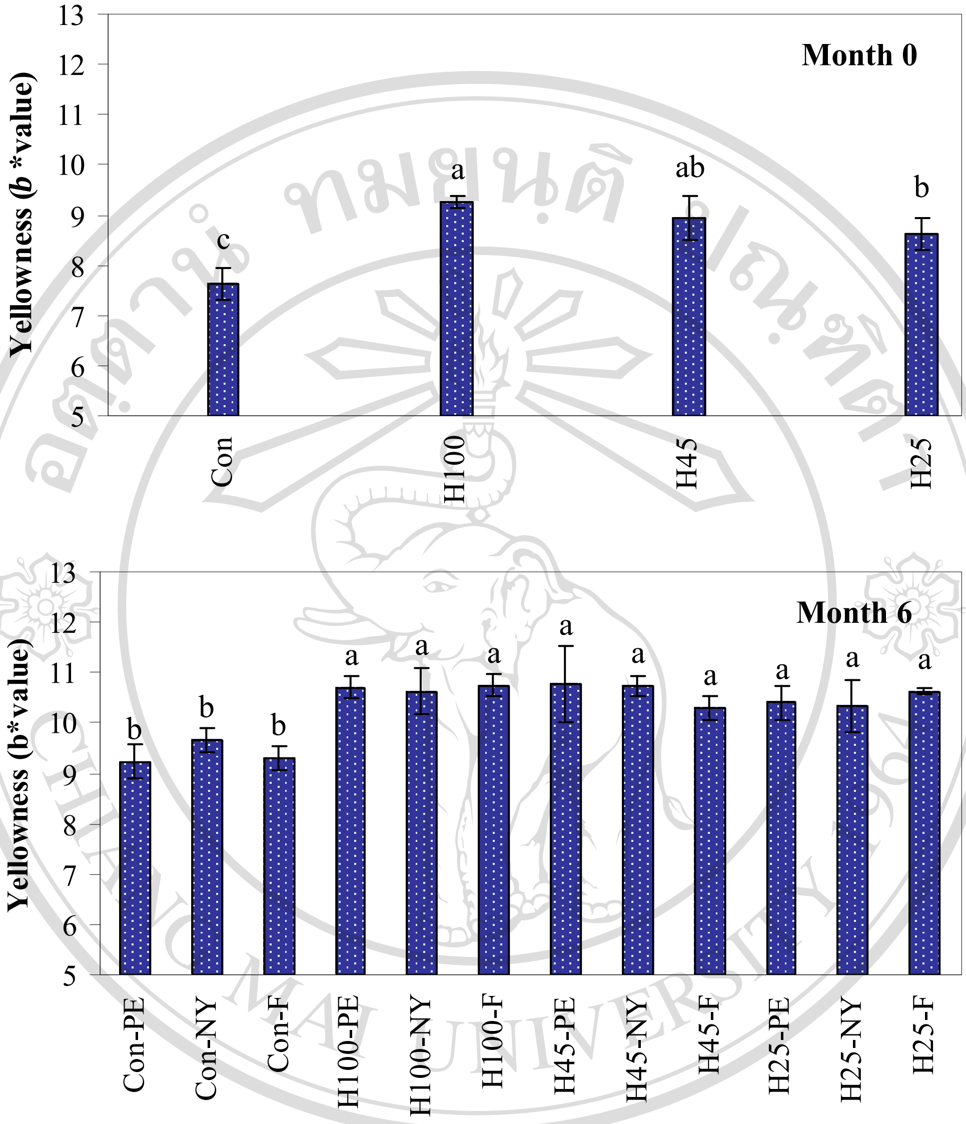


Figure 7.16 Change in yellowness (*b** value) of KDML105 milled rice after given different accelerated aging treatments and stored in different packaging types in ambient condition for a period of 6 months. Con; fresh rice: H100; heat 100°C–100 min: H45; heat 110°C–45 min: H25; heat 120°C–25 min: PE; polyethylene bag: NY; nylon laminated bag: F; aluminum foil laminated bag. Vertical bars (±SD) with the same letters are not significantly different by DMRT ($P<0.05$).

rice samples changed significantly from the beginning of storage time. As compared to the pasting curves just after given with aging treatments in Figure 7.12, the RVA curves of the stored samples dramatically changed. Peak viscosity increased at the initial phase of storage and decreased in the subsequent month while final viscosity continued to increase over 6 months (Figure 7.13). This indicated that the pasting property of rice could not be stabilized by the accelerated aging process. In contrast, hardness and adhesiveness, the main attributes of cooked rice, accelerated aging samples showed consistently higher hardness and lower adhesiveness values over the storage period as shown in Figures 7.14 and 7.15.

Change in yellowness (*b** value) of milled rice samples after receiving accelerated aging treatments and after storage for 6 months in polyethylene, nylon laminated and aluminum foil laminated bags under ambient condition were monitored (Figure 7.16). The accelerated aging samples were more yellow than fresh rice at the time just after given treatments and this difference was consistent over the storage period. These results suggested that aging treatments did not have an effect on stabilization of rice color as observed by the increase in their *b**values along the storage time. Increase in yellow color of KDML 105 stored rice has been reported (Soponronnarit *et al*., 2008) and the increase was attributed to browning (Maillard) reaction.

7.4 Conclusions

This study revealed that accelerated aging treatments did not change morphology of the rice starch granule but did affect rice starch thermal property and protein property. These changes occurred at the molecular level of the rice starch granule components and exerted their influences on the physico-chemical properties that are related to cooking characteristics of the rice. The study also revealed that changes in the rice physico-chemical properties occurred consistently during storage and these changes could not be stabilized by the accelerated aging treatments. However, the loss of aroma quality could be decreased by suitable protective packaging and aluminum laminated bag packed with reduced pressure was the most efficient.