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EFFECT OF ANAEROBIC LEVEL ON THE GERMINATION OF WHITE AND BLACK STICKY BROWN RICE IN MEKONG DELTA, VIETNAM

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ABSTRACT

The objective of this study was to optimize and compare production condition of GBRto maximize bio-active compounds. In order to do that the white sticky rice (CLN) and black sticky rice (DH6) varieties from An Giang Province, Mekong Delta, Viet Nam were selected for this study because of their popularity and benefits. Optimum conditions for dehusk, steep and germination to maximize γ-amino butyric acid, ferulic acid and γ-oryzanol content in brown rice were determined. Both sticky sticky varieties were dehusked at various grain's humidity conditions which were 12, 13, 14, 15, 16 and 17%. The brown rice was soaked in various solutions which were pH 2, 3, 4, 5 , 6, 7 and distilled water at room temperature for 6 hours and germinated in various CO_2 concentration as 0, 3, 5, 7, 9% at 37^oC for 16, 20, 24, 28 and 32 hours. The results indicated that the optimum condition for dehusk depended on grain's moisture content which was from 13 to 14% for black sticky and from 12 to 14% for white sticky. The highest γ-amino butyric acid content was obtained when the rice was soaked in solution at pH 5 and germinated in 5% CO_2 32 hours for white sticky rice and 7% CO_2 32 hours for black sticky rice respectively. The γ-amino butyric acid content in white sticky rice and black sticky rice increased as 7.5 and 9.13 times when compared to brown rice. The content of ferulic acid and γ-oryzanol in GBRwere also increased after germination. Black sticky rice showed better for value for production of germinated brown rice.

KEYWORDS: γ-amino butyric acid, γ-oryzanol, ferulic acid, germinated brown rice.

INTRODUCTION

Mekong Delta is the largest rice producibility area in Vietnam. However, For the purpose of diversity of rice products, production of germinated brown rice (GBR) is one of the most important direction due to there are many functional compounds in the GBR that are very helpful for human health such as γ-amino butyric acid (GABA), γ-oryzanol (GORY) and ferulic acid (FERA). GABA is essential for human life, it alleviates stress activity of neurons, reduces the spread, inhibiting the transmission of harmful to brain [1]. It is anti-hypertensive agents, diuretics, steady heartbeat and reduces pain [2], adjust serum lipid level [3] and assist the secretion of insulin to prevent diabetes [4]. GABA helps prevent Alzheimer's disease [5], inhibit the proliferation of cancer cells [6]. Gory is a mixture of FERA esterified with conventional sterols or triterpene alcohols, among them, GORY is paid much attention due to have high antioxidant property [7], reduces the absorption of cholesterol and reduce atherosclerosis soon [8]. With FERA, is a common phenolic component of plant cell, it is one of the metabolites of lignin biosynthesis of phenylalanine and tyrosine from plant. In plant cell, FERA is found in two forms free and conjugated. The total of these two forms shows the levels of total FERA [9]. FERA's antioxidant properties may be due to the phenolic atom and a long side chains. FERA is beneficial in delaying or treatable disorder involving to the present of too many atoms of oxygen in the blood such as Alzheimer's disease, diabetes, hypertension, atherosclerosis. Furthermore, due to its special structure which is capable for strongly absorbing of UV so it becomes a protective agent for skin [10].

Germination of brown rice is the development of the embryo inside the seed. This is a complex process, starting from the water absorption of dry rice grains and ends the process of the axis extending the embryos. It depends on inside and outside conditions. Too low or too high temperatures are making all seed cannot germinate. Generally,

germination is better with higher room temperature in several Celsius degrees [11]. In Japan, scientist has studied the germination from 5 varieties of rice [1]; Optimization of germination process of three different varieties of Thailand to achieve the highest levels of bioactive compounds in seed is also done [12]. During germination, the GABA levels rise from 9.43 to 16.74 times, besides germination also increased from 1.12 to 1.43 times level of FERA, whereas phytate decreased after germination. Although, the yield of white sticky rice (CLN) and black sticky rice (DH6) is not so high comparing to other rice varieties in Mekong Delta, these varieties of sticky rice are still one of useful rice for human living in this area due to produce many traditional products. In addition, some studies determine that the black sticky rice in general exist many functional compounds like anthocyanin which is very useful for human health. So, the GBRof these varieties may be a good product to provide another channel for human choice and to use these varieties more effectively. So, the study on germination to maximize the bioactive compounds with the two varieties like CLN and DH6 sticky rice was done.

MATERIALS AND METHODS

Materials and germinated brown rice preparation

Two sticky rice varieties including white sticky rice (CLN) and black sticky rice (DH6) were used for this study. After harvested, the moisture content was from 28 - 30%. They were dried until 12.0 - 13% before stored at room temperature for 2-3 months before used for the experiment. In order to germinate the rice, firstly they were dehusked by Yanmar – ST50 (Yanmar company, Tokyo, Japan) which adjusted to suitable distance between 2 rulos of the machine depending to which variety to get the best pre-germinated brown rice. After that the brown rice was soaked into water for 6 hours until saturation (32% for CLN, 36% for DH6). The saturated brown rice was then incubated at 37^{o}C under different condition of CO_2 concentration (0-7%) for different time (16, 20, 24, 32 and 36 hours) and measure changes of various bioactive compounds like GABA, GORY and FERA. Standard chemicals such as glutamic acid, GABA, GORY and FERA were purchased from Merck, Germany, other chemicals and solvent for HPLC analysis were obtained from Sigma Aldrich.

Determination of chemical composition of materials

Determination of starch and amylose content was followed Bertrand method; Total protein content was determined by Kjeldal method [13]. Amylose content was determined following the method of Chrastil (1987) [14]. Total polyphenol determination was determined by Folin – Ciocalteau method [15].

Determination of GABA [12]

Weighed 2g of ground GBR and put it into the 50 ml flask, added 9 mL of demineralized water and shook for 90 minutes to sample extraction. Then added 1 ml sulfosalicylic acid to the mixture and centrifuged at 8000 rpm for 10 minutes. Next, transferred 100µl supernatant to 1.5 ml eppendort, put into 100µl $NaHCO_3$ 100 mM and 100µl solution of 4-dimethyl-4-sulfonyl chloride aminoazobenzen 4 mM which diluted in acetonitrile. The mixture was shaken and heated at 70°C for 10 minutes. Consequently, added 500µl ethanol and 500µl phosphate buffer pH 6.8 to the mixture. The mixture was then shaken well and centrifuged at 13000 rpm for 10 minutes. The supernatant was then filtered through 0.2 µl filter before analysed by HPLC equipment (SHIMADZU, Japan), C18 column. Absorption wavelength was 465 nm. Mobile phase was ammonium acetate buffer 25mM and acetonitrile with the ratio of 55: 45, flow rate 1 ml/min, column temperature was 55°C. GABA standard was used to construct the standard curve which used for calculation the concentration of GABA.

Determination of ferulic acid [9]

Weighed 0,2 g of ground GBR and put it into 50 ml flask, put 20 ml of 1M NaOH solution into and shook for 3 hours, then put 10 ml of HCl 2M into the mixture to neutralize. Got 1.5 ml of the mixture and put into eppendort and centrifuged at 13,000 rpm for 10 minutes. The supernatant was then filtered through 0.2 µl filter before analysed by HPLC equipment (SHIMADZU, Japan), C18 column, 320 nm wavelength absorption, the mobile phase consisted acetic acid (2.5% (v/v)) and acetonitrile (the ratio was 55: 45). Flow rate 1 ml/min, column temperature 40°C. FERA standard was used to construct the standard curve which used for calculation the concentration of FERA.

Determination of γ-oryzanol [7]

Weighed 0.5 g of ground GBR and put it into tube, put 10 ml acetone into, covered and shook by vortex for 10 minutes before centrifuged at 13,000 rpm for 10 minutes. The supernatant was then filtered through 0.2 µl filter before analyzed by HPLC equipment (SHIMADZU, Japan), C18 column, 330 nm wavelength absorption. Ratio of acetone mobile phase and acetonitrile was 40: 60, the flow rate was 1.5 ml/min, and the column temperature was 35°C. GORY standard was used to construct the standard curve which used for calculation the concentration of GORY.

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Statistical analysis

All experiments were triplicate analysis. The results were analyzed by using ANOVA analysis, LSD analysis using Statgraphic 16.1.18 and Exel 2007 software.

RESULTS AND DISCUSSION

Effect of pH value to the steeping stage

Table 1 Content of chemical and bioactive compounds in white and black sticky rice

Chemical and bioactive compounds of material	Sticky rice	
	CLN	DH6
Water content (%)	12.1 ± 0.17	12.5 ± 0.26
Protein (g/100g)	8.0 ± 0.26	7.4 ± 0.17
Starch (g/100g)	78.94 ± 1.01	77.1 ± 1.39
Amylose (g/100g)	5.72 ± 0.17	5.5 ± 0.26
γ-amino butyric acid (GABA) (mg/kg)	58.27 ± 0.24	52.93 ± 1.45
γ-oryzanol (GORY) (mg/kg)	379.8 ± 1.91	556.4 ± 0.61
Ferulic acid (FERA) (mg/kg)	264.69 ± 0.28	304.18 ± 0.25
Total polyphenol (mg/100g)	8.85 ± 0.38	80.1 ± 0.90

In order to steep preparing for germination, two sticky rice varieties including CLN and DH6 were dehusked to get pre-germinated brown rice. There two sticky rice varieties consist of high nutritional values as well as bioactive compounds (Table 1). To be able to germinate, seeds must be increased the moisture to provide enough water that necessary to activate the physiological processes, stimulate biochemical processes in seed germination. The absorption of water makes the grain become swell and break the seed coat structures [16]. Soaking the seeds for too long, the rice's soluble materials will be dissolved in aqueous immersion causes the loss of nutrients in the soaking solution. In addition, when soaking the seeds for long time, it may create good condition for the development of microorganisms which may damage the nutritional value of GBR product. According to Komatsuzaki (2007) [1], conventional rice seed with 30-35% moisture content and high moisture content (35-50%) will create favourable conditions for the growth of microorganisms. If the rice is soaked in water for long periods of time, polysaccharide, protein and some other nutritional compounds in rice will be reduced by solubility in water immersion solution. Besides, metabolism eliminates CO_2 and converts partly into ethanol, grain dust outside is also washout [17]. From the preliminary study, white and black sticky brown rice can be soaked for 6 hours to reach saturation. Hence, in this experiment, just pH value was changed from pH 2-7 to determine what optimal pH value for the germination process is.

Table 2: Changes of bioactive compounds when steeping at various pH values for 6 hours

pH	γ-amino butyric acid (GABA) (mg/kg) db		Ferulic acid (FERA) (mg/kg) db		γ-oryzanol (GORY) (mg/kg) db	
	CLN	DH6	CLN	DH6	CLN	DH6
2	84.31±0.79^{d}	86.56±0.85^{c}	315.15±1.57^{a}	348.81±1.23^{a}	458.20±2.76^{a}	588.40±3.01^{a}
3	81.04±1.24^{e}	77.54±1.78^{e}	310.36±1.06^{b}	340.64±1.81^{b}	436.45±1.92^{b}	581.67±2.53^{b}
4	70.43±0.81^{f}	69.69±2.06^{f}	302.42±0.74^{c}	330.88±2.84^{c}	435.41±1.84^{b}	567.57±2.23^{c}
5	127.93±2.12^{a}	121.85±1.26^{a}	298.15±1.39^{d}	323.58±1.24^{d}	421.80±2.31^{c}	566.35±1.81^{c}
6	95.63±1.05^{b}	85.00±1.03^{cd}	298.38±1.00^{d}	316.86±0.55^{e}	415.50±0.72^{d}	561.53±2.15^{c}
7	87.34±1.09^{c}	82.39±0.88^{d}	288.50±1.73^{e}	311.75±1.53^{f}	414.03±0.28^{d}	551.61±3.80^{d}
DW	89.34±0.52^{c}	93.45±0.71^{b}	281.60±1.99^{f}	301.40±0.54^{g}	410.92±2.47^{d}	561.98±2.55^{c}

Note: The different letters in the same column indicate significant difference statistically at the 95% confident level; DW: Distilled water

The results in Table 2 shows that after 6 hours of soaking, GABA concentrations in the original white brown rice increased from 52.19 mg/kg to 121.13 mg/kg, for purple sticky rice increased from 46.14 mg/kg to 112.59 mg/kg. In

the previous research results showed that the optimal conditions for the activity of Glutamate decarboxylase (GAD) enzyme was at 40°C and pH 5.5. The results also showed that when citrate soaked in pH 5, the concentrations of glutamic acid was the highest (1016.7 mg/100g) for the white rice and 987.1 mg/100g for purple sticky rice. The GAD enzyme activity was highest for white rice as 18.584 UI / g and for purple sticky as 19.606 UI/g. So, citrate buffer at pH5 was selected as optimal condition for the process of soaking grain to generate the highest GABA levels. According to [12], GABA level was the highest (14.48 mg/100g) when soaking in for 5 hours. GABA in germinated of barley grain increase to the highest when soaked in buffer solution with pH 6. When pH of environment decreases, the amount of H^+ ions increases which facilitate the synthesis of GABA, however, when pH is too low, it will cause the inhibition of GAD enzyme activity causes decreases the synthesis GABA from glutamic acid through GAD enzyme activity [5].

FERA content in initial CLN and DH6 was 264.69 and 304.18 mg/kg respectively. The results also showed that when soaked the seeds in different buffers, the levels of FERA increased. Because FERA is an acid so when soaked in acid solution, it promoted the biosynthesis of FERA stronger. As the result, after soaked the grain in a pH 2 buffer, the FERA content increased to 315.15 mg/kg with CLN and to 348.81 mg/kg with DH6. GORY increase slightly after germination and achieves maximum with purple sticky rice comparing to either white sticky rice or normal rice [18]. GORY content in 3 Thailand rice varieties showed that GORY content in Niaw Dam Peuak Dam black sticky rice was higher than Sangyod Phatthalung (red rice) and Chaing Phatthalung (white rice) [12]. The result from this study was also suitable with above researches. GORY content in initial white (CLN) and purple sticky rice (DH6) was 379.8 mg/kg and 517.8 mg/kg respectively. The result in table 2 showed that γ-oryzanol content increased lightly about 20% with CLN and 13% with DH6. GORY is a mixture of ferulic acid esterified with conventional sterols or triterpene alcohols. At low pH environment, this esterification process is deepening increases γ-oryzanol content. Besides, low pH levels FERA content showed the highest increase, as the basis for increasing levels of GORY. Results in Table 2 also show that after soaking at pH 5 for 6h (optimal conditions for the water content inside the seed reached saturation status and the ability to form much bioactive compounds), GABA levels increased more than 2 times the levels of GORY increased 1.2 times while FERA increased just under 1.1 times compared to the soaking beginning for both 2 varieties.

Effect of CO_2 content and incubation time to the changes of bioactive compounds during the germination

γ - amino butyric acid

After steeping in optimal pH value, the water saturated brown rice was germinated in different anaerobic level. The result shows in Figure 1 for GBR of white sticky rice and Figure 2 GBR of black sticky rice. The germ in both GBRwas almost similar. The results in Table 2 showed that, after incubated, GABA content increased to 7.5 time higher (437.08 mg/kg) with CLN and 9.13 times (483.15 mg/kg) with DH6. The increase of GABA followed the incubation time. The highest GABA content achieved when incubated at 5% CO_2 for 32 hours with CLN (437.08 mg/kg) and at 7% CO_2 for 32 hours with DH6 (483.15 mg/kg). Other studies show a suitable result with this research. Foe instant, the study on 3 rice varieties to find out the incubation time like Sangyod Phatthalung and Chiang Phatthalung (36 hours incubation) and NiawDam Peuak Dam (48 hours incubation) [12]. The increase is 16.47, 9.47 and 11.28 times comparing to brown rice. And, the study on the germination of Red Jasmine from Thailand showed that GABA increased to 7 times (6 mg/100g increased to 41 mg/100g) when incubated at 35ºC for 24 hours. It increase to the highest level when incubated under anaerobic condition (41 mg/100g increased to 81 mg/100g) is also done [19]. The biosynthesis of GABA was considered as a reaction of cell tissue response to stress caused by cell acidosis. GABA synthesis process will be accompanied by the consumption of H + during decarboxylation, thereby reducing cytoplasmic acidosis [20]. One another study shows that under anaerobic conditions it will cause the reduction of intracellular pH (0.4 to 0.8) due to a stress caused by a deficiency of oxygen [21]. Thus, the reduced intracellular pH due to hypoxic conditions will generate increased levels of GABA produced by stimulating activity of GAD enzyme glutamic acid synthesized from GABA [5]. The acidification will create rapid accumulation of GABA, however if the pH decreases due to the prolonged lack of oxygen reduces the pH buffering capacity of cellular solution (resilience of the natural pH of cell solution) by the H^+ ions are consumed more, which leads to decrease ability to germinate.

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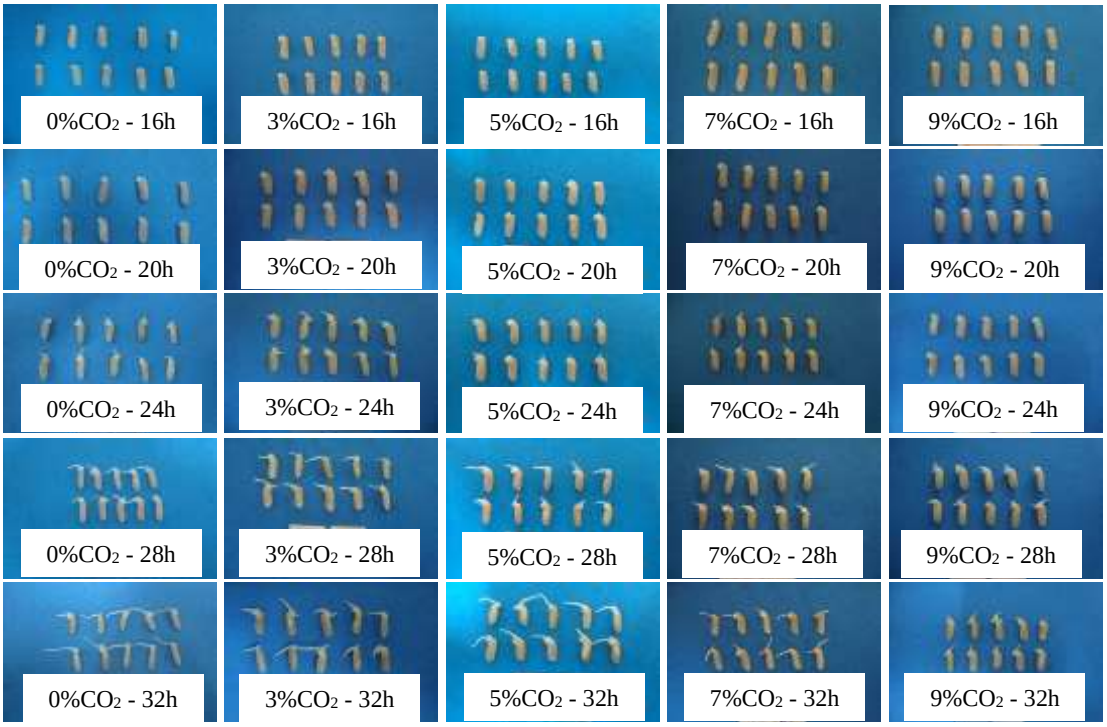

Figure 1. GBR of white sticky rice (CLN) in different incubation time and various anaerobic conditions

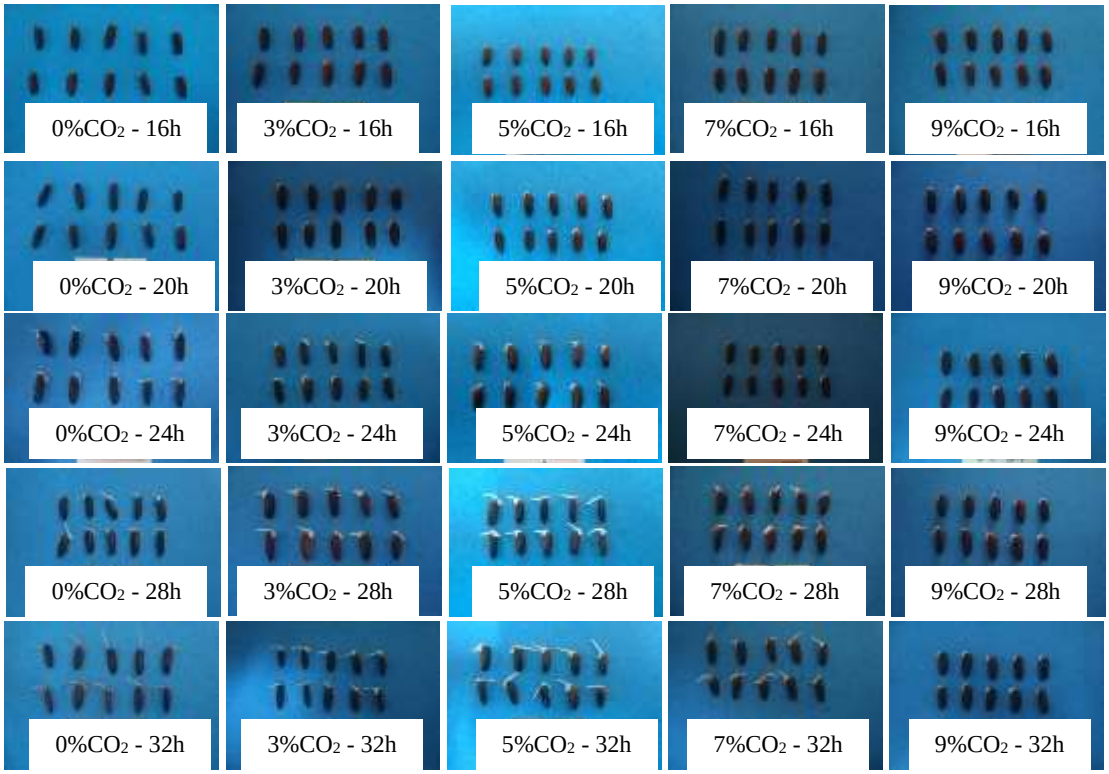

Figure 2. GBR of black sticky rice (DH6) in different incubation time and various anaerobic conditions

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Table 3. GABA content from CLN and DH6 when incubated in different CO_2 conditions

CO_2 (%)	GABA (mg/kg) db. CLN				
	16 hours	20 hours	24 hours	28 hours	32 hours
0	98.92±0.48^{Ce}	126.12±0.42^{Dd}	140.19±2.63^{Cc}	155.37±1.19^{Db}	161.12±0.98^{Ea}
3	110.04±0.66^{Be}	123.65±0.98^{Dd}	142.40±1.92^{Cc}	160.72±1.87^{Db}	176.09±1.80^{Da}
5	120.75±2.80^{Ae}	175.91±1.57^{Ad}	262.35±2.86^{Ac}	358.19±2.86^{Ab}	437.08±4.67^{Aa}
7	122.40±0.93^{Ae}	139.38±0.98^{Cd}	182.51±0.64^{Bc}	264.89±2.69^{Bb}	386.48±2.32^{Ba}
9	111.51±1.17^{Be}	153.45±1.16^{Bd}	183.32±3.55^{Bc}	248.72±5.20^{Cb}	341.26±9.09^{Ca}
CO_2 (%)	GABA (mg/kg) db. DH6				
	16 hours	20 hours	24 hours	28 hours	32 hours
0	101.71±2.39^{Ce}	111.70±2.18^{Dd}	132.41±1.99^{Dc}	147.34±3.22^{Eb}	163.8±2.22^{Ea}
3	98.57±3.07^{Ce}	131.97±4.82^{Cd}	148.17±0.47^{Cc}	176.49±2.93^{Db}	215.66±1.07^{Da}
5	111.01±1.46^{Be}	140.73±0.85^{Bd}	182.57±2.09^{Bc}	358.67±3.05^{Bb}	433.17±2.81^{Ba}
7	120.44±2.22^{Ae}	179.84±4.16^{Ad}	325.34±1.73^{Ac}	448.16±2.6^{Ab}	483.15±4.09^{Aa}
9	101.73±1.05^{Ce}	146.20±1.15^{Bd}	180.91±1.61^{Bc}	249.47±1.94^{Cb}	342.51±1.95^{Ca}

a-e: The different letters in the same row indicate significant difference statistically at the 95% confident level;
A-E: The different letters in the same column indicate significant difference statistically at the 95% confident level

The results also show that the concentration of CO_2 is tremendous influence the generation of GABA levels. CO_2 levels most suitable for white sticky CLN was 5% while 7& for DH6. GABA levels increased stronger when the germinating in closed jars than to sprout in the open bottle. CO_2 levels are too high or too low will affect the seed germination and GABA levels in rice germ. At low CO_2 concentrations of 0%, 3% failed to create a good anaerobic conditions for germination, so it do not stimulate the activity of enzyme GAD which make weak biosynthesize of GABA from glutamic acid [12]. On the other hand, if the composition of CO_2 too high 9%, it causes a shortage of oxygen for too long time so inhibits the biosynthesis of GABA. Indeed, the results show that glutamic acid content and GAD enzyme activity on two varieties of white sticky and purple sticky rice changed drastically after 32 hours of incubation for germination (data not Shown). Glutamic acid decreased deeply at 5% CO_2 after 28 hours (626.53 mg/100g) with CLN while 506.42 mg/100g for DH6. In parallel, GAD enzyme activity increases with following incubation time, after 28 hours incubated at 5% CO_2 GAD enzyme activity increased to 50.62 UI / g and 47.02 IU / g on white sticky and purple sticky rice respectively.

Ferulic acid

After germination, ferulic acid content increased 1.43 time with CLN and 1.52 times with DH6 (Table 4). This results is suitable with [12] when study the germination of three varieties such as Niaw Dam Peuak Dam (increase 1.12 times), Sangyod Phatthalung (increase 1.43 times), and Chiang Phatthalung (increase 1.37 times). After germination, FERA content in white sticky and purple sticky rice increased slightly. Ferulic acid concentrations increased with incubation time, however after 28 and 32 hour incubation, the ferulic acid content of no more significant differences. FERA content also change with the significant differences between the various levels of CO_2. Results also showed that, in conditions of 7% CO_2 incubation FERA showed the highest levels on both CLN and DH6. After 32 hours of such incubation condition, FERA content reached 378.33 mg/kg for CLN and 461.55 mg/kg for DH6. However, differences in levels of FERA in different CO_2 conditions are not too large. FERA is the biosynthesis of lignin from phenylalanine and tyrosine, so it is also affected by CO_2 content. When CO_2 levels is too

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low, no strong stimulation of enzyme activity during biosynthesis, whereas the CO_2 component is too high in the long run, it causes a deficiency of oxygen necessary for respiration, inhibit the germination process, leading to inhibition of the biosynthesis of FERA.

Table 4. FERA content from CLN and DH6 when incubated in different CO_2 conditions

CO_2 (%)	Ferulic acid (mg/kg) db, CLN				
	16 hours	20 hours	24 hours	28 hours	32 hours
0	285.88±2.32^{Cc}	287.43±1.48^{Cc}	317.98±4.10^{Db}	312,.97±5.25^{Db}	329.95±1.71^{Da}
3	332.57±2.94^{Ac}	341.56±3.08^{Aab}	335.65±2.81^{Cbc}	342.11±1.39^{Ca}	344.01±0.04^{Ca}
5	329.28±1.40^{Ad}	332.66±1.98^{Bd}	348.83±2.63^{Bc}	358.89±3.51^{Bb}	372.96±3.03^{Ba}
7	330.56±2.26^{Ac}	335.99±1.58^{Bc}	363.04±0.57^{Ab}	377.17±4.06^{Aa}	378.33±2.11^{Aa}
9	321.65±2.38^{Bd}	333.24±2.16^{Bc}	342.02±2.80^{BCb}	349.98±3.83^{BCa}	340.72±1.51^{Cb}
CO_2 (%)	Ferulic acid (mg/kg) db, DH6				
	16 hours	20 hours	24 hours	28 hours	32 hours
0	354.00±2,86^{Ac}	364.45±1.17^{Bb}	362.78±1.75^{Cb}	374.50±0.95^{Da}	372.29±2.08^{Ea}
3	317.86±1,07^{Cd}	321.39±1.73^{Dc}	363.94±0.34^{Cb}	381.06±1.34^{Da}	383.41±0.79^{Da}
5	307.61±2,21^{De}	343.58±1.51^{Cd}	367.24±1.47^{Cc}	391.13±3.75^{Cb}	443.29±4.85^{Ba}
7	356.35±3,00^{Ae}	379.46±6.26^{Ad}	403.71±4.33^{Ac}	446.29±3.21^{Ab}	461.55±5.90^{Aa}
9	330.82±1,56^{Bd}	363.47±3.39^{Bc}	383.90±3.04^{Bb}	413.53±5.32^{Ba}	406.61±2.91^{Ca}

a-e: The different letters in the same row indicate significant difference statistically at the 95% confident level;
A-E: The different letters in the same column indicate significant difference statistically at the 95% confident level

γ-oryzanol

The results in Table 5 shows the concentration of GORY slightly increased following the incubation time, but after 28 hours of incubation, the content of GORY virtually no significant changes at different conditions. In purple sticky rice, GORY content not differ significantly for the samples incubated in 5% and 7% CO_2 after 32 hours of incubation. A survey of influence germination to GORY content of rice varieties of Malaysia as Sabak, Silah and Hitam shows that GORY is slightly higher levels after 24 hours of incubation [22]. Similar to FERA, GORY shows the highest biosynthesis in acidity environment. Because it is also affected by CO_2 content in environment during germination. In addition, brown rice is soaked in pandanus solution and incubated in the dark for 24 hours, GORY content also increase, however, if changing to citronella of soaking in water, the concentration of GORY fell slightly [23]. In this study, for CLN, the best condition for incubation was 7% CO_2, for DH6, the highest levels of GORY was not significant difference in the two levels of 5% and 7% CO_2 after 32 h of incubation. When CO_2 levels was too low, not created a good anaerobic environment for germination process, the CO_2 content of 5, 7% made good anaerobic environment, these created best CO_2 levels that stimulated FERA biosynthesis and also increases the concentration of GORY. Because GORY is an ester compounds FERA with the sterol or triterpene of alcohol so it is directly related to the amount of FERA. The experimental results are consistent with the results on ferulic acid content above. Also, GORY content is no significant change before and after germination in 3 varieties Thailand as Niaw Peuak Dam Dam, and Chiang Phatthalung Phatthalung Sangyod [12]. The results are consistent with studies of Sungsopha et al. (2009) [24], levels of GORY in GBRincreased by 29.31% compared to non-germinated. Red

brown rice and brown rice of the variety of Khao Dawk Mali 105 which soaked in water for 6 hours and germinate in the dark for 24 hours, the concentration of GORY corresponding increase of 1.3 to 1.5 times higher than non-GBR [25].

Table 5. GORY content from CLN and DH6 when incubated in different CO_2 conditions

CO_2 (%)	γ-oryzanol (mg/kg) db, CLN				
	16 hours	20 hours	24 hours	28 hours	32 hours
0	504.09±2.26^{Bd}	508.16±3.04^{Aab}	512.52±6.00^{Bab}	509.92±3.78^{Cab}	515.56±1.07^{Ca}
3	429.51±1.99^{Dd}	453.00±3.93^{Cc}	448.41±5.63^{Cc}	467.97±2.40^{Db}	499.37±3.97^{Da}
5	477.93±6.72^{Ce}	491.77±5.49^{Bd}	508.10±0.30^{Bc}	543.82±1.00^{Bb}	531.35±2.18^{Ba}
7	515.28±2.91^{Ad}	516.42±1.43^{Ad}	536.12±1.74^{Ac}	583.71±3.82^{Aa}	573.27±3.72^{Ab}
9	423.43±4.24^{Dc}	435.07±5.78^{Dc}	450.61±3.11^{Cd}	471.32±4.98^{Da}	452.66±6.22^{Eb}
CO_2 (%)	γ-oryzanol (mg/kg) db, DH6				
	16 hours	20 hours	24 hours	28 hours	32 hours
0	630.20±2.40^{Bd}	640.30±1.51^{Bc}	652.06±3.25^{Cb}	645.83±0.76^{Dbc}	667.78±3.92^{Ba}
3	613.23±0.85^{Cc}	614.76±4.64^{Cc}	641.00±2.64^{Db}	648.63±1.03^{CDb}	656.56±3.99^{Ca}
5	682.00±2.90^{Ac}	719.25±7.34^{Ab}	722.10±2.52^{Bb}	783.97±6.96^{Ba}	784.29±2.66^{Aa}
7	688.55±2.29^{Ad}	727.65±5.90^{Ac}	744.63±5.02^{Ab}	797.42±3.38^{Aa}	786.10±5.77^{Aa}
9	581.43±5.03^{Dd}	607.06±2.76^{Cc}	613.81±4.34^{Ec}	658.08±3.10^{Ca}	623.74±1.60^{Db}

a-e: The different letters in the same row indicate significant difference statistically at the 95% confident level;
A-E: The different letters in the same column indicate significant difference statistically at the 95% confident level

CONCLUSION

During the germination, GABA increased fast while other bioactive compounds showed slowly increased especially in black GBR. With these results, black sticky rice should be used for production of GBR product.

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