Effect of modified atmosphere packaging on the quality of germinated parboiled brown rice

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Abstract Germinated parboiled brown rice (GPBR) is a healthy Thai food. To extend the quality of GPBR cv. KDML 105 with 8.06% moisture content, the effects of three packaging conditions on the characteristics of GPBR were investigated. GPBR in laminated (nylon/LLDPE) bags was sealed as follows: vacuum-sealed, 40% N₂-sealed with 60% CO₂ (60% CO₂+40% N₂), or 100% N₂-sealed before storage under ambient conditions (30±2°C, 70±5% RH) for 15 months. GPBR in all the packages exhibited delayed fungal proliferation and very low aflatoxin B₁ contamination (<0.82 µg/kg). The 100% N₂-sealed package was the best treatment to preserve GPBR quality, as indicated by the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity and free fatty acid content as well as the least rancidity and browning. As observed through a scanning electron microscope, compared with vacuum-sealed packages and those sealed with 60% CO₂+40% N₂, the lowest hardness texture and the least elongation of cooked rice in 100% N₂-sealed package were linked to a lesser compact GPBR endosperm ultrastructure.

Keywords: storage, parboiled rice, aflatoxin B₁, 100% N₂

Introduction

Rice is a staple food in Asia, where its daily consumption in the form of milled cooked rice is common. Alternatively, brown rice (BR) and germinated parboiled brown rice (GPBR), a traditional food in Thailand, are being increasingly consumed and appreciated as a healthy food. These forms of rice contain essential nutrition, particularly in their kernel's surface.

The presence of rice bran decreases the overall shelf life and quality of a rice grain mainly because of microbial growth, insect infestation, and chemical reactions. The unpleasant appearance of BR and GPBR kernels is attributed to their dark color. BR and GPBR contain high amounts of unsaturated fatty acid, protein, and reducing sugar and are susceptible to lipid peroxidation and oxidative deterioration as well as the browning reaction (1). This reaction (Maillard reaction) results in the dark brown appearance of the kernels, which leads to high non-marketability (1,2).

Lipid peroxidation and oxidative deterioration result in rancidity and high acidic content; this decreases the value of BR and GPBR. Additionally, this very high nutritive ingredient such as unsaturated fatty acid, protein and vitamins on their kernel surface enhances microbial growth, thereby leading to biodeterioration and contamination of mycotoxin. Aflatoxins are often reported in grains

with rich oil contents and in food products such as peanuts (3–5), almonds, pistachios, and walnuts (6).

Deterioration of BR and GPBR during shelf storage in a supermarket can be prevented by using suitable packages and packaging techniques. Vacuum packaging has been used to maintain quality losses in several foods. It allows extremely low O₂ availability in the package and is also a recommended BR preservation technique for supermarkets. Boonchoo *et al.* (7) reported that BR kept in a vacuum-sealed package exhibited delayed quality losses compared with rice kept in an ambient-air package (conventional technique) during storage.

Modified atmosphere packaging with varying portions of gasses is also a popular technique to prolong the shelf life of fresh produce. Additionally, flushing gases in a package is a common technique used for various processed foods to fulfil different purposes. Inert gases such as N₂ are generally used to avoid unwanted chemical reactions degrading food in the package (8) because it is known to reduce lipid deterioration (9).

CO₂ is used for fumigation to eliminate insect pests and fungal contamination (10) and is particularly recommended for organic rice. In a study, *Aspergillus flavus* and aflatoxin B₁ (AFB₁) contamination in BR was suppressed using 100% CO₂, and CO₂ did not cause any significant change in the proximate composition of protein and lipid

but reduced free fatty acid accumulation during storage (11). Ponsen (12) reported that using 100% CO₂ or 60% CO₂+40% N₂ delayed fungal contamination in sweet corn seeds compared with ambient air and led to greater percent germination during storage for eight months. Reportedly, gas ratios in the package can be altered to suit a food product in the package (13). Because of the low solubility of N₂ in water, the presence of N₂ can prevent packages comprising CO₂-absorbing products from collapsing as well as extend the storage stability of the products. Therefore, reducing the amount of CO₂ in an atmosphere using N₂ probably prevents packages from collapsing and delays fungal proliferation.

The efficacy of the concentrations of CO₂ and N₂ for the preservation of BR and GPBR in laminated plastic bags during ambient storage has not been thoroughly investigated. This research aimed to compare the quality and AFB₁ contamination in GPBR during shelf storage in laminated bags via vacuum sealing, 60% CO₂+40% N₂ sealing, or 100% N₂ sealing.

Materials and Methods

GPBR cv. KDML 105 was procured from Head Rice (Thailand) Co., Ltd, Bangkok, Thailand. GPBR was portioned into 1 kg and packed in 120-μm-thick nylon/linear low-density polyethylene (nylon/LLDPE) bags (12.5×30×5 cm³) and sealed using three techniques: vacuum-sealing (control), 60% CO₂+40% N₂ sealing, or 100% N₂ sealing using a vacuum packer (S225; VAC-STAR AG Switzerland, Sugiez, Switzerland). The experiment was arranged in a completely randomized design with three replications per treatment. The GPBR samples were stored at ambient conditions (30±2°C; 70±5% Relative Humidity, RH) for 15 months. The temperature and RH were monitored daily during the period of the experiment using a data logger (Nature Pro, Nature Eye, Thai Victory Co., Ltd., Bangkok, Thailand). The temperature and RH values calculated were the averages of the respective daily values. Quality measurements were monitored during storage. All chemicals used in this study were of analytical grade unless stated otherwise.

Moisture content (MC) MC was determined following the method of the American Association of Cereal Chemists (AACC) (14) with a minor modification. Five grams of GPBR were weighed in an aluminum can and then placed in a hot-air oven (Mettler, Bavaria, Germany) at 130±3°C for 1 h. The cans were moved to a desiccator until the temperature cooled down to ambient levels. The weight of the dried samples was recorded, and the percentage of MC was calculated and expressed on a wet weight basis.

Color The color of GPBR was measured with a color meter (CR-400; Konica Minolta, Osaka, Japan), described using the International commission on illumination (CIE) color system, and displayed as L^* ,

a^* , and b^* values. The color difference (ΔE or ΔE_{ab}) value was calculated using the following equation:

$$\Delta E = \sqrt{(L_n^* - L_0^*)^2 + (a_n^* - a_0^*)^2 + (b_n^* - b_0^*)^2}$$

Total fungal contamination The blotter method was used to measure total fungal contamination. One hundred kernels were placed on the blotter and incubated at room temperature for three days. Fungal contamination on the GPBR surface was visually observed and reported in percentage.

Aflatoxin B₁ (AFB₁) contamination AFB₁ was analyzed according to the method proposed by Chinaphuti *et al.* (15) with a slight modification. Briefly, 20 g of ground sample was placed into the flask and 100 mL of 70% methanol (1:5) were added. The mixture was agitated using a shaker at 300×g for 30 min and allowed to stand for 5 min. This mixture was then filtered through a Whatman No. 4 filter. The filtrate was diluted with 0.01-M phosphate buffer (1:20). The extract was analyzed using a DOA-Aflatoxin ELISA Test Kit (Department of agriculture-Aflatoxin enzyme linked immuno sorbent assay test kit), which contained 50 μL of the standard or sample in a coated well and 50 μL of an enzyme conjugate in every well with slight shaking. The mixture was then incubated at room temperature (37°C) for 30 min under dark conditions. The contents of the well were dumped into the appropriate waste container. The wells were thoroughly washed 3–5 times with the buffer and tapped on absorbent paper towel. Then, 100 μL of the substrate were added to the wells and incubated at room temperature in the dark for 5–10 min. This step indicated the qualitative result, for which the test well contained a 100-μL stopping solution. The absorbance of every well was measured at 450 nm using a micro ELISA Reader (Sunrise, Tecan, Switzerland).

Free fatty acid (FFA) content FFA was analyzed according to the AACC (16) method with a minor modification. Twenty grams of powdered GPBR were extracted with 50 mL of 99.8% benzene for 30 min; moreover, 20 mL of the rice extract were mixed with 20 mL of 95% ethanol. FFA was determined by titration with 0.01-M KOH using phenolphthalein as the indicator.

Thiobarbituric acid (TBA) value TBA was determined according to the method proposed by Hayashi *et al.* (17) with a slight modification. Briefly, 250 mg of powdered GPBR were mixed with 0.5 mL of 1.15% potassium chloride, 3 mL of 1% phosphoric acid, and 1 mL of 0.67% 2-thiobarbituric acid and then boiled for 45 min. The boiled solution was cooled and mixed with 4 mL of *n*-butanol. The absorbance of the supernatant at 520 and 535 nm was measured using a spectrophotometer (UV 1800; Shimadzu, Kyoto, Japan); moreover, 0.5 mL of 10-nmol/mL 1,1,3,3-tetrathoxypropane was used as the standard. The TBA value was calculated as follows:

TBA value (nmol/g dried weight (dw))

$$= \frac{(A_{535 \text{ sample}} - A_{520 \text{ sample}})}{(A_{535 \text{ standard}} - A_{520 \text{ standard}})} \times 40$$

Total phenolic content (TPC) TPC was determined via the method of Singleton *et al.* (18) using the Folin–Ciocalteu reagent; 2.5 g of powdered GPBR was mixed with 5 mL of 80% ethanol. This solution was centrifuged at $8,000 \times g$ for 40 min (Sorvall, RC5C plus, Kendro Laboratory, Weaverville, NC, USA). Then, 0.02 mL of the sample was mixed with 1.6 mL of distilled water, and 0.1 mL of the Folin–Ciocalteu reagent and 0.2 mL of 20% sodium carbonate were added. This solution was incubated at 40°C in a water bath for 30 min. Absorbance was measured at 765 nm with gallic acid as the standard, and the results were expressed as gallic acid equivalents (GAE).

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity The DPPH radical scavenging activity was determined using a free-radical DPPH (19) with slight modifications. Powdered GPBR (2.5 g) was mixed with 5 mL of 95% ethanol. The solution was centrifuged at $8,000 \times g$ for 40 min. Then, 0.15 mL of the GPBR extract was mixed with 2.85 mL of the DPPH working solution and kept in the dark for 30 min. Absorbance was measured at 515 nm. The DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_{\text{working solution}} - A_{\text{sample}})}{(A_{\text{working solution}})} \times 100$$

Ferric reducing antioxidant power (FRAP) FRAP was determined according to the method of Benzie and Strain (20). Powdered GPBR (2.5 g) was mixed with 5 mL of 80% ethanol. The solution was centrifuged at $8,000 \times g$ for 40 min. Then, 0.05 mL of the GPBR extract was mixed with 2.95 mL of FRAP reagent and kept in the dark for 30 min. The absorbance was determined at 593 nm. 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was used as the standard.

Elongation ratio The elongation ratio was determined according to the method of Juliano and Perez (21) with a minor modification. Ten rice kernels were set aside from 20 rice kernels per replication. The length of the kernels was measured using a digital caliper. The kernels were then put in a test tube (15 mm) containing 3 mL of distilled water. The length of 10 cooked rice kernels was measured. The elongation ratio was calculated as the ratio of the average length of cooked rice kernels to the average length of raw rice kernels.

Texture of cooked rice The cooking procedure of GPBR was previously tested to obtain the optimum volume of water and the length of time for cooking according to a local commercial method and that proposed by Chen *et al.* (22) with a minor modification. Briefly, whole grains of GPBR (25 g) were placed in an aluminum can

(12-cm diameter); then, distilled water (45 mL) was added. Subsequently, the cans were placed in a steamer. During steaming for 40–50 min, the cooked rice was monitored by taking 2–3 cooked rice kernels every 5 min. The rice samples were pressed between two pieces of glass petri dishes. A rice kernel without a white core was deemed sufficiently cooked at an average time of 45 min and thereafter used for hardness measurement. The hardness of the cooked rice was analyzed according to the method of Patindol *et al.* (23), using the uniaxial single compression method by a texture analyzer (TX-XT2 Plus; Texture Technologies Corp., Scarsdale, NY, USA). Cans of cooked GPBR were individually transferred and placed in a plastic box before covering with a lid. They were then stored in a foam box containing a thermal plastic bag filled with hot water. A single layer of 20 cooked rice kernels from the middle of the can was immediately arranged on a flat aluminum plate (100-mm diameter), and compression was set as follows: pre-test speed 0.50 mm/s, test speed 1 mm/s; post-test speed 10 mm/s, and target distance 4 mm. The maximum compression force (N) was used as an indicator of cooked-rice hardness. The temperature of the tested rice kernels was $50 \pm 3^\circ\text{C}$, as measured using an infrared thermometer (High Temperature IR Thermometer 42545, Extech Instruments, West Nashua, NH, USA). Each experimental unit was prepared in duplicates. Five measurements of each sample were analyzed for hardness, and the values were averaged.

Scanning electron microscopy (SEM) analysis The SEM analysis of GPBR kernels was monitored according to the method of Tulyathan *et al.* (24) with a minor modification. GPBR kernels were broken into two parts, and the microstructure of the endosperm coated with gold was then observed using SEM (JSM-6400; JEOL, Tokyo, Japan) at $\times 5,000$ magnification and an accelerating voltage of 10 kV.

Statistical analysis The data were subjected to analysis of variance (ANOVA), and the significant differences between the means were determined by Fisher's least significant difference (LSD) at $p \leq 0.05$ using the general linear model (GLM) procedures of the SPSS (Version 17.0, SPSS Inc. Chicago, IL, USA). The data given in percentages were subjected to arcsine transformation before ANOVA.

Results and Discussion

The amount of gases in a package has a specific effect on pest infestation and shelf life of food (25). This was also observed in our study.

MC The composition of the gases in the bags did not markedly affect the MC of GPBR during storage (data not shown) because it was comparable to the initial value (7.3–8.4% MC). This is due to the restrictive property preventing water and gas transmission through the laminated bag.

Color The superficial color of rice is an important factor in terms of visual quality for consumers. The color of GPBR slowly changed (Table 1). Brightness (L^*) of GPBR packed in the vacuum bag was the lowest. The packages with 100% N_2 and 60% CO_2 +40% N_2 maintained the a^* value (redness) of GPBR. At the 15th month, the a^* value of GPBR in the vacuum bag was the highest. Yellowness (b^*) of GPBR slightly increased but did not vary except for the sample in the vacuum bag. Compared with the initial values, stored GPBR showed a light brown appearance corresponding to slight increases in the b^* and a^* but not L^* values. Significant changes in the a^* , b^* and L^* values were observed in GPBR stored in the vacuum package. In the vacuum-sealed bag, the color change (ΔE) was 4.13, but it was only 1.98 and 2.21 in the 100% N_2 -sealed and 60% CO_2 +40% N_2 -sealed bags, respectively (Table 1). Generally, an occurrence of a dark brown color in stored grains and nuts is related to the non-enzymatic browning reaction (Maillard's reaction) (26). This reaction is particularly accelerated by heat, moisture, and O_2 . Compared with other test packages, we observed significantly lower L^* but higher a^* values in GPBR kept in the vacuum package (Table 1). This implies that the vacuum packaging contains more O_2 in an interparticle void volume than the packages sealed with 100% N_2 or 60% CO_2 +40% N_2 , thereby enhancing the color change of the kernel. Boonchoo *et al.* (27) reported that compared to the vacuum-sealed package, a higher O_2 content in the air in a sealed package accelerated the non-enzymatic browning reaction in the BR kernel. This response is similar to that in almond kernels, which had an in-package O_2 of 1.8%; this resulted in enhanced yellowing through the browning reaction (28). The use of 100% N_2 was promising in terms of maintaining the color of GPBR for 15 months. Similarly, 100% N_2 was

the most effective in delaying the color change (darkening) of faba bean (*Vicia faba* L.) compared with 100% CO_2 and 100% O_2 (2). In contrast to the quantitative color data, the change in color was slight, as shown by the ΔE value, which is comparable to the initial scores. This means that good GPBR appearance is maintained for at least 15 months when using these three packing methods, in particular, the 100% N_2 -flushed and 60% CO_2 +40% N_2 packages.

FFA content and TBA value A reduction in the FFA content was observed during storage (Table 2). It varied in GPBR kept under 60% CO_2 +40% N_2 and 100% N_2 during 3–9 months of storage. A gradual reduction in FFA was exhibited by GPBR in vacuum-sealed bags. Flushing with 100% N_2 delayed the loss of FFA because it was the highest at the end of 15 months. The TBA level increased with time except for the sample under 100% N_2 , for which it increased in the 3rd month; after this, the TBA values did not vary. The highest level (more than five times the initial value) was observed in vacuum-sealed GPBR, whereas the 100% N_2 -sealed product delayed TBA synthesis in GPBR, thereby showing the least amount, which was three times higher than the initial value (Table 2). The atmosphere in the package influences the hydrolysis and oxidation reactions of lipids, subsequently resulting in oxidative rancidity in oily food products. Thus, FFA content is linked to the development of rancidity, as indicated by the TBA values in this study. Additionally, the O_2 in the package enhanced oxidative rancidity. After 15 months, the highest TBA value for GPBR was detected in the vacuum package, suggesting that 60% CO_2 +40% N_2 and 100% N_2 more efficiently minimized lipid oxidation and delayed rancidity development compared with other gas compositions. The present results are in agreement with the

Table 1. Surface color of GPBR cv. KDML 105 packed in nylon/LLDPE bags and sealed using three techniques before storage under ambient conditions ($30\pm 2^\circ C$, $70\pm 5\%$ RH) for 15 months

Treatments	Storage time (months)			
	0	3	9	15
L^*				
Vacuum	58.31 $\pm$ 0.25 ^{A1)}	57.16 $\pm$ 1.36 ^A	56.30 $\pm$ 1.49 ^{AB}	54.58 $\pm$ 0.94 ^{BB}
60% CO_2 +40% N_2	58.31 $\pm$ 0.25	58.17 $\pm$ 1.13	57.18 $\pm$ 0.65	56.99 $\pm$ 0.71 ^a
100% N_2	58.31 $\pm$ 0.25 ^A	56.45 $\pm$ 1.39 ^{AB}	55.09 $\pm$ 1.05 ^B	57.36 $\pm$ 1.46 ^{AA}
a^*				
Vacuum	1.84 $\pm$ 0.43 ^C	2.43 $\pm$ 0.16 ^B	2.31 $\pm$ 0.16 ^B	2.90 $\pm$ 0.09 ^{AA}
60% CO_2 +40% N_2	1.84 $\pm$ 0.43	1.64 $\pm$ 0.54	2.17 $\pm$ 0.16	1.94 $\pm$ 0.40 ^b
100% N_2	1.84 $\pm$ 0.43	1.62 $\pm$ 0.12	1.97 $\pm$ 0.55	1.86 $\pm$ 0.26 ^b
b^*				
Vacuum	25.54 $\pm$ 0.32 ^C	26.23 $\pm$ 0.37 ^B	26.37 $\pm$ 0.18 ^{AB}	26.80 $\pm$ 0.18 ^A
60% CO_2 +40% N_2	25.54 $\pm$ 0.32	25.78 $\pm$ 1.82	26.05 $\pm$ 0.32	26.55 $\pm$ 1.31
100% N_2	25.54 $\pm$ 0.32	25.34 $\pm$ 0.71	25.21 $\pm$ 1.02	26.27 $\pm$ 1.18
ΔE				
Vacuum	NA	1.77 $\pm$ 0.69 ^B	2.40 $\pm$ 0.87 ^B	4.13 $\pm$ 0.62 ^A
60% CO_2 +40% N_2	NA	1.90 $\pm$ 1.01	1.44 $\pm$ 0.35	2.21 $\pm$ 0.50
100% N_2	NA	2.15 $\pm$ 1.11	3.28 $\pm$ 1.20	1.98 $\pm$ 0.42

¹⁾Values are means $\pm$ standard deviations (SD) ($n=3$); Means within a column^(a-b) and within a row^(A-C) separated by superscripts indicates statistical significance according to Fisher's least significant difference test ($p\leq 0.05$); NA, not applicable.

Table 2. Chemical attributes of GPBR cv. KDML 105 packed in nylon/LLDPE bags and sealed using three techniques before storage under ambient conditions (30±2°C, 70±5% RH) for 15 months

Treatments	Storage time (months)			
	0	3	9	15
FFA (mg KOH/100 g dw)				
Vacuum	2.25±0.00 ^{A1)}	1.81±0.08 ^{ab}	1.66±0.06 ^c	1.29±0.03 ^{cd}
60% CO ₂ +40% N ₂	2.25±0.00 ^A	1.95±0.05 ^{ab}	2.11±0.21 ^{ab}	1.54±0.12 ^{bc}
100% N ₂	2.25±0.00 ^A	1.52±0.14 ^{bc}	1.75±0.04 ^{bb}	1.74±0.08 ^{ab}
TBA (nmol/g dw)				
Vacuum	22.19±2.85 ^D	50.08±0.48 ^{bc}	64.10±1.66 ^B	112.00±2.94 ^{aA}
60% CO ₂ +40% N ₂	22.19±2.85 ^C	45.04±1.90 ^{cb}	54.59±2.66 ^B	82.25±10.98 ^{ba}
100% N ₂	22.19±2.85 ^B	61.85±0.95 ^{aA}	58.56±8.84 ^A	69.75±2.33 ^{ba}
TPC (mmol GAE/100 g dw)				
Vacuum	25.50±1.60 ^B	19.00±2.24 ^{bc}	31.95±1.04 ^A	32.86±0.76 ^A
60% CO ₂ +40% N ₂	25.50±1.60 ^B	23.51±1.15 ^{ab}	33.03±1.69 ^A	33.94±1.94 ^A
100% N ₂	25.50±1.60 ^B	24.59±1.06 ^{ab}	31.37±4.76 ^A	33.48±2.32 ^A
FRAP (mmol Trolox/100 g dw)				
Vacuum	13.97±1.58 ^B	11.74±0.30 ^C	17.15±0.78 ^A	17.96±0.84 ^A
60% CO ₂ +40% N ₂	13.97±1.58 ^B	12.96±0.27 ^B	17.46±0.62 ^A	17.45±1.22 ^A
100% N ₂	13.97±1.58 ^B	12.58±0.94 ^B	16.27±1.16 ^A	17.44±0.90 ^A
DPPH radical scavenging activity (%)				
Vacuum	14.88±0.41 ^A	12.44±0.72 ^{ab}	11.46±1.16 ^{bc}	10.35±0.99 ^{bc}
60% CO ₂ +40% N ₂	14.88±0.41 ^A	9.82±0.77 ^{bc}	12.79±0.79 ^B	9.64±1.32 ^{bc}
100% N ₂	14.88±0.41 ^A	9.69±0.36 ^{bc}	12.81±1.27 ^B	12.66±0.61 ^{ab}

¹⁾Values are means±SD (n=3); Means within a column^(a-c) and within a row^(A-D) separated by superscripts indicates statistical significance according to Fisher's least significant difference test ($p \leq 0.05$).

reports on the storage of raw whole unpeeled almond (28) and raw pistachios (29). Generally, N₂ is an inert gas and unreactive at ambient temperature and pressure. Consequently, there is reduced reaction in the GPBR kernels, as indicated by the lowest FFA value. Furthermore, the reduction of fungal development in the 100% N₂-sealed bag slowed the hydrolysis reaction of lipids; this is the reason for the low FFA levels in samples (Table 2). Ponsen (12) found that when the fungal population was low, the FFA and rancidity in sweet corn seed were similarly reduced. Conversely, CO₂ is soluble in water, in which it reversibly forms H₂CO₃ (carbonic acid), which is a weak acid because its ionization in water is incomplete. CO₂ is also a weak electrophile. It reacts with FFA, subsequently generating unpleasant agents that result in slightly higher TBA levels in GPBR packed under 60% CO₂+40% N₂ compared with that packed under 100% N₂ (Table 2). Thus, fewer changes in FFA and TBA values were observed in GPBR kept under 100% N₂, followed by 60%CO₂+40% N₂ (Table 2).

Total phenolic content (TPC), ferric-reducing antioxidant power (FRAP) and DPPH radical scavenging activity TPC also increased after storage for 9 months. In the 3rd month of storage, it significantly reduced in vacuum-sealed GPBR compared with the other two samples (Table 2). In all treatments, the TPC significantly increased at the 9th month and remained unchanged after 15 months. The packages did not significantly affect the FRAP value after storage for 15 months (Table 2). The DPPH radical scavenging value gradually

reduced during storage (Table 2). Vacuum-sealed GPBR showed the highest DPPH radical scavenging value after storage for 3 months. At the end of storage, GPBR packed in 100% N₂ showed the highest DPPH value compared to the two other samples. The amount of gases did not affect the TPC of GPBR at nine and 15 months (Table 2). Htwe *et al.* (30) reported that a controlled atmosphere containing different portions of 0, 5, and 10% O₂ balanced with N₂ in plastic containers did not significantly influence the total phenolic compounds of colored rice during 4 months of storage. They also reported that the amount of O₂ affected the amount of free, soluble conjugated phenolics but not insoluble, bound phenolics in the rice samples. In the case of faba beans, the samples flushed with O₂ exhibited high losses in phenolic constituents after 12 months (2). In this study, the increase in the TPC of GPBR may be attributed to an increase in the amount of soluble phenolic compounds degraded from bound or conjugated phenolics during storage (30). An increase in TPC has a positive relation to the FRAP value but not to the DPPH scavenging activity (Table 2). The reason for this varying response may be that DPPH is more active in reducing soluble compounds (31), whereas FRAP can reduce polyphenol compounds. The gases in the package played no role in the significant increase in TPC and FRAP values; however, the 100%-N₂ treatment significantly retained the DPPH radical scavenging capacity during storage for 15 months. Htwe *et al.* (30) reported that the concentration of O₂ influences the degradation of radical scavenging activity and that 0% O₂ or 100% N₂ are more

Fig. 1. SEM of GPBR cv. KDML 105 under ambient conditions ($30\pm 2^{\circ}\text{C}$, $70\pm 5\%$ RH), GPBR at 0 months (A); vacuum-sealed at 3 (B), 9 (C), and 15 (D) months; 60% CO₂+40% N₂-sealed at 3 (E), 9 (F), and 15 (G) months; 100% N₂-sealed at 3 (H), 9 (I), and 15 (J) months.

effective in maintaining DPPH radical scavenging capacity. In contrast, Ghirardello *et al.* (32) found that the TPC and antioxidant capacity of hazelnut (*Corylus avellana* L.) kernels gradually decreased; however, these two parameters did not differ in the two storage techniques (e.g., vacuum-sealing or with 100% N₂). Similarly, gradual losses in TPC, FRAP, and DPPH activity increased as a function of storage time; however, flushing with 100% N₂ or CO₂ retarded these losses (33). These contradicting observations can be caused by crop species (34).

Cooking quality and microstructure of endosperm The calculated elongation ratios and hardness values are shown in Table 3. The initial elongation ratio of GPBR was 1.09, and it tended to increase to 1.12 and 1.13 in the vacuum-sealed and the 60% CO₂+40% N₂-sealed samples after nine months. After 15 months, the various in-package atmospheres did not significantly influence the elongation ratio. The hardness of cooked GPBR steadily increased, and GPBR kept in the

vacuum bag exhibited the highest value, followed by the 60% CO₂+40% N₂-sealed and 100% N₂-sealed samples, respectively. The microstructure of the GPBR endosperm gradually changed as a function of the storage period (Fig. 1). The large cavities initially observed in the endosperm disappeared in GPBR kept in vacuum after storage for three months, and it showed a smooth texture in comparison to the endosperms kept under 60% CO₂+40% N₂ and 100% N₂. The changes in the texture of cooked rice kernels, such as their water absorption, hardness, and elongation ratio, were influenced by physicochemical changes in the rice kernel endosperm during storage (35). The elongation ratio and hardness of cooked GPBR increased with storage time (Table 3). A low pressure in the vacuum package enhanced the compact structure of the endosperm (Fig. 1). In the case of 60% CO₂+40% N₂-sealed package, the endosperm was observed to be tight after three months, indicating a low pressure in the package. Consequently, a compact structure of endosperm was observed in GPBR kept in both vacuum-sealed and

Table 3. Elongation ratio, hardness, total fungal contamination, and AFB₁ contamination of GPBR cv. KDML 105 packed in nylon/LLDPE bags and sealed using three techniques before storage under ambient conditions (30±2°C, 70±5% RH) for 15 months

Treatments	Storage time (months)			
	0	3	9	15
Elongation ratio				
Vacuum	1.09±0.02 ^{BC1)}	1.07±0.01 ^C	1.12±0.03 ^{AB}	1.14±0.03 ^A
60% CO ₂ +40% N ₂	1.09±0.02	1.13±0.04	1.13±0.01 ^a	1.13±0.02
100% N ₂	1.09±0.02 ^B	1.08±0.02 ^B	1.08±0.02 ^{bB}	1.13±0.01 ^A
Hardness (N)				
Vacuum	32.66±3.77 ^C	42.05±6.22 ^{AB}	45.60±6.99 ^B	89.38±4.79 ^{AA}
60% CO ₂ +40% N ₂	32.66±3.77 ^B	33.54±3.94 ^{bB}	47.56±9.07 ^B	78.02±4.42 ^{BA}
100% N ₂	32.66±3.77 ^C	40.39±6.03 ^{AB}	37.60±8.91 ^{BC}	67.29±1.03 ^{CA}
Total fungal contamination (%)				
Vacuum	2.33±1.53	0.80±0.75	1.00±1.00	1.00±1.73
60% CO ₂ +40% N ₂	2.33±1.53	1.20±0.50	1.67±0.58	1.00±1.73
100% N ₂	2.33±1.53	0.59±0.80	0.33±0.58	0.67±1.15
AFB ₁ contamination (µg/kg)				
Vacuum	0.40±0.25	0.77±0.62	0.57±0.36	0.82±0.32
60% CO ₂ +40% N ₂	0.40±0.25	0.69±0.43	0.81±0.37	0.79±0.45
100% N ₂	0.40±0.25	0.85±0.25	0.67±0.20	0.70±0.40

¹⁾Values are means±SD (n=3); Means within a column^(a-c) and within a row^(A-C) separated by superscripts indicates statistical significance according to Fisher's least significant difference test ($p \leq 0.05$).

60% CO₂+40% N₂-sealed packages (Fig. 1). This hastened the significantly higher elongation ratio of cooked rice in these two techniques, as observed at nine months of storage (Table 3). Additionally, a significantly greater hardness of cooked rice was revealed in those two samples compared with the 100% N₂ sample, whose hardness was the least. This suggests that 100% N₂ can maintain the cooking quality of GPBR (Table 3). Notably, in the vacuum-sealed and 60% CO₂+40% N₂-sealed packages, the pressure decreased, thereby hastening physicochemical changes, especially the compactness of the endosperm in GPBR; this led to significant changes in the quality of cooked rice.

Total fungal contamination and Aflatoxin B₁ (AFB₁) contamination

The different package atmospheres did not significantly influence fungal counts, which were reduced in the GPBR kernels' surface and in AFB₁ content during storage (Table 3). The AFB₁ content varied from 0.40–0.82 µg/kg during storage, which was lower than the Food and Agriculture Organization (FAO) regulation data. This was due to the low MC of the grain, which consequently delayed mycelial growth. Thus, the extremely low MC in GPBR during storage influenced fungal proliferation and AFB₁ production in the samples regardless of the atmosphere in the package.

Consequently, GPBR in all the packages remained stable for 15 months. The 100% N₂-sealed package showed the best treatment for preserving GPBR quality, as indicated by its surface color and DPPH radical scavenging activity as well as lower rancidity, fungal contamination, and AFB₁ compared with the other two packages. Moreover, the lowest hardness and least change in the elongation ratio of cooked rice exhibited by the 100% N₂ sample was attributed

to a lesser compact starch ultrastructure in the endosperm.

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