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Changes in quality properties of anthocyanin, protein and amylose contents in colored rice grains during storage

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Abstract

Rice (*Oryza sativa* L.) is the world's most important cereal crop and is in great demand to supply stable food for over half of the worldwide population, particularly in Asia countries. Aside from nutritional value, pigmented rice is being considered a promising source of antioxidant molecules and bioactive compositions, which increasingly received much attention from researchers and food producers around the world. This study aims to determine the quality modification of "Cam", a colored - paddy rice under the different storage conditions. The treatments were arranged in a completely randomized design and repeated 15 times each with packaging factors including poly amid bag (PA), polypropylene bag (PP), and Jute bag (JB). The variations of anthocyanin, protein and amylose contents during storage were determined. The results have revealed that there were no significant changes in "Cam" rice- anthocyanin and protein contents during 3 months of stock for all samples, but a slight decrease of these contents after 6 months of storage and a great decline of those for 9 and 12 month-stored samples. Meanwhile, the rice samples' amylose content showed a lower decrease, especially for those stored in PA bags during 9 months of stock. As a result attained, this study has found the optimal to store pigmented rice paddy rice at 25°C under the vacuumed-packed PA packaging.

Keywords: Colored rice, amylose, anthocyanin, protein, changes, "Cam" rice storage.
Abbreviations: PA_ Poly amid bag; PP_ polypropylene bag; JB_ Jute bag; SD_ Standard deviations; LSD_ Least significant difference; ANOVA_ Analysis of variance.

Introduction

Rice (*Oryza sativa* L.) is the major cereal crop in the world, supplying stable food for over half of the world population, especially in Asia countries (Wei et al., 2007). It possesses an important source of dietary carbohydrates and plays a vital part in nutritious intake (Yang et al., 2006). A vast variety of rice has been cultivated globally including white rice and varieties of pigmented or colored rice. The pigmented rice is defined as a variety of colored grains such as back, red, brown and purple. The most popular rice species have a white pericarp (>85%), meanwhile colored variants have various pericarp pigments of green, black or red (Htwe et al., 2010). In developing countries, a great calorie intake is primarily from the consumption of rice for a diet, which resulted in minerals and vitamin deficiency for the majority of people. Therefore, it is required innovation and the growing of nutrient-rich rice species for additional health benefits. A great amount of dietary fiber, minerals, carotenoids, flavonoids and polyphenols have been found in the traditional colored rice varieties and a diet with pigmented rice grains helps to promote consumer health (Anuprialashmi et al., 2019; Hue et al., 2017). Bioactive compounds of colored rice cultivars showed antioxidant ability and radical-scavenging properties, which attract much interest for their prospect to bring and boost human health by decreasing the amount of reactive oxygen molecules and free radicals (Adom and Liu, 2002).

Generally, the structure of the grain of rice includes hull, pericarp, seed coat, nucellus, embryo, the endosperm. The endosperm consists of the aleurone layer and the endosperm proper. The aleurone layer encloses the embryo, has large quantities of oils, minerals, and vitamins (Juliano and Tuaño, 2019). Colour rice contains anthocyanins in certain layers of the rice kernel, which could be separated into anthocyanin-rich fractions used as functional colorants or functional food ingredients (Francavilla and Joye, 2020). Rice endosperm is an industrial by-product containing starch that is mainly composed of amylose and amylopectin (Totaro et al., 2018). The amylose/amylopectin ratio significantly affects rice starch digestion in the gastrointestinal tract. Low amylose content in rice was suitable to use product consumption (Denardin et al., 2012). Besides, rice endosperm also contains protein content, which played a major role in cooked rice texture. Rice protein contains lysine that is considered hypoallergenic and is therefore favorable for human consumption (Al-Doury et al., 2018).

"Cam" rice, or black aromatic rice, is a special rice variant figured out by Vietnamese researchers. "Cam" rice possesses a great content of anthocyanin (46mg/100g) and protein (10.5%) but poor amylose content (16.6%). Moreover, it contains more vitamins, minerals, dietary fiber than those of other rice species. Owing to its excellent taste and nutrient components, "Cam" rice has been vastly grown and consumed in this country. However, because of the seasonality of production and the continuity of consumption, most freshly harvested "Cam" grains must be stored and subsequently processed for consumers and industries. These rice grains are often kept unpolished grains to maintain their available nutrients, vitamins and minerals (Loan and Thuy, 2019).

A majority of processed rice quantities are consumed for daily diet, whereas only a tiny amount of rice is used as raw materials for food products and animal feed. Accordingly, it is importantly required to store rice grains for a period of time without quality damage before consumption. During the storage period, rice aging causes a series of alterations in physical, chemical and physiological properties (Zhou et al., 2016). Typically, its changes in physical attributes and chemical components influence the rice cooking and eating quality (Singh et al., 2006). During the storage time, modifications of stored rice quality depend largely on applied conversation techniques and environmental conditions (Rajendran, 2004). To the best of our knowledge, there are no published results of the influences of package types on the alteration of anthocyanin, protein and amylose contents in "Cam" rice for 12 months. Therefore, the objective of this study was to evaluate the quality changes of "Cam" rice packed in various types of bags, which determine the most appropriate type of package for rice storage. Our finding has provided a valuable suggestion for a rice packaging design for optimal storage conditions in order to prolong the shelf-life and maintain the nutritional value of varieties of pigmented rice.

Results and Discussion

Effects of storage conditions on anthocyanin of "Cam" paddy rice

Anthocyanins are an important group of phenolic and water-soluble pigments with high antioxidant activities. Fundamentally, a large amount of anthocyanin responsible for colored rice is found in rice coats and its liquid waste (Chaudhary, 2003; Xuan et al., 2018). Recently, the health positive effects of anthocyanins have been proved for antioxidant activities and anti-mutagenic and chemo-preventive properties contributing to the decline of incidence of chronic diseases including neuronal and cardiovascular diseases, cancer and diabetes (Jing and Giusti, 2007; Konczak and Zhang, 2004).

In this study, our attempts were to evaluate the effects of three types of packaging on the anthocyanin content of "Cam" rice during 12 months of storage. Strikingly, the total anthocyanins content of these samples of PP, PA and JB showed a different variation. Only a slight reduction in anthocyanin content was determined after 6 months for all samples. However, there has been a significant decrease in anthocyanin concentration after 9 and 12 months of stock and the decline depended on various types of bags. The vacuumed-packed sample in PA bags has remarkably remained a higher anthocyanin content than the others (Fig.1). The results have indicated that there is an insignificant modification of total anthocyanin concentration in rice packs. This is due to the anthocyanin compositions of black rice consisting of 88% cyanidin-3-glucosides, which are stable at 25°C within 4 months of storage (Htwe et al., 2010). Over 6 months of stock, anthocyanin contents greatly fluctuated, aside from the samples in PA bag remains their quality during 6 months owing to preventing effectively bio-physiological and biochemical activities.

Air oxygen concentration enhances a strong effect on rice grain quality, which induces direct and indirect oxidation of anthocyanins (Jing and Giusti, 2007). Besides, during the storage period, Vietnamese tropical climate with a high air relative humidity facilitates moisture absorption of grain and increases its respiration intensity leading to a raise of rice bulk's humidity, which damages the anthocyanin molecular structure by linking water to C2 of the flavylium cation to transform the colorless chalcone (El-Sayed et al., 2003). Therefore, the rice samples in permeable PP or JB permit room air to enter in bags as a result of destroying anthocyanin. This has been also confirmed by many previous studies of the great effects of packaging materials, storage temperature, and time on anthocyanin and polyphenol (Tananuwong and Lertsiri, 2010; Tananuwong and Tangsrianugul, 2012). Apart from the rice samples in PP and JB, the ones in the vacuumed-packed PA bag showed the lowest reduction of anthocyanin contents due to barrier properties of PA and vacuum condition, which helps lessen moisture absorption and eliminate air oxygen. Consequently, "Cam" rice bulk has been recommended to stock in the vacuumed-pack PA bags for 6 months without pigments change.

Effects of storage conditions on the total protein content

Rice protein is the second most abundant constituent with high quality as compared to different protein sources (Oko et al., 2012), in particular, "Cam" rice containing 10.5% of protein. During storage time, the protein content significantly and steadily reduced after over 3 months as shown in Table 1.

The results disclosed that for the initial 3 months at room storage condition, there were no significant changes in "Cam" rice protein content, however a great alteration ($P < 0.01$) was determined after 6, 9, 12 months and fluctuation speed varied depending on different stock conditions, approximately 2.8% in JB, and 2.6% in PP and 2.0% in PA, respectively. This is due to the Maillard reaction, which is non-enzymatic interaction of free amines of proteins and carbonyl groups of reducing sugars to form complicated intermediate compounds (Oessoe et al.,

Table 1. Modification of total protein content (%) of "Cam" paddy rice stored in various bags.

Storage months	0	3	6	9	12	Average
JB	10.5	10.1	9.1	8.5	7.7	9.2^{A}
PP	10.5	10.3	9.5	8.7	7.9	9.4^{A}
PA	10.5	10.4	10.3	9.7	8.5	9.9^{B}
Average	10.5^{a}	10.3^{a}	9.6^{b}	9.0^{c}	8.1^{d}	
F (bags)_ **; F (months)_ **						

Different superscript letters "A–B" at each column indicate significant differences ($p < 0.05$) total protein content of "Cam" paddy rice stored in the bags; Different superscript letters "a–d" at each row indicate significant differences ($p < 0.05$) total protein content of "Cam" paddy rice stored the time.

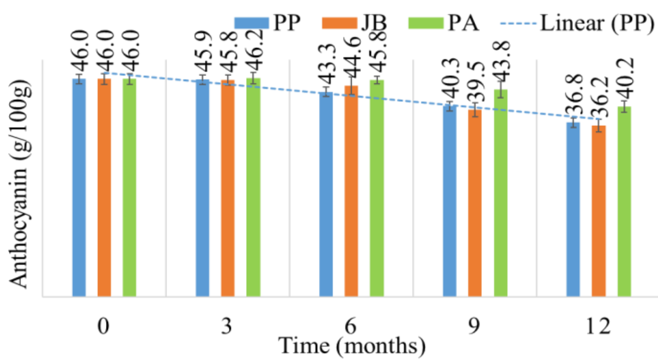

Fig 1. Decrease of total anthocyanin contents of "Cam" rice samples during storage in various types of packaging.

Table 2. Fluctuation in amylose content (%) of "Cam" rice packed in different storage bags.

F (Bag)	Months	Amylose (%)	Months	Amylose (%)	Months	Amylose (%)	Months	Amylose (%)	Months	Amylose (%)	Average
JB	-	16.6^{d}	3	16.4^{cd}	6	16.4^{cd}	9	16.3^{c}	12	15.1^{a}	16.16^{a}
PP	-	16.6^{d}	3	16.5^{cd}	6	16.4^{cd}	9	16.3^{c}	12	15.4^{a}	16.23^{ab}
PA	-	16.6^{d}	3	16.6^{d}	6	16.5^{cd}	9	16.3^{c}	12	15.8^{b}	16.36^{b}
Average		16.6^{C}		16.5^{C}		16.4^{BC}		16.3^{B}		15.43^{A}	16.3
F (interaction)_ **; F (bag)_ *; F (time)_ **											

A–B_ Different superscript letters at each column indicate significant differences ($p < 0.05$) amylose content of "Cam" paddy rice stored in the bags; a–d_ Different superscript letters at each row indicate significant differences ($p < 0.05$) amylose content of "Cam" paddy rice stored the time.

2014). Similarly, Kumar et al. (2017) also found a slight and steady decrease in protein content after storage time.

Changes in amylose content of "Cam" paddy rice

Amylose content is recognized as the most significant parameter for estimating rice cooking and processing behavior. Amylose content is directly associated with water absorption, volume, fluffiness, and separability of cooked grains and is inversely engaged in cohesion, tenderness, and glossiness (Zhou et al., 2002). Stock condition and time show varied impacts on the amylose content of "Cam" rice (Table 2). Initially, "Cam" rice contains 16.6% of amylose and after 6 months of stock, there was no significant modification in amylose content in all bags of PA, PP, JB (Table 2), but a slight decrease of amylose concentration was analyzed after 9 months. Until after 12 months, it is shown a great reduction in their amylose contents. In fact, the study of Abeysundara et al. (2015) reported no significant changes in amylose content of various rice species during 13 weeks of storage, but important variations in amylose concentration of stored rice samples of 40 weeks ($p<0.05$). Indeed, the chain length distribution of rice amylose can be changed over time under the storability of rice due to a minor increase in the percentage of short chains from DP6 to 15 (Huang and Lai, 2014). Moreover, Labuschagne et al. (2014) proved that starch and amylose content of maize grains after 6 and 12 months of stock at 30°C were significantly decreased ($P \leq 0.05$). This may explain that for the initial days of stock, rice germ of grains retains maturing stage and contains a considerable content of simple sugars involving continual metabolic activity. However, during storage as aging, its germ is stabilized, while simple sugars are exhausted, as implied rice grains are forced to use amylose in its germ for metabolic reaction causing a slight decline of amylose content at the early stage period of stock and following that, the degradation of amylose is relatively speedy.

Materials and Methods

Plant materials

Grain samples_ "Cam" rice crop was grown in Cai Lay district, Tien Giang province, Vietnam. It was harvested in December 2018. These rice samples of 24% moisture content were dried to 13% for preventing microbial growth. The samples of 300g of the paddy rice were vacuum-packed in poly amid (PA) bag or enclosed in PP (Polypropylen) bag or Jute bag (JB), and stocked at room temperature of 25°C for 12 months. Triplicate samples were processed for each specific storage condition (various packed bags and storage times of 0, 3, 6, 9 and 12 months). After a specific storage time, three bags of the rice samples were randomly selected for chemical analysis including the concentrations of anthocyanin (mg/100g), protein (%) and amylose (%).

Determination of anthocyanin content

The total anthocyanin content was determined according to the spectrophotometric pH differential method (Lee et al., 2005). Briefly, an aliquot (0.3 mL) of anthocyanin sample was mixed with pH 1.0 (potassium chloride buffer, 9.7 mL) and pH 4.5 (sodium acetate buffer, 9.7 mL) solutions, respectively, and equilibrated for 30 min at room temperature in the dark. The absorbance of the mixture was measured at 525 and 700 nm using a UV-Vis spectrophotometer. The total anthocyanin content was calculated as cyanidin-3-glucoside equivalents as in the following equation_

Anthocyanin content (mg/100g) = $\frac{A\ x\ M_w\ x\ D_F\ x\ V\ x\ 100}{\varepsilon\ x\ l}$

where A = [A525 − A700)pH1.0] − [(A525 − A700)pH4.5], Mw is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, V is the volume of the extract (mL); ε is the molar extinction coefficient (26,900) of cyanindin-3-glucoside, and l is the cell path length (1cm).

Determination of protein content

The total protein content was determined by the Kjeldahl method (AOAC, 2010). The protein content of the rice flour was analyzed using the Kjeldahl method and expressed using the conversion factor N × 6.25.

Determination of amylose content

Dry rough Cam rice was dehulled and milled using a commercial milling machine. The flour samples were passed through a 100-mesh sieve, packed in poly amid bag, and stored at 4°C until used. Amylose content was measured by the modified method (Juliano et al., 1981). Rice flour was defatted immediately using hexane with the Soxhlet apparatus. Then 100 mg of rice flour was put in a conical flask, to which 1 mL of 95% ethanol and 9 mL of 1 mol/L NaOH were added. The suspension was kept at ambient temperature for 16-24 h, and then distilled water was added to make a 100 mL solution. A 5 mL aliquot of the solution was transferred to a 100 mL volumetric flask, and to adjust pH, 1 mL of 1 mol/L acetic acid was added. Then 2 mL of 0.2 g/ L iodine solution (I2_ 2 g/KI_ 20 g/L) and distilled water were added to make exactly 100 mL. Spectrophotometer measurements were made at 620 nm after the above starch-iodine solution was incubated for 20 min at ambient temperature. Standard curves were generated using the mixture of potato amylose.

Statistical analyses

The collected data were calculated and shown in the study through means and standard deviations (SD) by Microsoft Excel version 2012. All data were subjected to analysis of variance (ANOVA) by the Statistical Analysis System software. When a significant treatment effect was observed, the Least Significant Different **(**LSD**)** test was used to compare means at $p<0.05$.

Conclusions

In summary, the changes in quality properties of the valuable components including anthocyanin, protein and amylose contents in "Cam" paddy rice stored in various conditions have been evaluated and determined during 12 months storage at room temperature. The results have revealed that the anthocyanin, protein, amylose contents of vacuumed-packed PA "Cam" rice grains were greatly remained during 6 months in PA and 3 months for the grains packed in PP and JB. Therefore, pigmented rice varieties, particularly "Cam" paddy rice should be stored at room temperature in vacuumed-packed PA packaging for its optimal quality. Our findings have provided useful information to prolong the shelf-life and maintain the nutritional values of varieties of pigmented rice for rice producers and industries.

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