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A method for reducing rancidity in germinated brown rice flour

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Abstract

Germinated parboiled brown rice (GPBR) is the rice that has been soaked, germinated, and steamed in the husk before drying and then dehusked. The objective of this research was to study the effect of slightly acidic soaking water (pH 5.6) on the inactivation of lipase activity, hence, retarding the rancidity in GPBR. The shelf life of germinated brown rice flour (GBF) was also determined. Results were compared with GPBR produced by the process of the local community (soaking in tap water, pH 7.0). We suggested soaking rough rice in slightly acidic water for 36 h followed by germinating, and steaming to 16 h, and 15 min, respectively. This has been shown to decrease the lipase activity and retard the development of PV and TBA value as compared to the process of the local community. Moreover, the process of soaking in the slightly acidic water for 36 hours promoted GABA content in GPBR to the highest value of 25.70 mg/100 g dry matter. Shelf life of GBF produced from both processes was slightly different, varying between 64-67 days at 30°C. When storage temperature increased, different shelf lives of GBF produced from such processes were documented. Soaking rough rice in the slightly acidic water is therefore one method to prevent lipase activity. Other strategies to improve GBF storage stability should focus on methods to inactivate lipoxygenase and lipase activity, thus halting or slowing the early stages of lipid degradation and retard rancidity.

Keywords**:** *rancidity, germinated parboiled brown rice, shelf life*

บทคัดย่อ

ข้าวฮางงอก (GPBR) คือ ข้าวที่ผ่านกระบวนการแช่น้ำ การเพาะงอกทั้งเปลือก และนึ่ง ก่อนที่จะนำไปอบแห้ง และกะเทาะเปลือก วัตถุประสงค์ของงานวิจัยเพื่อศึกษาผลของกระบวนการแช่น้ำในสภาวะกรดอ่อน (pH 5.6) ในกระบวนการผลิตข้าวฮางงอกเพื่อยับยั้งกิจกรรมเอนไซม์ Lipase และเพื่อที่จะชะลอการเกิดกลิ่นเหม็นหืนในแป้งข้าวฮางงอก (GBF) งานวิจัยนี้ได้ศึกษาอายุการเก็บรักษาแป้งข้าวฮางงอก และเปรียบเทียบผลการทดลองที่ได้กับแป้งข้าวฮางงอกที่ผลิตโดยวิสาหกิจชุมชน (ที่แช่น้ำที่ pH 7.0) จากการทดลองพบว่า การแช่น้ำในสภาวะกรดอ่อน เป็นเวลา 36 ชั่วโมง บ่มเป็นเวลา 16 ชั่วโมง และ นึ่งด้วยไอน้ำเป็นเวลา 15 นาที จะช่วยลดกิจกรรม Lipase และการเกิดสาร Peroxide value และ TBA value ซึ่งเป็นดัชนีชี้วัด ในการเกิดกลิ่นเหม็นหืน นอกจากนั้นกระบวนการแช่น้ำในสภาวะกรดอ่อนเป็นเวลา 36 ชั่วโมงจะช่วยทำให้เพิ่มปริมาณสาร GABA สูงสุดในข้าวฮางงอก (25.70 มก/100 กรัม น้ำหนักแห้ง) อายุการเก็บรักษาแป้งข้าวฮางงอกทั้งสองกระบวนการผลิตแตกต่างกันเล็กน้อยอยู่ระหว่าง 64-67 วันที่อุณหภูมิการเก็บรักษา 30°C แต่เมื่ออุณหภูมิการเก็บรักษาสูงขึ้น แป้งข้าวฮางงอกที่ผ่านกระบวนการผลิตโดยข้าวฮางงอกที่แช่น้ำในสภาวะกรดอ่อน จะมีอายุการเก็บที่นานกว่าแป้งข้าวฮางงอกที่ผลิตโดยกระบวนการของวิสาหกิจชุมชน ดังนั้นกระบวนการแช่น้ำในสภาวะกรดอ่อนเป็นวิธีหนึ่งในการลดกิจกรรมเอนไซม์ Lipase เพื่อยืดอายุการเก็บรักษาแป้งข้าวฮางงอกจำเป็นต้องมีวิธีการอื่นๆ เพื่อช่วยลดกิจกรรมเอนไซม์ Lioxygenase และ Lipase เพื่อยับยั้งการสลายตัวของกรดไขมันตั้งแต่เริ่มต้น เพื่อชะลอการเกิดกลิ่นเหม็นหืนของผลิตภัณฑ์สุดท้าย

คำสำคัญ: *กลิ่นเหม็นหืน, ข้าวฮางงอก, อายุการเก็บรักษา*

1. Introduction

The Sakoltawapi Community Enterprise located in Sakolnakorn Province, Thailand produces germinated parboiled brown rice (GPBR) to satisfy the public need. GPBR is produced from paddy rice through pretreatments of soaking and germination and steaming before drying, and dehulling. Germination of brown rice is known to increase levels of nutrients such as gamma-aminobutyric acid (GABA) as well as soften the texture of brown rice and give it a pleasant flavor and odor (Tian, Nakamura, & Kayahara, 2004). GPBR therefore is marketed as a health food product returning profit to the community each year.

Brown rice flours prepared from germinated brown rice have been documented to have better

nutritional values such as increased GABA, oryzanol, vitamin B complex as well as some minerals and essential amino acids as compared to those from ungerminated brown rice flours. Similar to whole wheat flour, brown rice flour is increasingly popular as research continues to reveal the benefits of brown rice over milled rice and the food industry offers more options to consumers.

According to the community, it is necessary to produce a variety of products from germinated brown rice in order to expand the circulation of germinated brown rice in the market. GBF has been selected and is being introduced to the market for the bakery industry with the expectation of adding more value to the germinated brown rice as well as to add more income to the community. However, the problems facing the community are the short shelf life and rancid odor of GPBR and GBF products, making the products unacceptable. As a result, an improvement is needed in the production process to reduce rancidity while retaining the product quality without adding extra expenses to the community. Therefore, the purpose of this research was to improve the production process in order to decrease the rancidity in GPBR which will be processed to GBF with increased nutritional quality so that it can be further used in the bakery industry.

Factors contributing to the nutritional values of GPBR during germination are temperature, humidity, length of soaking and germination time. By soaking the rice at 40°C for 8-24 hours, the amount of GABA is significantly increased. The process of soaking the rice at 35°C can increase the amount of GABA to 24.9 milligrams per 100 grams compared with the normal soaking method which contains only 10 milligrams per 100 grams. It was also found that germination affects the texture of brown rice (Komatsuzaki et al., 2007; Saikusa, Horino, & Mori, 1994).

In brown rice, rancidity is caused by hydrolytic degradation of lipids located in the aleurone layer and the germ. Lipase (E.C. 3.1.1.3) is the key enzyme involved in enzyme-catalysed lipid triacylglycerols degradation to non-esterified fatty acids, and diglycerides, mono-glycerides, eventually undergoing further oxidization by enzymes called lipoxygenase (LOXs, linoleate oxygen oxidoreductase, E.C.1.13.11.12) and by autoxidation. These reactions not only result in free radicals but also rancidity, bad taste, and loss of nutrition. The hydrolytic reactions can be minimized by cold storage, good transportation, careful packaging, and sterilization, but oxidative rancidity, including autoxidation, is not stopped by lowering the temperature of food storage (Doblado-Madonado, Pike, Sweley & Rose, 2012; Champagne & Hron 1994; Zhang et al., 2007; Galliard, 1986).

2. **Objectives**

The objective of the research is to study the effect of slightly acidic soaking water (pH 5.6) on the inactivation of lipase activity, hence, retarding the rancidity in GPBR. The shelf life of GBF from GPBR processed by the community's process compared to the improved process is also determined.

3. Materials and methods

3.1 Germinated parboiled brown rice (GPBR) production

Rough rice (Khao Dawk Mali 105) obtained from the Sakoltawapi Community Enterprise was used in this experiment. The process of germinating parboiled brown rice was done at Rangsit University laboratory adhering to the Sakoltawapi Community Enterprise protocol. The procedures were as follows: soaking samples with filtered tap water (pH 7.0) for 48 hours, germinating at room temperature for 16 hours, then steaming by water vapor for 15 minutes. The germinated parboiled rice was dried until the moisture content decreased to 10-12% then milled to obtain the germinated parboiled brown rice (GPBR). The other process developed by the researchers was soaking the rice in weakly acidic water. The pH of soaking water was 5.6 adjusted with 0.1 N citric acid. Aside from this change the remaining steps were similar to the community as mentioned above. GPBR samples, produced by the 2 processes, were taken periodically for chemical analysis; 12 hours to 48 hours, 4 hours to 48 hours, and 5 minutes to 15 minutes during soaking, germination, and steaming, respectively. The optimum time from such process was selected according to the chemical analysis indicating the lowest index of rancidity, and then the next process of GPBR production was done. Gamma-aminobutyric acid content of rice was determined during soaking for 48 hours.

3.2 Germinated parboiled brown rice flour (GBF) production

The GPBR obtained from the selected optimum time from all processes of the 2 practices was grinded using a Hammer mill then passed through an 80-mesh sieve to obtain germinated parboiled brown rice flour (GBF). Samples were kept in zipped plastic bags awaiting shelf life determination.

3.3 Chemical analysis of GPBR

GPBR samples, produced by the 2 processes, were taken at each step. Chemical analysis was determined as follows: lipase activity (Hatzinikolaou et al., 1999), peroxide value (PV) (Horwitz, 2002), thiobarbituric acid value (TBA) (modified from the method of Jayasinghe, Gotoh, Aoki, & Wada, 2003), and gamma-aminobutyric acid content (GABA content (modified from the method of Karladee & Suriyong, 2012).

3.4 Shelf life of GBF

The GBF samples kept in zipped plastic bags were stored at 4°C (control), 30°C, 40°C, and 50°C in temperature controlled and light protected chambers for 12 weeks. The TBA of the samples was determined weekly. Odor evaluation of stored GBF samples was assessed by a trained panel of experts (six people) at Phatumthani Rice Research Center using the method of Ratio Profile Test.

3.5 Statistical analysis

The experiment was designed by using the CRD model. There were two factors considered, which were the time in each particular step of producing GPBR (soaking, germinating, and steaming) and the pH (5.6 and 7.0) of soaking water. Samples subjected to each treatment were evaluated for lipase activities, PV, TBA, and GABA. All measurements were made in 3 replicates. ANOVA was performed using SPSS program (version 20) and means were compared by Duncan's Multiple Range Test (DMRT) at $p<0.05$.

4. Results and discussion

4.1 Soaking, germination, and steaming process

The results in Table 1 show that lipase activities in rough rice soaked in tap water and acidic water gradually increased with soaking time. At 48 h of soaking, lipase activities were significantly different in both soaking water pH conditions ($p\leq0.05$). The increase in lipase activities found during germination followed a similar trend as those found in soaking. The longer germination was allowed to proceed, the higher increase in lipase activity was observed. However, germinated rough rice soaked in weakly acidic water showed less significant increases in lipase activity than those soaked in tap water. The results were in agreement with that of Qingci, Wei, Yong, and Chongy (1994) which indicated that lipase activity of rice bran had the pH optima of 7.5-8.0. Changing the pH outside of this range causes lipase activity to decrease. Therefore, a low acidic condition (pH 5.6) in soaking water before germination was hypothesized to slow down the lipase activity.

Even though a longer germination time could promote lipase activity, we allowed the germination time to continue for 16 h similar to Sakoltawapi Community Enterprise protocol since there was evidence that germination time can improve nutritional and physical properties in rice (Ohtsubo, Suzuki, Yasui, & Kasumi, 2005). In germinated grains, starch, non-starch polysaccharides and proteins are decomposed and turned into oligosaccharides and amino acids resulting in easy digestion. According to Kaosa-ard and Songsermpong (2012), germination time of 72 h contributed to the higher GABA content and the softer texture of cooked germinated brown rice than brown rice.

Table 1 Lipase activities (mU/ml) of germinated Khao Dawk Mali 105 brown rice produced by different pH of soaking water

Processing step	Time (h)	Soaking pH	
		pH 7.0	pH 5.6
Soaking	12	1.129$^{b(a)}$ ±0.28	1.186$^{b(a)}$ ±0.02
	24	1.380$^{b(a)}$ ±0.30	1.225$^{b(a)}$ ±0.30
	36	1.604$^{ab(a}$) ±0.33	1.513ab(a)±0.25
	48	2.135$^{a(a)}$ ±0.23	1.812a(b)±0.16
Germinating	4	1.531$^{b(a)}$ ± 0.68	1.695ns(a) ± 0.63
	8	2.521$^{ab(a)}$ ± 0.01	1.840ns(a) ± 0.44
	12	3.034$^{a(a)}$ ± 0.70	1.842ns(a) ± 0.54
	16	3.155$^{a(a)}$ ± 0.60	1.860ns(a) ± 0.64
Steaming (min)	5	2.329$^{a(a)}$ ±0.09	1.729a(b)±0.24
	10	1.092$^{b(a)}$ ±0.32	1.037b(a)±0.20
	15	0.948$^{b(a)}$ ±0.27	0.785b(a)±0.28

a, b Means followed by the same letters within the same column, (a), (b) means followed by the same letters within the same row are not significant different at 0.05. Data are expressed as sample mean ± SD (n =3).

When the germinated rice was steamed at 100°C, the lipase activities were decreased and almost inhibited when steaming was continued for 15 minutes. Results were similar to millet flour which was steamed for 15 minutes causing the lipase activity to reduce, hence increasing its shelf life (Meera, Bhashyam, & Ali, 2011). There are different types of heat treatment stabilization procedures: moisture retaining heating, added moisture heating, dry heating in atmospheric pressure extrusion cooking, and microwave cooking. In commercial processes, heat treatment (90°-130°C), including extrusion cooking, at natural moisture levels or less severe heating (>80°C) after water/steam addition, which is the preferred method to inactivate lipases in rice bran (Malekian et al., 2000). A previous study showed that hydrolytic rancidity of rice bran can be prevented by microwave heating and that the recommendation for microwaved rice bran is 4-5°C in zipper-top bags (Ramezanzadeh et al., 1999).

Table 2 Peroxide values of germinated Khao Dawk Mali 105 brown rice soaked for 48 hours produced by different pH of soaking water

Time (h)	Soaking pH	
	pH 7.0	pH 5.6
12	13.465$^{c(a)}$± 2.74	1.318$^{c(b)}$ ± 0.17
24	18.388$^{b(a)}$ ± 2.28	2.979$^{bc(b)}$ ± 1.44
36	22.005$^{a(a)}$ ± 1.41	4.386$^{ab(b)}$ ± 0.72
48	25.188$^{a(a)}$ ± 0.84	5.510$^{a(b)}$ ± 1.10

$^{a, b}$ Means followed by the same letters within the same column, $^{(a), (b)}$means followed by the same letters within the same row are not significant different at 0.05. Data are expressed as sample mean ± SD (n =3).

Table 2 shows the peroxide values (PV) increased during soaking rice in both conditions. Nevertheless the values were much higher in rice soaked in tap water than in weak acid water. The peroxide value gives a measure of the extent to which an oil sample has undergone primary oxidation. Free fatty acids formed by hydrolysis of lipids with the activation of lipases are also precursors of hydroperoxides. The hydroperoxides are intermediates, eventually changing to aldehydes and ketones as well as other breakdown products causing rancidity. This was the reason that the PV in rice during germination was not measured but the increased in TBA values was noticeable (Table 3).

Table 3 TBA (mg malonaldehyde eq/kg sample) of germinated Khao Dawk Mali 105 brown rice produced by different pH of soaking water

Processing step	Time	Soaking pH	
		pH 7.0	pH 5.6
Germinating	4	$2.028^{c(a)} \pm 0.16$	$1.193^{b(b)} \pm 0.09$
(h)	8	$2.494^{b(a)} \pm 0.15$	$1.293^{b(b)} \pm 0.03$
	12	$3.070^{a(a)} \pm 0.27$	$1.223^{b(b)} \pm 0.02$
	16	$3.225^{a(a)} \pm 0.18$	$1.420^{a(b)} \pm 0.14$
Steaming	5	$2.851^{b(a)} \pm 0.83$	$2.721^{b(a)} \pm 0.00$
(min)	10	$3.475^{b(a)} \pm 0.33$	$3.329^{a(a)} \pm 0.01$
	15	$4.007^{a(a)} \pm 0.36$	$3.380^{a(b)} \pm 0.03$

[a, b] Means followed by the same letters within the same column, [(a), (b)]means followed by the same letters within the same row are not significant different at 0.05. Data are expressed as sample mean ± SD (n =3).

Further, our results showed that soaking at weakly acidic water for 36 h increases GABA content to the highest level of 25.70 mg/100g dry matter (Figure 1). Due to the mild acidic conditions, the enzyme GAD works effectively and catalyzes the change of glutamate to GABA and CO_2. Zhang et al. (2007) found that the enzyme GAD in rice embryos functions optimally at pH 5.5-5.8. In our previous study, we found that at pH 5.6 soaking water, Jasmine germinated brown rice had the highest concentration of GABA (Punkum, 2009). The results were similar to other data which indicated that GABA content increased steadily, reaching its highest levels of 17.87 mg/100g dry matter at 24 h incubation, and then decreasing continuously afterwards to 1.36 mg/100 g dry matter at 48 h (Karladee & Suriyong, 2012). In germinated Jasmine brown rice, GABA was highest after soaking for 24 h at 35°C (Wichamanee & Teerarat, 2012).

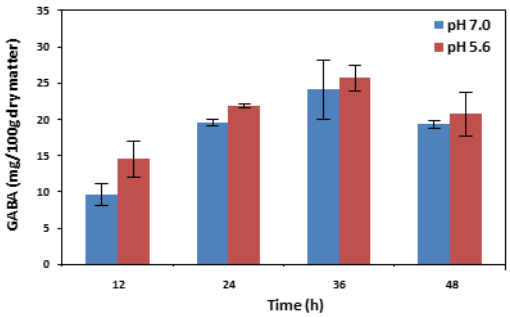

Figure 1 Gamma-aminobutyric acid (GABA) (mg/100g) in rough rice during soaking for 48 hours at 35°C

4.2 Shelf life of germinated brown rice flour (GBF)

As shown in Figure 2, TBA levels significantly increased during storage at the 3 temperatures (30°, 40°, and 50°C, $p \leq 0.05$). A closer examination of the linear plot revealed that the rate of increase of TBA is constant throughout the storage periods, (r = 0.96**-0.99**), the plot represented a zero order reaction. In GBF, it was noticed that the rate of increased in TBA was increased when storing samples at higher temperature. However, the kinetic parameters of the changing TBA value of GBF produced from GPBR soaked in pH 5.6 water were slightly less than those at pH 7.0, especially when temperature increased from 30°C to 50°C (Table 4).

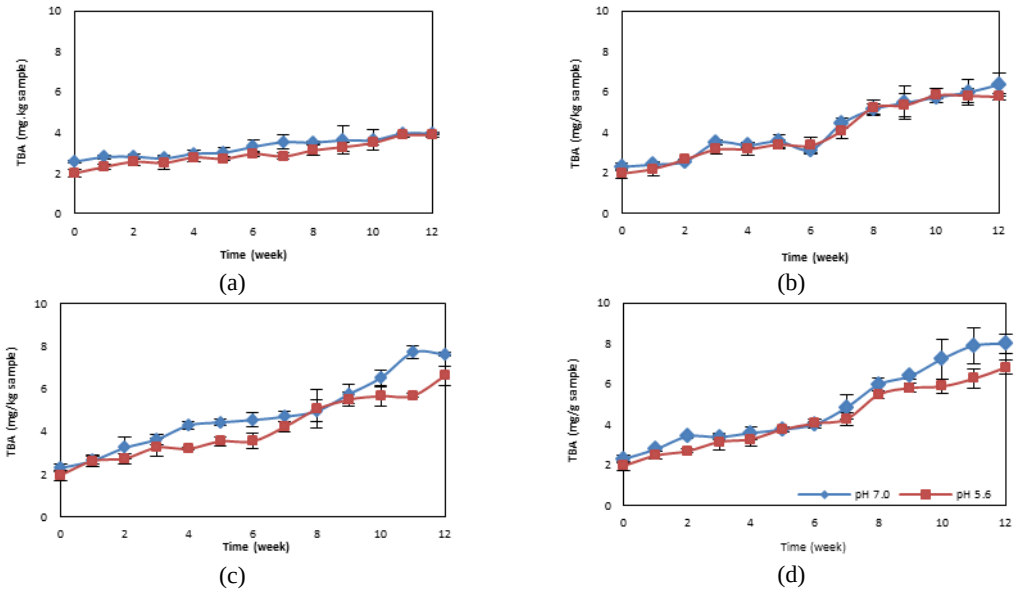

Figure 2 TBA at different storage temperatures for germinated rice flour produced from GPBR soaked at pH 5.6 and pH 7.0 (a=4°C (control), b=30°C, c = 40°C, d = 50°C)

Table 4 Kinetic parameter (k) of TBA at different storage temperatures for germinated rice flour produced from GPBR soaked at pH 5.6 and pH 7.0

Soaking pH	Storage Temperature (°C)	k ($mg.kg^{-1}.week^{-1}$)
pH 5.6	4 (control)	0.119 (0.96)*
	30	0.357 (0.97)
	40	0.369 (0.98)
	50	0.407 (0.99)
pH 7.0	4 (control)	0.142 (0.95)
	30	0.359 (0.96)
	40	0.431 (0.97)
	50	0.495 (0.97)

* Number in parenthesis indicated correlation values (r)

According to the odor evaluation, there was a correlation of sensory evaluation (scaling) with TBA values (r^2 = 0.567*) (Figure 3). The panel decided to reject the stored GBF at week 8 (Figure 4). Using the correlations in Figure 3 and 4, then, the TBA value at week 8 was calculated to be 5.27 mg/kg. Therefore, it was possible to estimate the shelf lives of both germinated flour samples at the storage temperatures of 30°C - 50°C (Table 5).

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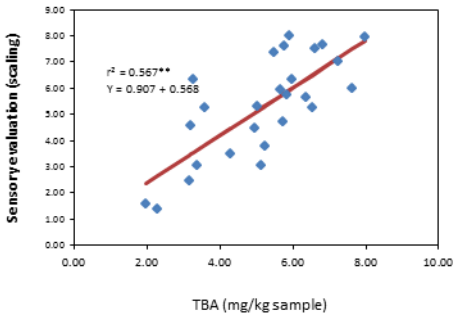

Figure 3 Correlation between sensory evaluation and TBA values in stored GBF

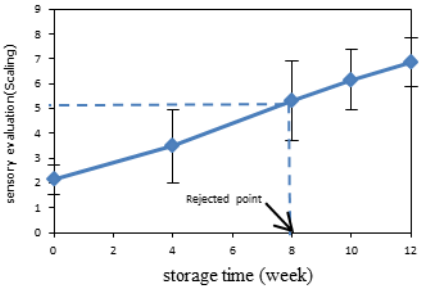

Figure 4 Correlation between sensory evaluation and storage time of GBF

Table 5 Shelf life evaluation (days) of GBF stored at 4°C (control), and 30°C-50°C

Storage temperature (°C)	pH 5.6	pH 7.0
4 (control)	160	156
30	67	64
40	64	50
50	59	48

The shelf lives of GBF produced from GPBR after being soaked at pH 5.6 and pH 7.0 were not significantly different (64-67 days). Nevertheless, the shelf life starts to shift noticeably when storing both samples at higher temperatures (Table 5).

Although lipase was inhibited during the GPBR production by lowering pH level to weak acid (pH 5.6) and using the steaming process, the rancid smell of GBF still occurred. Hydrolytic rancidity reaction stimulated by the remaining lipase in rice bran can explain the continuing odor. Once processed into GBF and stored, the hydrolytic reaction occurs by the stimulation of moisture content and physical disruption during grinding in the process of making flour. Moreover, there is also an oxidative rancidity reaction from the lipoxygenase in the rice bran and germ. Lipoxygenase catalyzes the oxidation of methylene-interrupted unsaturated fatty acids and their ester such as linoleic and linolenic acids. This reaction is called autoxidation. Autoxidation occurs with non-enzymatic reactions from fat and oxygen. These lead to compounds called hydroperoxides that later change to ketone, lactones and furans (McWilliams, 2005). The rancid smell in GBF occurs from these reactions: lipolytic rancidity stimulated by lipase and autoxidation stimulated by lipoxygenase and also another type of non-enzymatic reaction. However, the GBF produced from the improved process (soaking in the weak acid water) retards the lipase activity and allows the GBF to have longer shelf life at higher storage temperature than that processed from the community.

5. Conclusion

An improvement in the production of GPBR by soaking the rice in weak acidic water helps reduce lipase activity, decreasing the amount of TBA values and significantly increasing the amount of

GABA. Therefore, an optimal condition recommended to the local community for GPBR production is soaking at low acidic water at pH 5.6 for 36 hours and germinating for another 16 hours.

However, the shelf life of GBF produced from the recommended process remains nearly the same as those produced from the community process when stored at a low temperature. Other strategies should be included in the production, such as storing in low O_2, and low temperature, using proper packaging and protecting moisture content during storage. It is necessary to further study these conditions to slow or even eliminate rancidity in GBF while also retaining GBF quality.

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