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Influences of Processing Conditions, Thickening Agents and Storage on Quality and Gamma-amino Butyric Acid Content of Germinated Brown Rice Milk

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ABSTRACT

Germinated brown rice has recently been recognized as a nutritious and functional food for human health and rice milk is known as one of the most convenient and favorable products from the germinated brown rice. In this study, effects of soaking time, ratio of rice to water, thermal treatments, different thickeners and storage temperature on gamma-amino butyric acid (GABA) content and quality characteristics of germinated brown rice milk (GBRM) were investigated. The results showed that the soaking with ratio 1:10 of rice to water for 8 hours produced the highest GABA content (304.70 µg/ml) compared to other soaking treatments. The heating treatment using 90ºC was the most suitable for retaining the GABA content in the obtained GBRM. Regarding the effect of thickening formulations, the mixture of guar gum and xanthan gum created the highest stable GBRM product with the stability approximately 100% after 7 days of storage. In addition, the storage at 5ºC was exhibited as the most suitable among the investigated storage conditions for the preservation of GABA content, stability and microbial quality of the GBRM.

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1. Introduction

Rice (*Oryza sativa* L.) is the most important cereals product and is the major staple food for most parts of the world [1]. There has been a trend in the current modification of this product by germinating to achieve more nutritional and functional values. Germinated brown rice (GBR) is the unpolished rice that has been germinated for gaining more nutrition. The germinated brown rice has a better texture and flavor, most importantly is the high level of gamma-aminobutyric acid (GABA) [2]. Germinated brown rice is used in many different cuisines such as Japanese and Korean. The nutritional content of the germinated brown rice appears to be higher than its normal white counterpart. The production of germinated brown rice is conducted by soaking the brown rice in warm water of 30-40ºC. The rice is thereby swollen with water and the endogenous enzymes such as phytase, lipase, α-amylase and β-amylase are activated for the purpose of germination [3]. Germinated brown rice has been known for many benefits to human health due to the higher nutritional composition than regular brown rice such as vitamins, antioxidants, sugars and especially the amount of GABA [4]. There are different methods for increasing the level of GABA content in germinated brown rice via improving yield of the germination process such as controlling the soaking time and temperature [5], biological modification [6], gaseous treatment [7].

GABA is an amino acid which acts as a neurotransmitter in the human brain. GABA has been proven to have calming effect, promoting the development in the children brain, healing and curing effect in overactive children and autism [8]. Many research have point out that the addition of GABA to the human brain help reduce stress, anxiety [4], [8], [9]. Recently, there has been an increasing trend of using the germinated brown rice to develop many food products which are easier for human consumption. Germinated brown rice milk (GBRM) is one of the most ideal ways for a convenient product but it was found that the thermal processing and the preserving of GBRM may cause the loss of GABA and the degradation in the quality of the rice milk [10]. Heat treatment has been known to be the main cause for the loss of GABA during processing. A research conducted by Jirasatid &

Nopharatana (2021) [11] showed that GABA is sensitive to heat: the higher temperature and longer heating time lead to the greater the loss in GABA. There were also concerns that the rice to water ratio and the extraction time can also affect the retrieved GABA content as well as other nutrients in the extracted GBRM. In addition, GABA has also been known to have chemical interaction with other chemical component within the rice itself, but these interactions mostly occur due to the catalyst components such as enzymes or lipid oxidation [12].

For stabilizing beverage products especially bran milk, thickening agents are commonly used to provides the thicker characteristic by increasing the viscosity of the products as well as act as an emulsifying agent and a stabilizer [13]. For example, using of xanthan gum (a harmless thickener for human health) could significantly increase in the viscosity of beverage products [14]. Pectin has also been widely used for developing juice drinks with low juice content or sugar-free soft drinks [15]. In addition, another common thickener having good emulsifying properties, especially suitable for oil-in-water emulsion systems (Guar gum) is widely used in emulsion flavor stabilizers. It also has good film forming properties, used as a thin film to help preserve the flavor of foods and prevent oxidation. Gum guar has the ability to thicken, emulsify, and contribute to swelling, as a stabilizer to maintain the uniformity of food products [16].

In Vietnam, some germinated brown rice milk products have been developed and available in the market and there have been a few studies on this type of rice product. These studies mostly focus on the manufacturing capacity and increasing yield for producing the GBR and GBRM [17] – [19] while the investigations for improving and preserving quality GABA content of GBRM during processing and preservation is limited. Thus for retaining the GABA content, determination of the appropriate conditions for processing, suitable formulation and preservation of the produced germinated brown rice milk is necessary. This study investigates effects of parameters including soaking time and ratio of rice, preserving temperature and different formulations of thickening agents (xanthan gum, guar gum, pectin) on the quality characteristics of the product.

2. Materials and Methods

2.1. Materials

The germinated brown rice used for this study was Vibigaba obtained at Gao Mam Vibigaba retailer (District 10, Ho Chi Minh City). Xanthan gum, Guar gum, pectin, acid boric, phenol, sodium hypochlorite, borax and others were purchased from Bach Khoa chemical store (District 10, Ho Chi Minh City). Enzyme amylase was purchased from Angel Biotic. Inc retailer (District 1, Ho Chi Minh City). All chemicals are analytical grade.

2.2. Experimental methods

Investigation of soaking time:

Soaking is an important step during the production of GBRM, as the unprocessed GBR has a hardened structure which is unsuitable for achieving the highest milling efficiency. Soaking the rice in warm water at different time for soaking was surveyed to determine the most suitable time for soaking the rice. The soaking times of 2, 4, 6, 8, 10 and 12 hours under ambient temperature were investigated for the level of GABA in the germinated brown rice.

Investigation of rice to water ratio:

The ratio between water and rice for milling can affect the GABA content of the GBRM as water act as the main ingredient to contain the GABA content after the filtration process. The soaking time in this experiment was 8 hours. Three ratios of rice to water of 1:5, 1:10 and 1:15 (g/ml) were investigated for the level of GABA in the GBR.

Sterilization and storage study of GBRM:

GBRM samples were prepared and stored in 100ml Duran sterilization bottles. These tubes were sterilized in 15 minutes at 121^oC under the pressure of 2 bars by using an autoclave. The samples were then cooled to the room temperature and stored at 5^oC, 10^oC, 25^oC. To determine the most suitable preservation conditions for the GBRM, the change in pH and microbial count of the GBRM was investigated during the storage. Samples are taken and measured every 24 hours after the rice milk samples being sterilized.

2.3. Analytical methods

Method for determination of GABA content using spectrophotometry

GABA in the samples was analyzed according to the method described by [11]. Briefly, 0.1 ml the extract containing GABA was mixed with 0.2 mL of 0.2 M borate buffer having (pH of 9.0) and 1 ml of 6% phenol reagent in a test tube. Then 0.4 mL of 7.5% sodium hypochlorite solution of was added and the test tube was boiled for 10 minutes until the solution color change to blue. The solution was cooled under running tap water and the absorbance was measured at 630 nm wavelength using an UV-VIS spectrophotometer. The GABA content of the sample was calculated based on the absorbance against the standard curve the GABA standard.

Determination of viscosity of GBRM

The determination of viscosity of the GBRM with addition of xanthan gum, guar gum, pectin and the mixtures of those thickeners (xanthan gum-guar gum, xanthan gum-pectin and guar gum-pectin with the ratio of 1:1) at different concentration 0.1% g/ml, 0.3% g/ml and 0.5% g/ml was carried out by using a Viscometer (DVEELVTJ0, Brookefield, USA). The measurement cylinder used was S61 and S62, the result was expressed in mPa. The viscosity of the GBRM was measured at 3 different speeds including 50 rpm, 60 rpm and 100 rpm.

Determination of stability of GBRM

The stability of GBRM was determined by calculating the precipitate after 7 days and measure the time for diffusion to begin. This method was based on Shimoyamada et al. (2008) [20] with minor modification by replacing the 10ml cylinder with the 100ml cylinder. The stability of the product was calculated by the equation:

$$\text{Stability (\%)} = \frac{Vp}{100} \times 100 \tag{1}$$

Where V_p is the volume (ml) of precipitated fraction observed in the cylinder containing 100ml of GBRM.

pH determination

30ml GRBM samples was prepared by using a sterilized 10ml pipette to take sample. For determining the pH, use a pH-meter by first calibrating the pH-meter and measure the change of pH value of GBRM by dipping it into the sample. This process was repeated daily until the end of preservation.

Determination of bacterial count

For determining the total aerobic bacteria in the GBRM, use the RAC Petrifilm provided by 3M. Use a sterilized micropipette to extract 1ml of the rice milk and insert it on the petrifilm. Let the solution be spread evenly throughout the surface of the petrifilm and let it rest in 1 minute for the gel formation to be built. This process was repeated daily until the total bacteria count is above the criteria. The result was observed and counted. The total bacteria count was determine until the GBRM excess the safe level for consumption (total bacterial count under 10^2 CFU/ml) [21].

2.4. Statistical analysis

All experiments were carried out in 3 replications. The results were expressed as the mean value ± standard deviation. Statistical tests with analysis of variance and the Duncan's Multiple Range Test (DMRT) at a significance level of 95% were used to analyze the difference among the means.

3. Results and Discussion

3.1. Determination of the suitable soaking time and ratio of rice for achieving GABA

Figure 1 presents the GABA content of the germinated rice samples soaked for different time of soaking time (from 0h -12h) and ratio of rice to water (1:5; 1:10; 1:15). Overall, the level of GABA can only be detected after soaking for 4h at all ratios, GABA content was least spotted at 4 hours of soaking due to the fact that the germinated brown rice has a hard outer layer which make it hard for the milling process to achieve the GABA content from the inner kernel of the rice. At the ratio of 1:5, this is the ratio which the most GABA content (125 µg/ml at 4h to 517 µg/ml at 12h) achieved due to the concentration between water and rice in the solution is the lowest in comparison to the two other ratios. While soaking the rice at the ratio of 1:15 the GABA content achieved is the lowest due to the concentration of GABA is minor by the volume of the solution. The ratio of soaking at 1:10 result in GABA content ranging between 66 µg/ml at 6h and 376 µg/ml at 12h. Although the GABA content of this soaking ratio was lower than that of the ratio 1:5 from 4h to 10h, at the end of soaking (12h) its GABA content reached statistically comparable value as the highest GABA achieved from ratio 1:5. Thus the soaking ratio of 1:10 could be considered to have the best effect on both scientific research and fit for the economical because it is suitable for the production scale to achieve a higher volume of solution for producing GBRM.

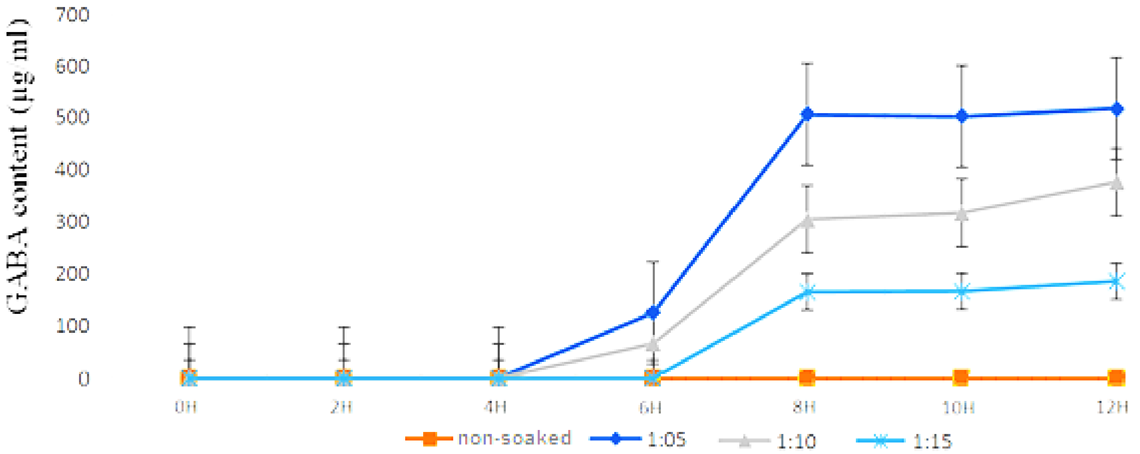

Figure 1. *GABA content (µg/ml) of GBR affected by soaking time and ratio of rice to water*

Soaking is the first main act for producing of rice milk. As soaking helps the rice to intake moisture content in the kernel, this expands the starch matrix within the rice and expand it, revealing the inner rice germ and softening the hard outer layer for wet milling [22] – [23]. This intake of water during soaking requires an amount of time for the soaking process to reach its suitable parameters. Exposure of the rice by soaking can help increase the milling efficiency thus enhancing the GABA retrieval rate in the production of GBRM. While germinated brown rice is enriched with GABA, a partial of this content is still within the rice residue after being filtrated through a cloth bag.

3.2. Effects of heating time and temperature on the GABA content of the germinated rice milk

The amount of GABA retain in GBRM after being heated at different temperatures of 70°C, 80°C, 90°C for 10, 20, 30 minutes is presented Figure 2. The GABA content was least affected when treated under the temperature of 90°C. The amount of GABA when thermal treated at 70°C (422.86 - 435.77 µg/ml) and 80°C (404.17 – 435 µg/ml) showed insignificant GABA loss. In comparison among the thermalizing processes, treatment at 70°C showed the lowest loss of GABA.

The loss GABA during thermal treatments mainly related to the chemical interaction between GABA and other components within the GBRM. Le et al. (2020) [24] found that the GABA content of rice milk was lost during thermal processing at 70°C and 90°C. As GABA is an amino acid which acts in the non-enzymatic browning process with sugars. For the GBRM, the GBRM's starch granules are hydrolyzed with α-amylase into simple sugar. In addition to this, the GBRM is mixed with 5% D-glucose for the purpose of mimicking the sweetness of the commercial rice milk. This addition affects the non-enzymatic browning process by having GABA as the amino acid to interact with simple sugars, thus making a reducing trend in the GABA content visible. Consequently, the heating of GBRM at 80^{o}C for 30 minutes was selected as the most effective parameter for thermalizing the GBRM as this process has a minor loss on the GABA content but also act as pasteurization for the rice milk.

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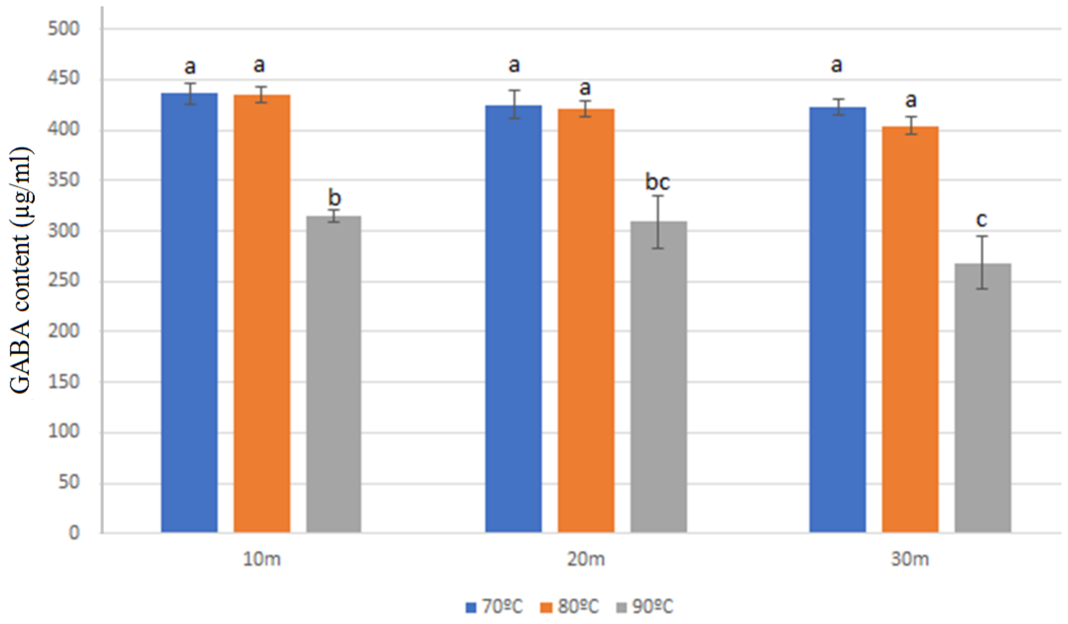

Figure 2. *GABA content (µg/ml) of GBRM heated with different temperatures and times*

3.3. Influence of different thickening formulations on the stability of GBRM

The stability of the GBRM depends on the particle size and concentration of the starch granules and other particles within the GBRM solution. Figure 3 illustrates the stability of the GBRM determined after 7 days of storage at 5°C. The use of hydrocolloids at any concentration showed an increase in the stability of the GBRM, although the use of 0.1% (w/v) of hydrocolloids showed no significant differences to the control sample.

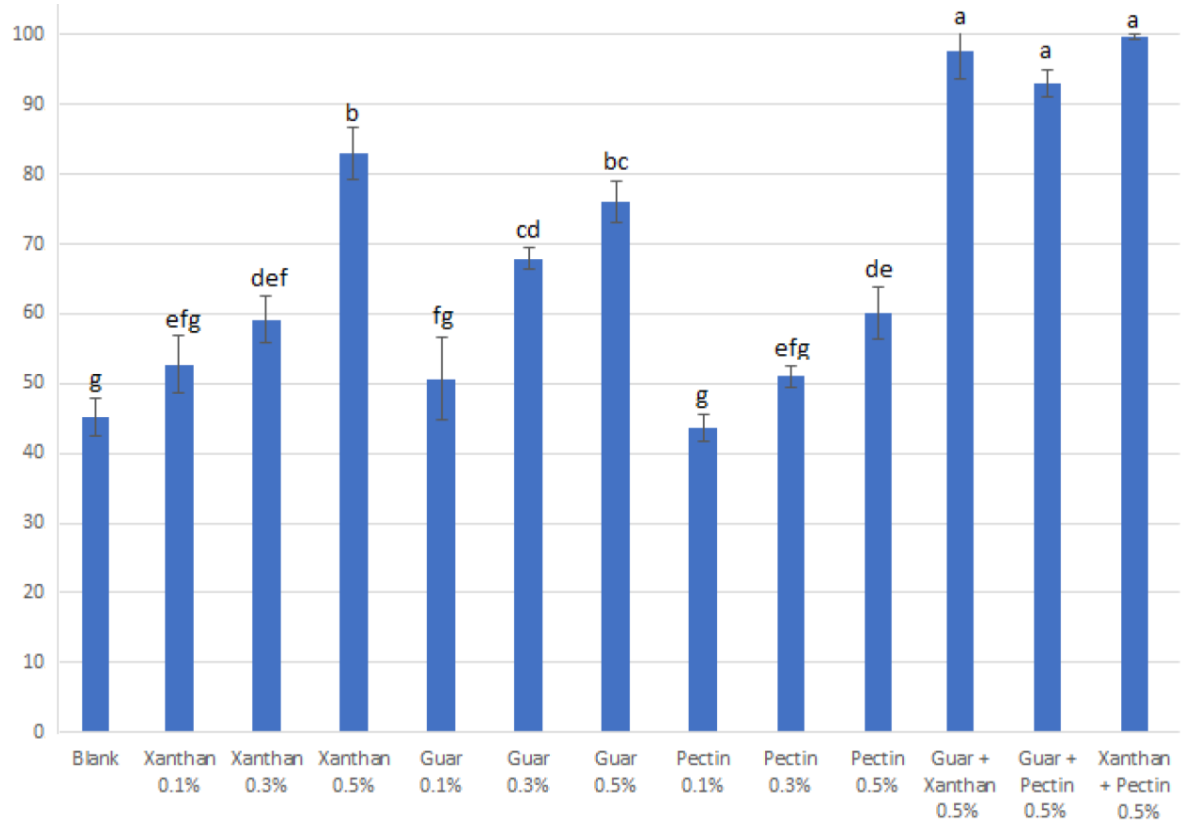

Figure 3. *Stability (%) of GBRM using different thickening agents*

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A trend of stability increases in the sample used thickeners at a concentration from 0.3% (w/v) to 0.5% (w/v) can be spotted which could be considering that this is the appropriate use for enhancing the stability of rice milk. While the use of xanthan gum at 0.5% (w/v) showed the highest stability (83%) compare to other thickeners. However, the use of a mix of hydrocolloids shows a higher result in stability. The addition with mixture of xanthan gum with pectin (ratio 1:1) resulted in the greatest stability of approximately 100% while the mixture of guar gum and pectin (ratio 1:1) resulted in a stability of 93% and xanthan gum with guar gum (ratio 1:1) resulted in a stability of 97%, respectively.

In the food and beverage industry, bran milk or oat-based milk like rice milk is known to be an emulsion which comprises of a variety of constituents that are separated by different phases. As the rice milk is ensure to separate after a period of preservation, which could cause a rejection by undesirable appeal by the consumer [25]. For this reason, applying hydrocolloids as thickeners to help enhancing the quality of the rice milk to prevent and reduce separated layer for sensory appealing is necessary for the evaluation of the rice milk product.

The use of hydrocolloids with the rice milk also increases the viscosity of the final product, the germinated brown rice milk incorporated with thickeners have a longer duration of keeping its stability during preservation [26].

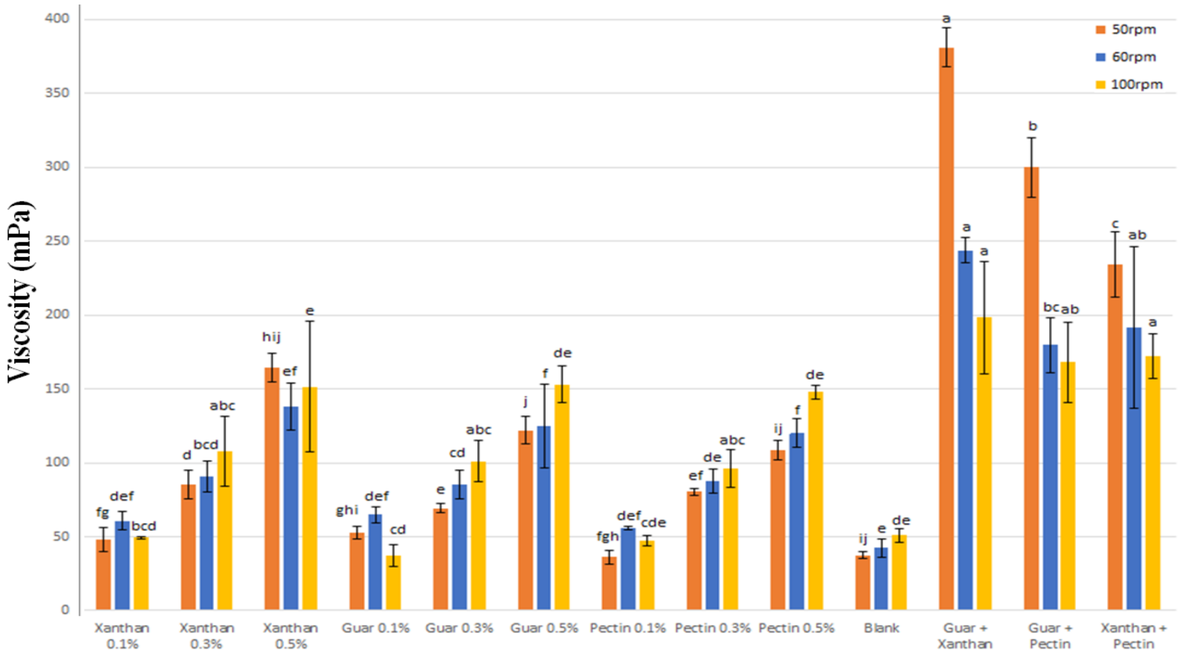

Figure 4. *Viscosity (mPa) of GBRM using different hydrocolloids and concentrations*

The data in Figure 4 shows that the viscosity of the rice milk increased along with the concentration of hydrocolloids used. Although the concentration of 0.5% of each hydrocolloid showed increase in viscosity when measured at three different speeds of rotation, the highest viscosity belonged to the combinations of those. The viscosity of the addition with mixtures of thickeners showed no significant differences when measuring at high speeds but it was significantly higher compared to the use of individual hydrocolloids. The greatest viscosity to be measured was the use of xanthan gum and guar gum which greatly enhanced the viscosity of the rice milk to over 200 mPa. This increase in viscosity can also be the result of the gel formation between guar gum and xanthan gum with the starch in the rice milk [27]. The rheological characteristics of a fluid depend on its chemical component, temperature of processing and other processing factors [28]. Viscosity slowed the movement of particles and droplets in still liquids, increasing the physical stability of GBRM by reducing the rate of destabilization [29] – [30]. In this study, the use of hydrocolloids greatly enhanced the viscosity of a product. Thus the increasing trend of the viscosity may contribute to the greater stability of the rice milk.

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3.4. GABA loss, pH change and microbial quality of GBRM during preservation

As shown in the Table 1, preservation of the GBRM at 5ºC, 10ºC, 25ºC show a GABA loss of 34.93, 42.42 and 43.44 µg/ml, respectively, after 7 days of storage. The GABA loss between different temperatures of storage was difference between each temperature of preservation but these losses are insignificant different. This loss in GABA can show that temperature of storage plays no effects on the GABA loss [24]. For this, the conclusion can be given is that GABA rice milk can be stored at different temperature of storage while doing no harm to the GABA content with in the rice milk.

Table 1. *The GABA loss of GBRM preserved at different temperature for 7 days*

Storage temperature (ºC)	5	10	25
GABA loss (µg/ml)	34.93 ± 7.85^{a}	42.42 ± 20.83^{a}	43.44 ± 11.33^{a}
Loss percentage (%)	11.20	11.11	9.22

Different superscript letters show significant differences among the means within each row ($p < 0.05$).

NA: Not applicable

One of the most important factors to determine the consumption for GBRM is the total microbial count. Amongst the three temperatures surveyed for the temperature of storage for GBRM, cold storage at 5ºC show the most effective to inhibit the growth of microorganisms. As shown in Table 2, during 7 days of storage at 5ºC, the microbial content reached the overgrowth of microorganism at the last day of preservation (141±20.52 CFU/ml) while storage at 10ºC and room temperature reach the last day of preservation earlier when the total count was over 100 CFU/ml.

Table 2. *Microbial count of GBRM preserved at different temperature for 7 days (CFU/ml)*

Storage time (day)	**Storage temperature**		
	5°C	10°C	25°C
0	0.00 ± 0^{f}	0.00 ± 0^{f}	0.00 ± 0^{f}
1	2.00 ± 2.00^{f}	12.33 ± 7.02^{f}	17.67 ± 9.61^{def}
2	4.33 ± 4.04^{f}	28.67 ± 6.03^{cdef}	44.33 ± 5.86^{cde}
3	16.67 ± 2.52^{ef}	47.67 ± 6.11^{cd}	121.67 ± 25.58^{a}
4	27.33 ± 8.39^{cdef}	85.00 ± 8.72^{b}	NA
5	51.33 ± 4.51^{c}	149.00 ± 12.53^{a}	NA
6	83.33 ± 9.07^{b}	NA	NA
7	141.00 ± 20.52^{a}	NA	NA

Different superscript letters show significant differences among the means ($p < 0.05$).

NA: Not applicable

Most bacteria in the rice milk are aerobic bacteria, typically *Bacillus cereus*, these microorganisms mainly grow in the condition of 8°C to 55°C in aerobic condition. Rice milk is a nutrient rich solution containing starch, sugars which are the main source of food for these bacteria to consume them and convert it into toxins which is the main cause of spoilage and food poisoning. According to the TCVN 4884-1: 2015 (4833-1:2013), the total aerobic bacteria count must be lower 100 CFU/ml as the acceptance level. If the total aerobic bacteria count is higher than this number, the product has already been spoiled and is marked as unsafe for commercial consumption [21].

Table 3. *pH value of GBRM preserved at different temperature for 7 days*

Storage time (day)	Storage temperature		
	5°C	10°C	25°C
0	$6.62±0^{a}$	$6.62±0^{a}$	$6.62±0^{a}$
1	$6.43±0.06^{ab}$	$6.53±0.06^{a}$	$6.32±0.10^{ab}$
2	$6.24±0.12^{abc}$	$6.27±0.09^{abc}$	$6.02±0.19^{cd}$
3	$6.05±0.19^{abcd}$	$6.20±0.04^{abc}$	$6.01±0.04^{cd}$
4	$5.86±0.26^{bcde}$	$6.15±0.10^{bc}$	NA
5	$5.67±0.31^{cde}$	$6.03±0.02^{bcd}$	NA
6	$5.50±0.41^{de}$	NA	NA
7	$5,29±0.44^{e}$	NA	NA

Different superscript letters show significant differences among the means ($p < 0.05$).

NA: Not applicable

The results in Table 3 exhibit that the overall trend of pH value of the germinated brown rice milk shows a decrease as the time of preservation goes on. While storage at 5°C, the pH value dropped from 6.62 to 5.29 in 7 days of preservation while the storage of GBRM at 10°C had a minor drop from 6.62 to 6.03 and the same minor decrease for preserving the GBRM at room temperature 25°C from 6.62 to 6.01 in 3 days of preserving. The reason for the preservation at temperature higher than 5°C end earlier is due to the growth of microorganisms being keep above their inhibition temperature. At 5°C of preservation, most harmful microorganisms are inhibited and some growth at a slow rate [31]. Through this parameter, the GBRM can be ensured to reach the food safety standard for consumption.

4. Conclusions

In this study, the effects of soaking treatments on the GABA content of germinated rice and the influences of different processing parameters on the quality, stability, pH value, microbial count were investigated. The soaking for 8 hours with ratio of rice to water 1:10 was found as the most suitable for the industrial production of germinated brown rice milk as it extracts the favorable GABA content with minimal time and cost. The addition of thickening mixture of guar gum and pectin resulted in the best GABA preservation while mixed xanthan gum and guar gum formulation resulted in the best stability and viscosity of the GBRM. The results suggest that those technological parameters can be applied for the formulation and production of GBRM with minimal loss of GABA while still retain the stability of the GBRM. The storage study of GBRM suggests that most suitable temperature to ensure the preserving quality for GBRM is 5°C as this caused minimal GABA loss and ensured the safety for consumption of GBRM. However, as this study only focuses on the effects of thickening formulation, treatment temperature and storage at 5 to 15ºC for 7 days, further researches on using other food additives or low temperature pasteurization and the extensive storage studies are recommended to minimize the loss of GABA in the GBRM.

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