

The properties of GABA (Gamma-probioticin nutritional drinks

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ABSTRACT

The quantities of γ -aminobutyric acids (GABA) and total sugar of the germinated brown rice (GBR) and germinated brown rice drinks supplemented with probiotic and oligofructose (GBRD-PO) were measured by high-performance liquid chromatography. The physicochemical properties (i.e., total soluble solids, total solids, moisture, pH, total acidity, and apparent viscosity) and sensory properties of the GBRD-PO were investigated during 21-days storage. The survival of probiotics was counted. The results showed that the quantities of GABA, total sugar, glucose, and maltose depended on the types and cultivars of the germinated rice. The quantities of GABA and total solids increased but the sucrose and pH decreased in the GBRD-PO during chilled storage. The survival of probiotics averaged 8.26 log CFU/ml throughout the storage. The sensory properties of GBRD-PO were in an acceptable range. This research is a prototype for the development of GABA quantities in the fermented rice drinks supplemented with synbiotics for health.

Keywords: γ -aminobutyric acid, germinated brown rice, rice drink, probiotic, oligofructose

INTRODUCTION

Germinated brown rice (GBR) is an innovative food. Its major bioactive compounds increase during the germination process. GBR contains levels of high vitamins, minerals, fiber, antioxidants, polyphenols, inositols, ferulic acids, phytic acids, γ -oryzanol, prolylendopeptidase inhibitors, and γ -aminobutyric acids (GABA) (Cho & Lim, 2016; Kayahara, Tsukahara, & Tatal, 2001; Wu, Yang, Toure, Jin, & Xu, 2013). GABA is a four-carbon non-protein amino acid. It is a significant component in most microorganisms, plants, and vertebrates. The biosynthesis pathway of GABA is accomplished by GABA shunt. In GABA synthesis, L- glutamate is catalyzed to form GABA by glutamate decarboxylase (GAD). The quantities of GABA were dependent on L-glutamate concentration and GAD activities (Rashmi, Zanan, John, Khandagale, & Nadaf, 2018; Shelp, Bown & McLean, 1999). It has been shown that environmental stresses promoted the accumulation of GABA

in plant tissues (Bown & Shelp, 2016). In mammals, GABA is a major neurotransmitter in the central nervous system (Bouche, Lacombe, & Fromm, 2003; Olsen, 2002). It has multiple biological functions which help to prevent Huntington's chorea, Parkinson's disease, Alzheimer's disease, anxiety, depression, panic, and insomnia (Gajcy, Lochyński, & Librowski, 2010). Therefore, the germinated rice can be used as a bio-active composition in functional foods, such as fresh germinated colored rice noodle, germinated red rice yoghurt, and GBR bread (Anawachkul & Jiamyangyuen, 2009; Cornejo, Caceres, Martínez-Villaluenga, Rosell, & Frias, 2015; Ohtsubo, Suzuki, Yasui & Kasumi, 2005; Thao, 2018). Several researchers have studied the enhancing GABA, ferulic acid, γ -oryzanol, tocopherols, phenolic compounds, anthocyanins, and antioxidant capacities of germinated rice and GBR. (Banchuen, Thammarutwasik, Ooraikul, Wuttijumngong & Sirivongpaisal, 2010; Chaiyasut et al., 2017; Lin, Pao, Wu & Chang, 2015; Ng, Huag, Chen & Su, 2013; Singh, Simapisan, Decharatanangkoon & Utama-ang, 2017).

Few researchers studied GBR beverages mixed with chlorophylls and GBR beverages mixed with cereals (Assumption University, 2015; Division of Rice Research and Development, 2016). Recently, GBR products such as rice balls, rice-burger, soup, baked goods, wines, meat, vegetables, legumes, beverages, and dairy products are widely enjoyed by consumers (Diana, Quílez, & Rafecas, 2014; Patil & Khan, 2011). However, there has been no research on

the development of the germinated brown rice drinks supplemented with probiotic and oligofructose (GBRD-PO) and its properties. This product does not contain the cow's milk which is suitable for the lactose intolerant. This plant drink can be easily absorbed by the body.

A “probiotic” is a food supplemented with viable bacteria. It has several benefits for the body, adjusting the balance of colon microorganisms, modulating immunological response, and protection from diseases, such as antibiotic-associated diarrhea, and colon cancer. Furthermore, probiotics prevent infections of intestinal pathogens by creating antimicrobial agents and improving host health (de Vrese & Schrezenmeir, 2008; Havenaar & Huis in't Veld, 1992; Markowiak & Śliżewska, 2017; Šušková et al., 2010). Moreover, oligofructose is a non-digestible sugar which can be fermented by the intestinal bacteria. Therefore, it is considered a dietary fiber for the body. It helps to improve the ecology of microorganisms in the gut (de Vrese & Schrezenmeir, 2008; Ziemer & Gibson, 1998). In this research, oligofructose was used as a prebiotic together with probiotics in the GBRD considered as a synbiotic drink. Synbiotics have been demonstrated to alter, modify, and reinstate the pre-existing intestinal microflora during the passage through the upper intestinal tract and it promote increasing survival, stimulate growth, and activate probiotic metabolism (Pandey, Naik, & Vakil, 2015).

The objectives of this research were to study the quantities of GABA and total sugar of the germinated paddy rice (GPR), GBR, and GBRD-PO and the physicochemical, biological and sensory properties of GBRD-PO during chilled storage.

MATERIALS AND METHODS

Materials

Thai Hom Mali (HM) brown rice (white), cleaved soybean, white sesame, and pure refined sugar were purchased from a supermarket in Bangkok, Thailand. Hom Lanna (HL) paddy rice and HL brown rice (purple) were purchased from the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Thailand. Oligofructose (Orafti P95, Tienen, Belgium) with a degree of polymerization of 2-8 was received from the DPO (Thailand) Co., Ltd. *Lactobacillus rhamnosus* TISTR 047 (TISTR 047) was purchased from the Department of Bio-Science, Thailand Institute of Scientific and Technological Research.

Preparation of starter

TISTR 047 was inoculated in a sterilized flask of de Man, Rogosa and Sharpe (MRS) broth and incubated (Memmert UN 110, Germany) at 37 °C for 24 hours. The standard curve was plotted for the probiotic numbers and the optical densities at 600 nm using UV/VIS spectrophotometer (Biochrom Libra S22, EU). The starter probiotics were prepared by incubating TISTR 047 (37 °C, 18 hours); the numbers of starter bacteria were assayed by comparing with the standard curve for preparing fresh culture. They were then centrifuged (Digicen 21 R, Spain) at 3,000xg (4 °C, 5 minutes), washed with 0.85% sterilized saline solution, and adjusted at 8 log CFU/ml into the drinks (Srisuvor, Chinprahast, Prakitchaiwattana & Subhimaros, 2013).

Preparation of GBRD-PO

HL paddy rice, and HM brown rice, HL brown rice were washed and soaked in water using a rice-to-water ratio of 1:3 (w/v) (paddy rice, 24 hours; brown rice, 4 hours). HM and HL brown rice were incubated for 6-8 hours. They were washed again, then incubated (paddy rice, 48 hours; brown rice, 14 hours) until sprouting 0.5-1.0 mm length. The soybeans were soaked in water for 6-8 hours and washed with water. The sesame seeds were roasted until brown and fragrant. All ingredients (Table 1) were blended with hot water in a high-speed blender (5 minutes). The mixture was boiled (70 °C, 10 minutes), filtered, and pasteurized (70 °C, 5 minutes). When the mixture cooled to 37 °C, it was inoculated with 8 log CFU/ml of TISTR 047 starter (Srisuvor et al., 2013). The GBRD-PO was filled into 100 ml of polypropylene bottles; then refrigerated (4 °C, 21 days).

Table 1. Ingredients of the GBRD-PO

Ingredients	Quantities (%)
GBR-HM	4.29
GBR-HL	1.84
Soybean	1.23
Sesame	1.23
Sugar	2.52
Oligofructose	3.00
Water	85.89
Total	100.00

From: Division of Rice Research and Development (2016); Srisuvor (2019)

Analysis of physicochemical properties

The quantities of GABA and different types of sugar, i.e., total sugar, fructose, glucose, sucrose, maltose, and lactose in the GPR, GBR and GBRD-PO were analyzed using TAS 4003 and AOAC method 982.14, respectively using high-performance liquid chromatography (Shimadzu LC-6A, Japan) (AOAC, 1995; TAS, 2012a). The quantities of GABA and total sugar in the GBRD-PO were analyzed at 0 and 21 days of storage. The physicochemical properties of the GBRD-PO were examined on 0, 7, 14 and 21 days of storage. The total soluble solids (TSS) were measured by a hand refractometer (Atago 2373 WO5, Japan), the total solid (TS) and moisture were analyzed by using a hot air oven (Memmert, UN 450, Germany), the pH was measured by a pH meter (SCHOTT Lab 850, Germany), the total acidity (TA) was assayed by the titration method (AOAC, 2012), and the apparent viscosity was measured using viscosity meter (Brookfield LVDV-II+P, USA).

Analysis of probiotic survival

The enumerations of probiotic cells were made cell spreading on the MRS agar at 0, 7, 14 and 21 days of storage by plate count technique. Total plates were incubated in an anaerobic jar (Merck) at 37 °C for 72 hours.

Analysis of sensory properties

The GBRD-PO at 0 and 21 days were coded with 3 digit random numbers, served at 10°C, and evaluated by 45 semi-trained panelists (students of Department of Food Technology and Nutrition) for the preference test in the appearance, color, odor, flavor, taste, texture, and overall satisfaction. The sensory evaluations used preference scores with 7-point hedonic scale (7- like extremely, 6- like moderately, 5- like slightly, 4- neither like nor dislike, 3- dislike slightly, 2- dislike moderately, and 1- dislike extremely (Larmond, 1982).

Analysis of statistics

The Completely Randomized Design was used for the experimental design of physicochemical and biological properties, and the Randomized Complete Block Design was used for the evaluation of sensory properties. They were carried out over three replications. These results were assayed by the analysis of variance. Means were compared by Duncan' New Multiple Range Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Quantities of the GABA and total sugar

Table 2. Quantities of GABA and types of sugar in the GPR, GBR, and GBRD

Categories	Quantities (mg/100g, WB)						
	GABA	Total sugar	Fructose	Glucose	Sucrose	Maltose	Lactose
GPR-HL	6.96	-	-	-	-	-	-
GBR-HM	0.20	Nd ^a	Nd ^a	Nd ^a	Nd ^a	Nd ^a	Nd ^a
GBR-HL	8.91	2,050	Nd ^b	650	Nd ^b	1,400	Nd ^b
GBRD-PO	0.27	4,090	Nd ^b	Nd ^b	3,060	1,030	Nd ^b

PONd^a<100 mg/100g, Nd^b<600 mg/100g

Table 2 shows the quantities of GABA and different types of sugar in the germinated paddy rice of HL variety (GPR-HL), germinated brown rice of HM variety (GBR-HM), germinated brown rice of HL variety (GBR-HL), and GBRD-PO. The quantities of GABA in the GPR-HL and GBR-HL were 6.95 and 8.91 mg/100g WB, or 9.21 and 9.57 mg/100g, DB, respectively (data not shown). These results correlate with Anawachkul and Jiamyangyuen's (2009) assertion that the GABA quantities of the GPR and germinated red rice were 1.5-13.1 and 2.6-21.3 mg/100g, DB, respectively at different germination time. The results showed that the germinated rice had a higher GABA quantities than the GPR due to the soaking of the rice germ in water. It meant that the germination ratio of rice was higher than the paddy rice due to water absorption and hydrolytic enzyme activity. The quantities of GABA increased as the time of germination was prolonged. Karladee and Suriyong (2012) reported that the quantities of GABA during incubation at 0 and 24 hours were 3.96-17.87 mg/100g, DB. At 24 hours, Khao Dawh Mali (white) and Kum Doi Saket (purple) rice displayed the highest GABA quantity (23 mg/100g, DB) of all 21 varieties. Tantakul (2009) showed that the GABA quantities of low-amylose rice (31-37 mg/100g) were higher than high-amylose rice (21-29 mg/100g), and the GABA quantities of glutinous rice (29-72 mg/100g) were higher than low-amylose rice. The conclusion was that the quantities of GABA depended on the types and cultivars of the germinated rice. This correlates with Chua, Koh, & Liu's (2019) assertion that the factors of high GABA quantities were the rice cultivars, steeping conditions, provided nutrients, germination duration, and novel techniques.

In this study, the GABA quantity in the GBR-HM was low since the rate of germination was less than 70% and it was

polished for more than 2 weeks (TAS, 2012b). Later results showed that the GABA quantity in the GBRD-PO was 0.27 mg/100g WB, because this drink contained GBR-HL at only 1.84% of total ingredients. Besides, the pasteurization of GBRD-PO may cause a decrease of GABA quantities from the initial GBR-HL. This result was consistent with the decrease of GABA quantities in all the cooking processes of germinated grains and yoghurt - containing paste of germinated red rice including canned GBR (Anawachkul & Jiamyangyuen, 2009; Jongyingcharoen & Cheevitsopon, 2016; Tiansawang, Luangpituksa, Varayanond, & Hansawasdi, 2016). Therefore, the pasteurization of the GBRD-PO should use temperatures at ≤ 100 °C for 1020 minutes because the high temperature could inactivate GAD and protease inhibiting the raise of GABA (Parnsakhorn & Langkapin, 2018). Contrarily in GBR-HM, the quantity of GABA increased slightly when it was produced as GBRD-PO. The result may be due to the production of GABA through multiple GABA synthetic pathways. GABA may be synthesized by chemical synthesis from various bio-based or biological synthesis from microorganisms (Koubaa, Delbecq, Roohinejad & Mallikarjunan, 2019; Rashmi et al., 2018;). Therefore, increasing the quantities of GABA in the pasteurized drink, fermented food, or commercial food could be obtained by supplying probiotics with high GABA- producing ability (Anawachkul & Jiamyangyuen, 2009). Further study should investigate the steeping conditions, germination duration, and provided nutrients of rice to produce high GABA before the probiotic fermentation. It has been suggested that the amount of GABA used to decrease anxiety should be 300-600 mg/day (Thorne Research, Inc., 2007).

The total sugar, glucose, and maltose of the GBR- HL were found to be in reasonable quantities. The fructose, sucrose, and lactose of the GBR were undetected since they are usually found in fruits or milk rather than rice. However, the total sugar and sucrose were high in GBRD-PO because sucrose creates sweetness and is a carbon source for the probiotics. Lastly, the quantities of glucose and maltose slightly dropped in the GBRD-PO because the monosaccharide and disaccharide could be fermented by probiotics.

Physicochemical properties of the GBRD-PO

Figure 1 and 2 show the physicochemical properties of the GBRD-PO during chilled storage. The drinks showed no significant difference in TSS, apparent viscosity, moisture, or TA. The 7-21 day samples had more TS than on the production day. This may be due to the fact that the rice starch was hydrolyzed by β -amylase on α -1, 4 glycosidic linkages at the non-reducing ends to produce maltose (Evans, Li & Eglinton, 2009). This result correlates with the increased quantity of maltose on day 21 (Table 3) and it is associated with maintaining probiotics survival (Figure 3) by using sugar as a carbon source. The pH of the 0-day storage was higher than the 14-21 days since the probiotics fermented some glucose and sucrose in the drinks to produce lactic acids, decreasing the pH (Bonestroo, Kusters, de Wit & Rombouts, 1992; Costa, Fonteles, de Jesus & Rodrigues, 2013).

The effects of chilled storage of the GBRD-PO on the quantities of GABA and total sugar are presented in Table3. The quantity of GABA on day 21 increased about 2 times from the production day. This may be due to the fact that GABA is synthesized via GABA shunt, and the L-glutamate is catalyzed by GAD to form GABA (Rashmi et al., 2018; Shelp et al., 1999). Also, this drink was produced by supplementing TISTR 047, which could convert the L-glutamate to form GABA by GAD during incubation. Enhancing GABA production of the probiotics depends on the probiotic species, co-incubation strains, culture medium, L-glutamate concentration, GAD activity, pH, temperature, and time (Kim, Lee, Ji, Lee & Hwang, 2009; Lee, Shim, Yao, Kim & Kim, 2018; Li, Qiu, Huang & Cao, 2010; Shan et al., 2015). The quantities of sucrose decreased slightly by day 21 since it was used for the metabolism and activities of the probiotics (Costa et al., 2013). However, the maltose quantities of the GBRD- PO increased slightly by day 21. This may be due to the fact that β -amylase converted the starch in the drink into maltose (Cho & Lim, 2016).

Probiotic survival in the GBRD-PO

Figure 3 displays the survival of TISTR 047 in the GBRD-PO during 0-21 days. They averaged 8.26 log CFU/ml ($p > 0.05$). They can survive throughout the chilled storage. Probiotics live in the gut of the mammal host (de Vrese & Schrezenmeir, 2008). Their survival gradually reduced during chilled storage especially in non-dairy foods (Costa et al., 2013; Nematollahi, Sohrabvandi, Mortazavian & Jazaeri, 2016). However, combining the appropriate probiotics and prebiotics could improve the survival of probiotics in fermented products (Kailasapathy & Chin, 2000). Pimentel, Madrona, Garcia & Prudencio (2015) reported that oligofructose helped the survival of probiotics by preventing injuries from the environment and acidity during juice storage. Furthermore, Acevedo-Martínez, Gutiérrez-Cortés, García-Mahecha & Díaz-Moreno (2018) suggested that the 5% oligofructose could be used to protect cells from acidity in mango drink at 4 °C by providing a substrate for metabolism and it would help probiotics to survive in the gastrointestinal tract. There are several factors involved in enriching probiotic viability in food products, such as probiotic selection, inoculum level, microbial interaction, strain adaptation, food matrix, probiotic encapsulation, synbiotic combination, product formulation, processing conditions, packaging system, and storage temperatures (Karimi, Mortazavian, & Da Cruz, 2011; Terpou et al., 2019). In conclusion, these fermentation conditions are suitable for the survival of TISTR 047 at >8 log CFU/ml with no loss of viability during chilled storage at 4C.

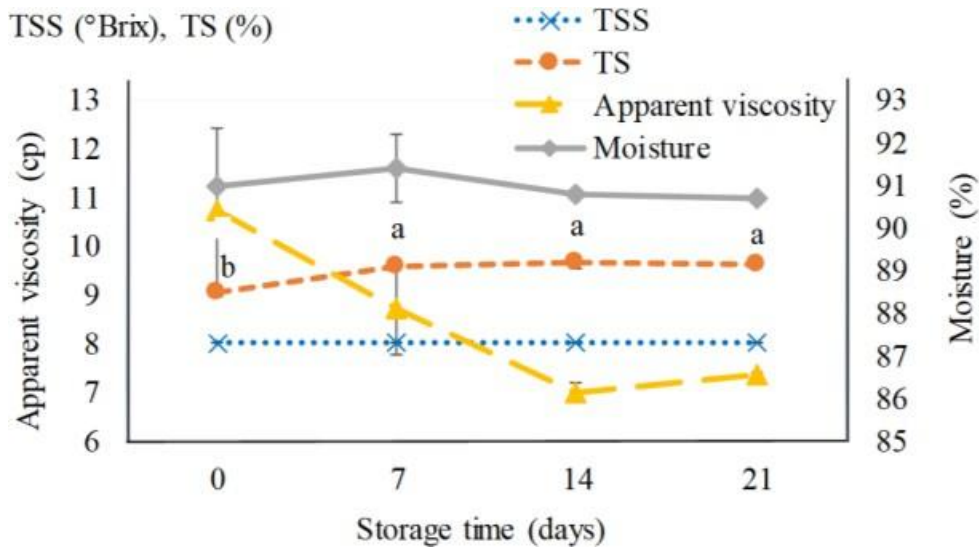


Figure 1. Total soluble solids (TSS), total solids (TS), apparent viscosity, and moisture levels of the GBRD-PO during chilled storage

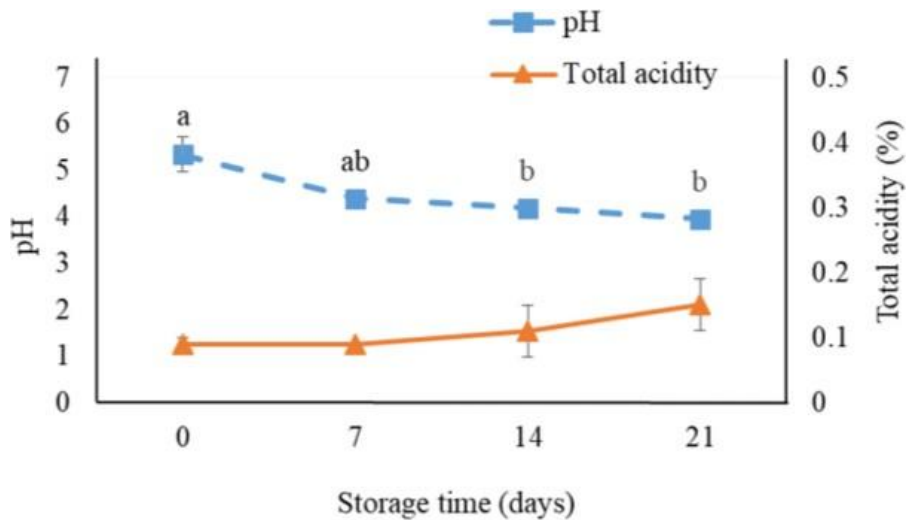


Figure 2. The pH and total acidity of the GBRD-PO during chilled storage

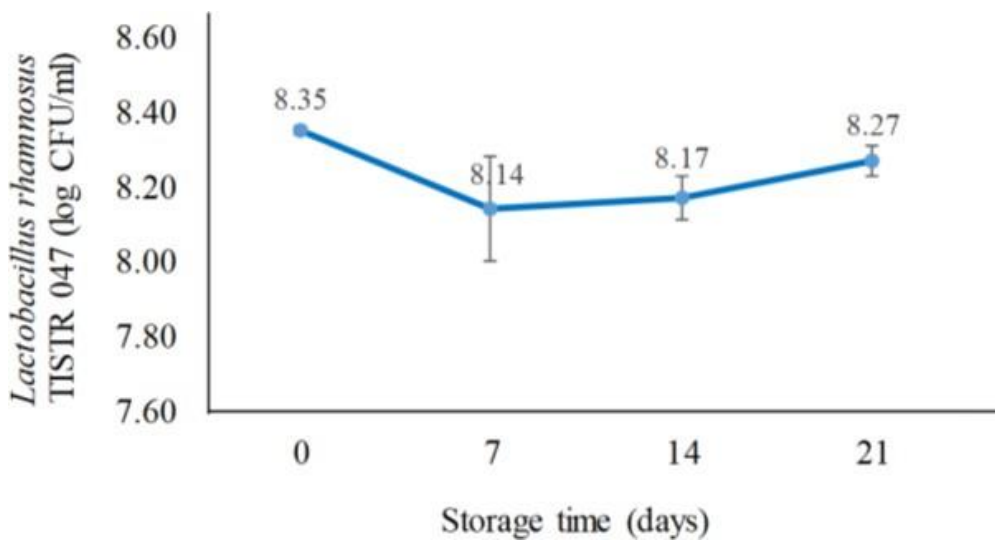


Figure 3. Survival of probiotics in the GBRD-PO during chilled storage

Sensory properties of the GBRD-PO

Figure 4 shows the sensory properties of the GBRD- PO during chilled storage. The preference scores on day 0 were all higher than on day 21, except for color. On the 21st day, the GBRD-PO had an increased sour odor and fermented rice taste causing a decrease in overall liking scores. These results correspond with the decrease of sucrose and pH (Table 3 and Figure 2). Supplementing probiotic and oligofructose did not influence the color of the GBRD-PO during chilled storage. The overall liking scores were in an acceptable range. However, this GBRD-PO needs to improve the sensory properties due to the fermented rice's sour odor and taste. The selection of probiotic species to produce less acid and better flavor is a way to improve the sensory properties of the product.

Table 3. Quantities of GABA and types of sugar in the GBRD-PO during chilled storage

Storage (days)	Quantities (mg/100g, WB)			
	GABA	Total sugar ns	Sucrose	Maltose
0	0.11 ± 0.00 ^b	4,910 ± 0.14	3,860 ± 0.12 ^a	1,050 ± 0.23 ^b
21	0.23 ± 0.00 ^a	4,485 ± 0.13	2,840 ± 0.13 ^b	1,645 ± 0.01 ^a

Fructose, Glucose, and Lactose < 100mg/100g

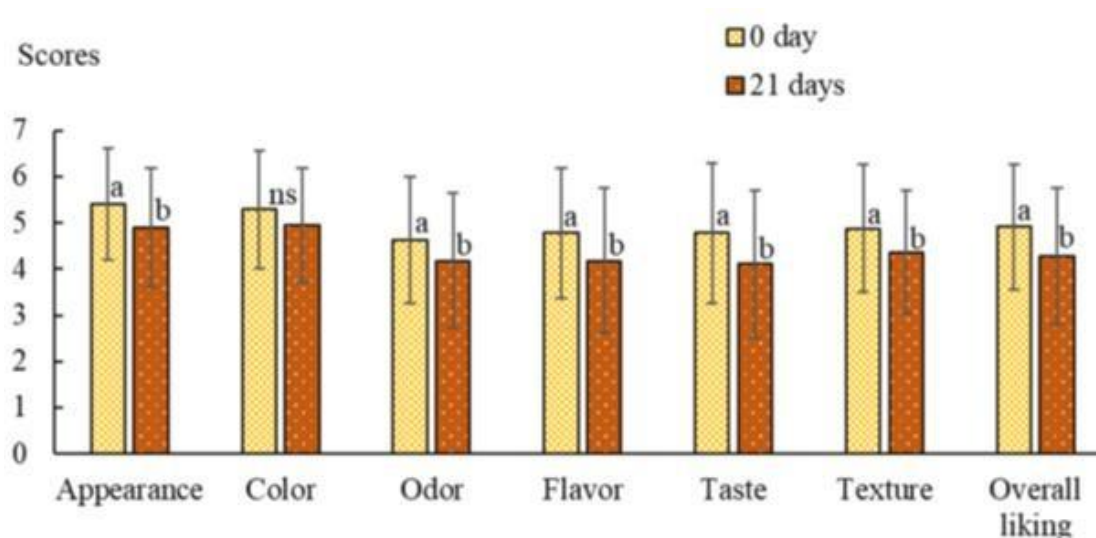


Figure 4. Sensory properties of the GBRD-PO during chilled storage

CONCLUSIONS

The quantities of GABA and total sugar in the GBR depend on the types and cultivars of rice. The GBRD-PO had an increase of GABA and TS quantities but a decrease of the sucrose and pH levels during chilled storage. The survival of probiotics was potentially suitable to be used in functional drinks (>8 log CFU/ml). It is an enriched drink from GBR which contains GABA as a synbiotic drink. Further studies are required to improve the GABA quantities and sensory properties of the GBRD-PO to be accepted as synbiotic fermented rice drink for health.

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