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The Perspective Effects of Various Seed Coating Substances on Rice Seed Variety Khao Dawk Mali 105 Storability II: The Case Study of Chemical and Biochemical Properties

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Abstract: The aim of this study was to investigate the effects of seed coating substances; chemical fungicide (CA) and biological fungicide polymers [chitosan-lignosulphonate polymer (CL) and eugenol incorporated into chitosan-lignosulphonate polymer (E+CL)] on chemical and biochemical changes of rice seeds cv. KDML 105, which have been studied during storage for 12 months. CA significantly affected the rice seed chemical properties and the associated seed deterioration. After 12 months storage, protein content decreased accompanied by declined of lipid content, increased free fatty acids and activated lipoxygenase enzyme. In the case of biological fungicide coated seeds, the antioxidative scavenging enzymes were ascorbate peroxidase and superoxide dismutase and a high antioxidant activity protected them. Moreover, the sugar content was positive correlated with seed germination and vigor. The biological coated seeds were found to maintain high sugar contents inside the seeds, which resulted high seed storability significantly. In contrast, under fungicide stress (CA), those compounds were lost that directly affected seed vigor during storage.

Key words: Seed coating, rice, KDML 105, seed quality, seed chemical property

INTRODUCTION

Rice seeds are known to be attacked by many pathogenic fungi, which is a major cause leading to seed deterioration and degradation of rice grain qualities. Treating seeds with fungicide to protect the seed from those organisms can improve stand quality, increase yields and increase return on investment (Thobunluepop *et al*., 2008). Although, fungal pathogen normally lives in closed association with the seed, so it is difficult to find chemicals that kill the fungus without harming the seeds. The toxicity of fungicide was ascribed to produce phytotoxic compounds that induced seed deterioration. Kunkur *et al.* (2007) reported that chlorine, bromine and iodine in fungicide compounds induced physiological deterioration of seeds. In seeds treated with the fungicide chlorine, a loss of cell membrane function was found which might be the main reason of high respiration and subsequently, deterioration of the seeds.

Seed storability depends on seed varieties, conditioning, treatments and storage conditions and most losses resulting from infestation by microorganisms (FAO, 1997). The metabolic changes occurring in the early stages of germination are the result of the activity of various enzymes, which are either present in the dry seed or very rapidly become active as the seed imbibes water. Generally, during germination, the increasing of enzyme activity, which are causally related to breaking down starch, protein, lipids and other storage materials as essential to the germination proceeds. Metabolic processes that occur upon imbibition are complex. They include three processes. First, the reserve compounds are broken down and then they are transported from one part to the other parts of the seed. Finally, new compounds are synthesized during growth. The large amounts of soluble sugars present in germinating seeds are apparently the result of the breakdown of reserve carbohydrates, such as starch and oligosaccharides (Salisbury and Ross, 1985). The ratio between the various sugars and oligosaccharides changes because of the activity of the enzymes discussed above. The great majorities of seed proteins are metabolically inactive and serve merely as

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food reserves used by the growing embryo during germination. Protein synthesis begins in the various embryonic organs immediately with the beginning of their growth. Thus, the dry seed is a well functional unit, which can carry out a large number of biochemical reactions if placed into aquatic medium.

Biochemical and physiological changes during storage are commonly termed aging. The natural aging process, which is caused by deterioration moves inexorably forward toward death. Moreover, seed deterioration was most affected under the unsuitable seed treatment conditions. Deterioration cannot be reversed or eliminated, once it has occurred. A smaller but quite significant proportion of the total loss results from respiration and gradual deterioration of viability, nutritive quality and the germination. The aged seed with loss of viability is associated not only with disturbances of the cell membranes, but also biochemical nutrients losses. Changes in their compositions and losses of enzymes activities is cause of rice seed deterioration (Enju *et al*., 1993). Thus, the aim of this study was to analyze the effects of seed coating substances on rice seed deterioration via biochemical changes during storage.

MATERIALS AND METHODS

The experiment was conducted at section of Seed Science and Technology, Faculty of Agriculture, Chiang Mai University, Thailand, Institute of Agricultural Chemistry, Georg-August University of Goettingen, Germany and Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University, during 2007-2008. Dry graded rice seeds (*Oryza sativa* L. cv. KDML 105) from one seed lot were supplied by Bureau of Seed Multiplication of Thailand. The split-plot design with four replications was applied. The main plot was seed treatments; chemical fungicide (CA) and biological fungicide polymers [chitosan-lignosulphonate polymer (CL) and eugenol incorporated into chitosan-lignosulphonate polymer (E+CL)] and control (CO). The sub-plot was the storage duration (12 months). The seeds of each treatment were randomly stored in plastic bag sealed in an incubation chamber, (KPB6395FL, Termaks, S/N 2-858 Germany), which controlled temperature at 30±2°C and relative humidity at 40±5%. Seeds were immediately examined, then, every month for 12 months to determine chemical and biochemical properties.

Determination of the chemical composition: All experiments were done using dehulled grains with 12±0.5% of seeds moisture content. Freeze-dried rice seeds were ground (UDY, Cyclone Sample Mill No. 2).

Determination of total protein content: The protein content was analyzed according to Kjeldahl method (AOAC, 2000). Protein content was calculated from nitrogen content, which was multiplied with 6.25.

Determination of total lipid and Free Fatty Acid (FFA) content: The gravimetric method was used to determine the total lipid content according to Lam and Proctor (2000) and some modification. Lipid content was extracted by vortexing 10 g of rice sample with 4 mL of isopropanol for 5 min. Then, added 5 mL of isopropanol into the sample and vortexed for 5 min. After that, the extract was centrifuged at 2500 rpm for 10 min. The weight of extracted lipids was determined after evaporating the solvent on an electric hot plate at 40°C.

FFA was analyzed according to Walde and Nastruzzi (1991) and some modification. Assay solution contained 0.375 mL of solution A (0.1 M Tris/HCL; pH 9.0), 0.125 mL of solution B (2 mM phenol red in 0.1 M Tris/HCl; pH 9.0) and 0.5 mL of solution C (50 mM Bis (2-ethylhexyl) sodium sulfosuccinate in isooctane). Then, 30 μL of isopropanol sample extract was mixed with 1 mL of assay solution in a 1-cm wide cuvette and was shaked for 1 min before measuring absorbance at 560 nm. FFA of each extract was obtained from a calibration curve. The calibration curve was prepared by dissolving oleic acid in isopropanol to produce oleic acid solution of 0.001 to 0.02% (w/w).

Determination of sugar content: Sugar content was analyzed by iodine titration of excess copper of Luff-Schoorl method (Alexander *et al*., 1985). Sample solution preparation: 5 g of ground sample was accurately weighed to nearest 1 mg and transferred to a 250 mL volumetric flask, which contained 200 mL distilled water. Five milliliters of Carrez solution I, [21.795 g of zinc acetate dehydrate (Zn $(CH_3COO)_2.2H_2O$) and 3 mL of glacial acetic acid were dissolved and made up to 100 mL with distilled water] and 5 mL of Carrez solution II, [10.76 g of potassium hexacyanoferrate II trihydrate (K_4 [Fe $(CN)_6$].$3H_2O$) in water and make up to 100 mL with water], were mixed after each addition and made up to 250 mL with water and then mix its well.

Luff-Schoorl reagent preparation: Citric acid solution (50 g of citric acid dihydrate ($C_6H_8O_7.2H_2O$) in 50 mL of water) was added to sodium carbonate solution (143.78 g of anhydrous sodium carbonate in 300 mL of warm water and allow to cool) in a 1 L volumetric flask with gentle swirling. Then, copper II sulphate pentahydrate solution (25 g of $CuSO_4.5H_2O$ in 100 mL of water) was added and made up to 1000 mL with water. The solution was allowed to stand overnight and then filtered.

Twenty five milliliters of Luff-Schoorl reagent and 25 mL of sample solution were transferred into 250 conical flasks and extracted at 80°C in ultrasonic bath (Bandelin, Germany) for 10 min. After that, the sample was immediately cooled for 5 min. Then, 10 mL of 30% (w/v) potassium iodide solution were added and immediately followed by 25 mL of 3 M sulphuric acid adding. Titration was done with 0.71 M sodium thiosulphate solution until the solution was almost colorless, then added a few milliliters of starch indicator (5 g of soluble starch; Sigma, St. Louis, MO, USA) slurred in 30 mL of water, boil for 3 min, allowed the mixture to cool and added water to make the 1 L solution). The titration was continued until the blue color disappears.

Finally, sugar content was calculated from the standard table by using the weight of glucose or the weight of invert sugar in percentage corresponding to the difference between the two titration readings, expressed in milliliter of 0.71 M sodium thiosulphate. Express the results in terms of invert sugar or D-glucose as percentage of the dry matter.

Determination of total antioxidant activity: Total antioxidant activity was determined by the diphenylpicrylhydrazyl (DPPH) radical scavenging effect according to Kim *et al.* (2002) and some modification. To obtain the concentration of crude methanolic extract, one gram of ground sample was dissolved in 50 mL methanol (Merk, Germany) and the ultrasonic solvent extraction was carried out in an ultrasonic bath (Bandelin, Germany) for 1 h. The sample solution was then filtered. The filtrate was evaporated with rotary evaporator (Buchi, Switzerland) at 40°C until dried. Crude extract was then weighed and calculated back to obtain the concentration in mg (crude) per mL (methanol).

To determine the antioxidant activity, 3 mL of methanol solution of each treatment at various concentrations (1.5-45 mg mL^{-1}) were added to 1 mL of 1×10^{-4} M DPPH (Fluka, Germany) in methanol. The reaction mixture was shaken vigorously. After leaving the mixture at the room temperature for 30 min, the optical density was measured at 520 nm using a UV-VIS spectrophotometer (SPECCORD 40). The radical scavenging activity of each sample was expressed by the ratio of lowering of the absorption of DPPH (%) and the absorption (ASB) (100%) of DPPH solution in the absence of test sample (control). The mean value was obtained from four replication experiments. The percent inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{1 - \text{Mean of sample ASB}}{\text{Mean of control ASB}} \times 100$$

The antioxidant activity of each sample was expressed in terms of fifty percent of effective concentration (IC_{50}) value, which required inhibition of DPPH radical formation by 50%. The IC_{50} value was calculated by plotting the inhibition percentage of each tested concentrations in the logarithmic graph. A logarithmic equation was used to calculate IC_{50} value.

Determination of ascorbate peroxidase activity (APX)
Enzyme extraction: Five gram of rice powder was weighed into a centrifuge tube. Five milliliters of extraction buffer (0.1 M phosphate buffer, pH 6.0) were added and mix well on shaker for 15 min. The mixture was then centrifuged at 12000 rpm at 4°C for 30 min. Supernatants were used for enzyme assays. All steps of extraction procedure were carried out at 1-4°C.

Soluble protein content of the extracts was determined by the method of Bradford (1976), which used a Bio-Rad protein assay kit with bovine serum albumin (Sigma, St. Louis, MO, USA) as the calibration standard (150-900 μg mL^{-1}) at 595 nm. Fifty microliters of extracted solution added to 2.5 mL Biorad before it was vortexed for 5 min. Then the mixture was shaken for one minute before measuring the absorbance at 595 nm.

APX was determined according to Nakano and Asada (1981), with some modifications. The reaction mixture (2.0 mL) contained 0.05 M phosphate buffer (pH 7), 0.1 mM EDTA, 5 mM ascorbate and 800 μL of crude enzyme extract. The reaction was initiated by adding 200 μL H_2O_2 (2 mM). The decreasing of absorbance from the oxidation of ascorbate at 290 nm was recorded using a spectrophotometer (Hewlett Packard 8453, Germany) at 1,200 s after the adding H_2O_2. The activity of APX was expressed as μmol/min/100 mg protein.

Determination of superoxide dismutase activity (SOD): The method of Oberley and Spitz (1985), with some modifications was used to determine the activity of SOD. One milliliter of reaction mixture for the determination of SOD activity contained 800 μL of reaction mixture (0.1 mM Xanthine, 0.056 mM NBT, 1.0 mM DETAPAC and 1 U CAT in 0.05 M phosphate buffer (pH 7.8)), 100 μL of enzyme extracted and 100 μL of 0.1 U mL^{-1} xanthine oxidase. SOD was evaluated by measuring the ability of the enzyme extract to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT). The reaction was initiated by illuminating the reaction mixtures at 26-28°C for 30 min and absorbance was read at 590 nm. One unit of SOD was defined as the enzyme activity that inhibited photoreaction of NBT to blue formation by 50%. SOD activity of the extracts was expressed as Δ activity mg^{-1} protein.

Determination of lipoxygenase activity (LOX)

Enzyme extraction: Three gram of rice powder from the seeds subjected to various duration of ageing were homogenized with 5 mL of extraction buffer (0.05 M sodium borate buffer, pH 9.0) and shaken for 30 sec. The mixture was centrifuge at 12000 rpm at 26-28°C for 5 min. For LOX assay, 5 mL of 96% (v/v). Ethanol were added and incubated at 26-28°C for 15 sec, then filtered. Afterwards, 3 mL of 70 % (v/v) ethanol were added to 50 μL of extract solution and absorbance was read at 234 nm using 70% (v/v) ethanol for blank. LOX activity of the extracts was expressed as Δ activity mg^{-1} protein (Meshehdani *et al*., 2006).

Calculation and statistical analysis: The data are presented as Mean±SD. The analysis of variance was performed for data analysis and differentiated with LSD test at $p<0.05$ using the software SX release 8.0 (Analytical software, Tallahassee, USA).

RESULTS

Total protein content: The differences of the total protein content between seed treatment variances are shown in Fig. 1. As expected and there was a reduction, which affected from both of seed treatment variances and the storage time. Therefore, the biological seed coating substances; CL and E+CL were non-significantly different when compared with CO. On the other hand, seed greatly deteriorated due to effect of CA, the results revealed that, gradual loss of total protein content was observed in CA and these differences were more accentuated after stored for 12 months.

Total lipid and free fatty acid content: Figure 2 showed the content of total lipid decreased sharply in CO and CA, particularly at the long storage time. On the other hand, a gradual decrease in total lipid content occurred in both of biological seed coating substances; CL and E+CL, which reached higher level of total lipid content. Later stored for 12 months, the content of total lipid slightly decreased in both coating substances.

Within 12 months of storage, measurable FFA was discernible in each of seed treatment variances (Fig. 3). The result showed that, FFA increased relatively rapid with higher rates in CO and CA than CL and E+CL. The increased was much higher after 6 months storage and reached maximum after stored for 12 months. CL and E+CL were more effective in keeping the minimum level of FFA content during storage.

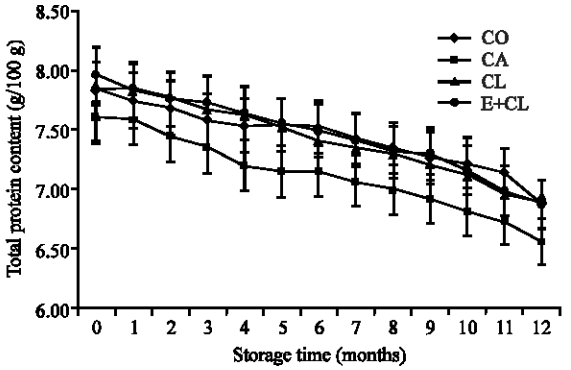

Fig. 1: The effect of seed treatment variants and storage duration on total protein content of rice seed during storage for 12 months. Values reported as Mean±SD of 4 replications of storage samples

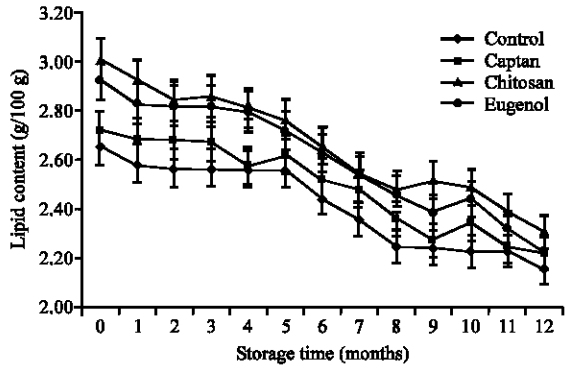

Fig. 2: The effect of seed treatment variants and storage duration on lipid content of rice seed during storage for 12 months. Values reported as a Mean±SD of 4 replications of storage samples

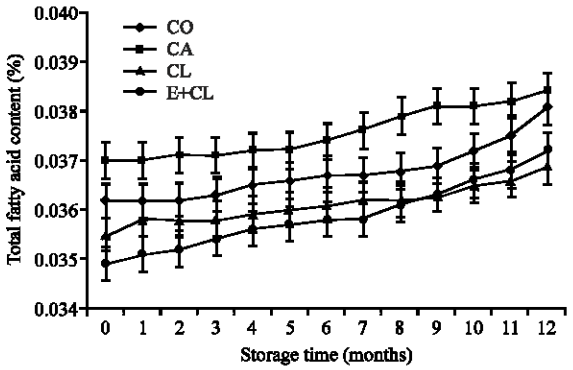

Fig. 3: The effect of seed treatment variants and storage duration on free fatty acid content of rice seed during storage for 12 months. Values reported as a Mean±SD of 4 replications of storage samples

Table 1: The effect of seed treatment variants on glucose, sucrose, lactose and maltose content changes during storage for 12 months

	Glucose (g/100 g DM)		Sucrose (g/100 g DM)		Lactose (g/100 g DM)		Maltose (g/100 g DM)	
	0	12	0	12	0	12	0	12
Treatment	---------- (months) ----------		---------- (months) ----------		---------- (months) ----------		---------- (months) ----------	
CO	6.42±0.29ab	4.12±0.72a	3.21±0.14ab	2.05±0.36a	9.51±0.40b	6.23±1.09a	10.16±0.44a	6.58±1.13a
CA	6.06±0.82b	2.97±5.77E-0.3b	3.03±0.44b	1.83±0.17a	9.89±0.40b	4.50±0.02b	10.97±1.03a	5.13±0.60a
CL	9.10±2.77ab	3.54±0.19ab	4.55±1.39ab	1.76±0.10a	11.82±1.76ab	5.34±0.29ab	13.85±4.41a	5.67±0.32a
E+CL	9.44±1.86b	4.11±0.80a	4.72±0.93a	2.05±0.40a	13.59±1.59a	6.20±1.20a	14.52±3.04a	6.59±1.26a

The different letters indicate the statistically significant difference by LSD at 5% level. Data are expresed as Mean±SD

Soluble sugar content: On the minor components, the soluble sugars are of particular interest because of their derived from starch, which concentrated in the endosperm of the rice seed. These compounds are also related to rice seed deterioration. The data are available on the concentration of glucose, sucrose, lactose and maltose. The changes observed suggested that at least two factors are affecting to soluble sugars content, one involves seed treatments and the other is storage time. Table 1 showed that CL and E+CL were contained appreciable amounts of glucose, lactose, maltose and sucrose content. In the case of CO and CA, showed dramatic affected to decrease on various types of those soluble sugar components. Although, after stored for 12 months, which showed clear patterns, the average of glucose, lactose, maltose and sucrose levels tended to be lowest in CA. On the other hand, CL and E+CL did not significant different with CO.

Changes in free radical scavenging enzymes; APX and SOD activities: The activities of all two enzymes; APX and SOD were decreased as affected of CA when compared with CO. On the other hand, CL and E+CL are always maintained much highest enzyme activities. Figure 4 shows the activity of APX. At the beginning of storage, the APX activity of CL and E+CL (0.895 and 0.885 Δ activity mg^{-1} protein, respectively) did not significant different with CO (0.880 μmol/min/100 mg protein). However, CA significantly decreased APX activity (0.829 μmol/min/100 mg protein). After storage, APX activity continuously decreased, especially on CA had significant affected on APX activity (0.033 μmol/min/100 mg protein) when compared as CO (0.050 μmol/min/100 mg protein). Nevertheless, CL and E+CL could maintained much highest of APX activity (0.121 and 0.122 μmol/min/100 mg protein, respectively). Figure 5 showed the changes in SOD activity as compared between seed treatments variances. The pattern of the curves was similar whatever the way of expression of results, which indicated that each treatment resulted in no significant difference. CL and E+CL showed high level of SOD activity. On the other hand, CA inhibited activity of SOD, which showed lowest SOD activity.

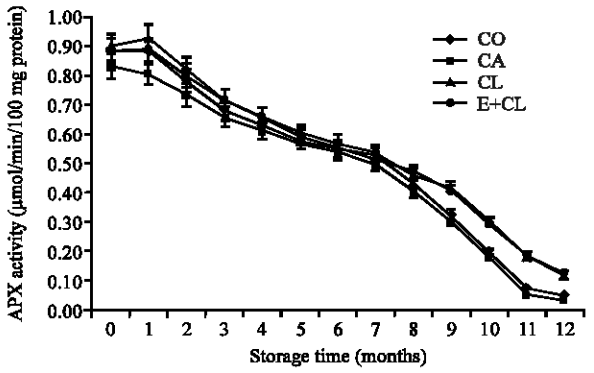

Fig. 4: The effect of seed treatment variants and storage duration on the activity of ascorbate peroxidase of rice seed during storage for 12 months. Values reported as a Mean±SD of 4 replications of storage samples

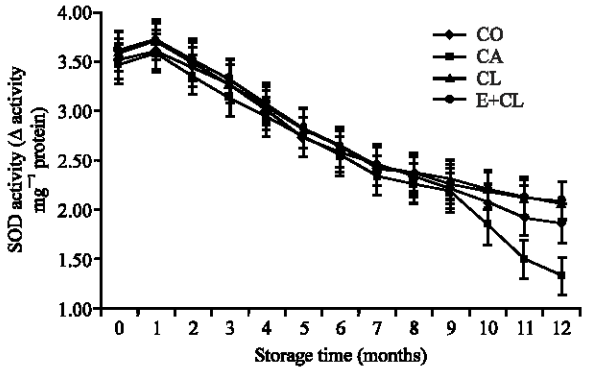

Fig. 5: The effect of seed treatment variants and storage duration on the activity of superoxide dismutase of rice seed during storage for 12 months. Values reported as a Mean±SD of 4 replications of storage samples

Changes in LOX enzyme activities: The results were showed that, CA increased significantly on LOX activity at the time before storage (0.0358 Δ activity mg^{-1} protein) when compared as CO (0.0226 Δ activity mg^{-1} protein). Although, CL and E+CL showed less activity of LOX (0.0204 and 0.0195 Δ activity mg^{-1} protein, respectively),

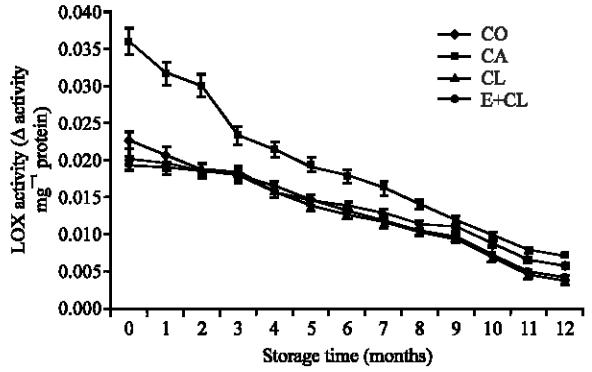

Fig. 6: The effect of seed treatment variants and storage duration on the activity of lipoxygenase of rice seed during storage for 12 months. Values reported as a Mean±SD of 4 replications of storage samples

Table 2: The effect of seed treatment variants on total antioxidant activity changes during storage for 12 months

	Antioxidant activity (IC_{50})**	
	0	12
Treatment	(months)	
CO	0.1177±8.89E-03ab	0.9662±0.03a
CA	0.1260±2.50E-03a	0.8934±0.07ab
CL	0.1145±4.80E-03b	0.8093±0.06b
E+CL	0.1174±2.51E-03ab	0.8256±0.07b

The different letters indicate the statistically significant difference by LSD at 5% level. **IC_{50} was expressed in terms of the 50% of effective concentration (IC_{50}) value, which required inhibiting DPPH radical formation by 50%. This IC_{50} parameter has the drawback value with the total antioxidant activity. Data are expresed as Mean±SD

which exhibited the same trend like untreated seed (Fig. 6). Subsequently, after storage, all seed treatment variances resulted in a significant decrease in LOX activity. LOX activity was slightly changed in CL and E+CL. On the other hand, LOX activity sharply increased in CO, but a significant transient in LOX activity was observed under CA.

Changes in total antioxidant activity: For the results of total antioxidant activity indicated in Table 2, at the beginning of storage, total antioxidant activity was significant difference between seed treatment variances. Total antioxidant activity was the highest in biological seed treatments; CL and E+CL. This results non-significant difference when compared as CO. Nevertheless, CA significant decreased the activity of total antioxidant. After stored for 12 months, CA affected seriously on total antioxidant activity and non-significant difference when compared as CO. Although, CL and E+CL can be maintained the highest level of total antioxidant activity.

DISCUSSION

In rice seeds, chemical and biochemical properties are commonly used criteria for measuring the seed deterioration. Most of the usually applied seed treatment substances directly affected the mobilization of food reserves in the seeds, which is essential for the growth of embryogenic axis during early seed germination. The present results showed that storage time, CA treatment and the interaction of seed treatment and storage time affected the biochemical degradation in rice seeds significantly higher than the biological seed treatment. In congruence with Kozlowski (1986), seeds injury was reported after the use of captan. Van Iersel and Bugbee (1996) described that the metabolism of captan is activated as aqueous slurry; it released tetrahydrophthalimide, tetrahydrophthalic acid and three molecules of chloride (Cl^-). The penetration of these compounds into the cell can cause phytotoxic effects.

In present experiments, CA treated seeds had higher amount of FFA and a higher LOX activity than biological coated seeds and seeds without treatment (CO). The experiments suggested that under the stress conditions and during storage, lipid in rice seeds are broken down by LOX into FFA. An interesting result of the present study was that the CA treatment induced lipid peroxidation, which was seen in an inverse relationship between lipid and FFA content, lipid and LOX and a positive correlation between LOX and FFA as well, that resulted of lipid peroxidatin mechanism. According to Narvaey-Vasquez *et al.* (1999), the deterioration of rice seeds is generally accompanied with lipid peroxidation. Rosahl (1996) who reported that CA induced LOX activity also found this relationship. The degradation of lipids in senescing membranes and the release of FFA initiate oxidative deterioration by providing substrate for LOX. This mechanism releasing Reactive Oxygen Species (ROS) from membrane phospholipids in aleurone layer of rice seeds in response to chemical stress conditions could induced cell senescence (McCord, 2000). The ROS have the potential to damage cell membranes and is likely to be a primary cause of deterioration of seeds (Sung and Chiu, 1995). Moreover, several comprehensive reviews have identified ROS mediated lipid peroxidation, enzyme inactivation, protein degradation, disruption of cellular membranes and damage to genetic (nucleic acids) integrity as major cause of seed deterioration (McDonald, 1999).

The CA treatment decreased also the total protein content in the seeds. However, the biological coated seed enhanced the relative synthesis of proteins of the seeds. Apparently, CA stress altered the turnover of proteins. According to Slavin (2003), ROS can activate proteins by

reacting with specific amino acids (amine group), which resulted in DNA degradation and impaired transcription which causes incomplete protein synthesis. CA had a multi-site activity, which inhibited the tubulin formation, affecting DNA and mRNA synthesis and metabolism (effects on cell division) as well as site I and II of electron transport pathways. Therefore, the degradation of protein content was assumed to be associated with the change of nitrogenous fractions and a decrease in true protein nitrogen. Dell'Aquila and Spada (1992) reported that CA inhibited amino acids synthesis, especially glutamine. Moreover, it acted like a glutamine synthetase inhibitor, which directly affected the total protein content. Hydrolytic enzymes were inhibited by CA, which might influence the protein turnover in germinating seeds. Xu *et al.* (1997) also reported that CA inhibited such enzymes that decreased the protein content during rice seed storage. However, it does not identify specific mechanisms. Therefore, future studies about the effect of CA stress on protein degradation could be interesting.

This study demonstrated a clear relationship between the detoxifying enzymes (APX and SOD), total antioxidant activity, the lipid peroxidation and the seed deterioration depending on seed treatments. APX and SOD as well as total antioxidant capacity were in biological coated seeds more active than in the CA treatment and CO. The experiments suggested that those are important factors for scavenging ROS, which induced the seeds tolerance to oxidative stress conditions. These findings are similar to that of Esfandiari *et al.* (2007). Additionally, the loss of seed viability was associated with the decrease of SOD, APX and antioxidant activity in the seeds. Asada (2000) found that higher activities of SOD and APX decreased the level of ROS in the cells and increased the stability of cell membrane, as well as activate the Calvin cycle that could maintain the seed viability as well.

During the early period of seedling growth, the main source of energy is derived from the sugars. This sugar is released after enzymatic hydrolysis starch that is stored in the endosperm of the rice seeds. The water was consumed and induced the starch hydrolysis and the sugars were converted to CO_2 and water via the respiration. These reactions led to the decrease of the sugar content in the seeds. The sugar content was also positive correlated with seed germination and vigor. The biological treated seeds had a higher seed quality throughout the storage period, because they could maintain high sugar contents inside the seeds. Glucose was derived from the endosperm in the amylolytic breakdown process. Following that, it was mobilized to the scutellum, where the sucrose synthesis occurred. After that, the glucose was transported to the embryonic axis for further seedling development.

The present results suggested that Maillard reaction might be a mechanism that declined seed viability. This reaction occurred after an initial simple non-enzymatic attack on amino groups of protein and nucleic acid/protein complexes by reducing sugars (Bradbeer, 1988). The AGE products (Advanced Glycosylation End-products) from Maillard reactions occur in both aged seed and deteriorated seed (Sun and Leopold, 1995). In addition, lipid peroxidation and sugar hydrolysis were coupled to the Maillard reactions during seed storage (Wettlaufer and Leopold, 1991).

CONCLUSIONS

The results clearly explained that seeds coated with CL and E+CL decreased to a lesser extend rice seed quality compared to CA treatment or no seed treatment (CO). The storage time was probably the main factor that affected biochemical degradation. However, the results indicated that CA treatment affected rice seed viability significantly, which was associated with biochemical deterioration. After 12 months storage, total protein content decreased and was accompanied by declined of lipid content, which was activated by LOX and produced more FFA . These mechanisms showed that an increasing of lipid peroxidation led to the rapid loss of seed viability. In the case of biological fungicide coating substances, the lipid peroxidation was alleviated by the APX and SOD enzymes and showed a high antioxidant activity. These mechanisms induced the seed tolerance to oxidative stress conditions. Moreover, the sugar content was positive correlated with seed germination and vigor. Preliminary evidence under fungicide stress showed that sucrose and larger oligosaccharides were lost but in the biological coated seeds their compounds were consistently presented and therefore, this treatment maintained high seed quality throughout the storage.

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