

Survival of Probiotic and Antioxidant Activity on Health Beverage from Fermented Purple Rice Supplemented with Probiotic

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Abstract—This study reported the survival of probiotic (*L. acidophilus* FBRL-B07) and antioxidant activity in health beverage from fermented purple rice supplemented with probiotic (HBFP). Centrifugation was designed to separate heavy rice granules from the beverage. HBFP made with different time of centrifuge and stored at refrigerator for up to thirty five days. As centrifuge time increased, antioxidant activity decreased but it had no effect on survival of probiotic. As storage time increased, *L. acidophilus* FBRL-B07 number decreased but antioxidant activity remained unchanged during storage. The results suggested that five minutes centrifugation could be used to obtain the highest antioxidant activity in HBFP. The product could be stored at refrigerator for more than thirty five days and still had more than 6 log cfu/ml of lived probiotic.

Keywords—fermented purple rice, survival, protic, health beverage, antioxidant activity

I. INTRODUCTION

Products supplemented with probiotics including *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium* are claimed as nutraceutical products. Most of these products are made from milks of various animals. Milks contain lactose and lactalbumin to which many adults, especially in Asians, are allergic. There have been efforts to manufacture probiotic products without milks. Purple rice contains many rich nutrients such as proteins, lipid, carbohydrates, fiber, minerals and vitamins as well as some bioactive compounds and antioxidants, especially anthocyanins, the pigment in purple rice [1]. Bioactive compounds such as γ - oryzanol, tocopherols, tocotrienols, phytosterols and phytic acid possess important role to enhance immune system and prevent diseases [2]. It has been reported to reduce cholesterol, triglyceride, low density lipoprotein cholesterol (LDL-C) [3] and increase high density lipoprotein cholesterol (HDL-C) in blood. Purple rice grains are in red or dark purple colors. The anthocyanins mainly founded in aleurone layer [4] and pericarp which are parts of rice bran [5]. Anthocyanins however are unstable in food products [6]. When added to food products, it will contribute to enhanced color as well as improve the product functionality. The aim of this research was to study survival of probiotic culture and antioxidant activity of fermented purple rice supplemented with probiotic (HBFP).

II. MATERIALS AND METHODS

A. Sample preparation

Rough purple rice of local cultivar rice samples (*Oryza sativa* L.) was obtained from Loei province, Thailand. Rough purple rice was dehusked by a custom made dehusker to obtain brown rice. Broken kernels were removed.

B. Bacterial strain and culture preparation

Freeze-dried of *Lactobacillus acidophilus* FBRL-B07 was activated in MRS (Man Rogosa Sharp) broth at 37°C for 48 h [7]. Single colonies were selected and grown on MRS agar at 37°C for 48 h. Sterilize peptone water was then used to dilute and obtain optical density (OD) of 1.8-1.9 at 600 nm.

C. Source of loog-pang-kao-mag

Samples of loog-pang-kao-mag (mixed and unidentified starter culture) were collected from Mahasarakham province's food market, Thailand. They were stored at room temperature until use.

D. Preparation of health beverage from fermented purple rice supplemented with probiotic (HBFP)

Purple rice was washed and soaked overnight at room temperature. It then was steam cooked and washed again with boiled water. A 0.3% w/w starter culture was added to cooked and dry purple rice. It was incubated at 37°C for 3 days. The purple rice was blended and filtrated with a cotton sheet. The filtrate was centrifuged at 5,000 rpm 0, 5, 10, 15 and 20 min and then added with 30% sucrose syrup in a ratio of 1:1. HBFP were stored in a refrigerator until test.

E. Determination of probiotic survival

Survival of *L. acidophilus* was determined by spread plate technique. HBFP samples were plated onto MRS agar and incubated at 37°C for 48 h. Survival study was performed every 7 days for 35 days.

F. Determination of antioxidant activity

The antioxidant activity of HBFP on the α , α - diphenyl- β -picrylhydrazyl (DPPH) radicals was estimated according to the method of [8]. A 3 ml of HBFP was mixed with 0.1 ml solution of DPPH radical in methanol. The

mixture was shaken vigorously and left standing in overshadow for 30 min, and the absorbance of the mixture at 517 nm [9] was measured.

G. Statistical analysis

Two-way analysis of variance (ANOVA) and Scheffe's multiple comparison test were performed to test the statistical differences in survival of probiotic and antioxidant activity using SPSS software (version 11.5). The significance level was $p < 0.05$. All tests and analyses were performed in triplicate.

III. RESULTS AND DISCUSSION

A. Survival of probiotic in HBFP

Appearance of HBFP was shown in Figure I. HBFP with 5 to 20 min centrifugation had similar appearance with significantly less rice granules on the bottom. Survival of probiotic in HBFP (Table I) was not affected by different centrifuge time (0, 5, 10, 15 and 20 mins). Centrifugation did not separate important nutrients for *L. acidophilus* FBRL-B07 from HBFP. However, storage time decreased ($p < 0.05$) survival of probiotic. Viable counts were high during 0-14 days and began to drop after on day 21. This may be due to nutrients in HBFP that could still support the growth of *L. acidophilus* FBRL-B07 [10]. These results were similar to

those reported by Vinderola and coworkers [11]. After 35 days of storage, HBFP still contained more than 6 log cfu/ml and thus believed to provide beneficial effects of probiotic.

B. Antioxidant Activity

The decrease in the absorbance of the DPPH radical caused by antioxidants is due to the scavenging of the radicals by hydrogen donation; this is a visible change from purple to yellow [12]. The antioxidant activity effects of HBFP on DPPH radical were shown in Table II. Storage time did not significantly affect the antioxidant activity of HBFP. As centrifugation time increased, the antioxidant activity of HBFP decreased. These results were similar to those reported by Yen and coworkers [13]. This suggested that centrifugation had separated some high molecular weight antioxidant compounds such as phenolic compounds from the HBFP. Phenolic compounds were found in the bran layer of rice [14]. The activity of antioxidants corresponds to the number of hydrogens available for donation by hydroxyl groups [15]. In general, the polyphenol concentrations were positively correlated with antioxidant activity due to their hydrogen-donating abilities. Near linear correlations between DPPH radical scavenging activities and polyphenolic compound concentrations in various fruits and vegetables have been reported [16].

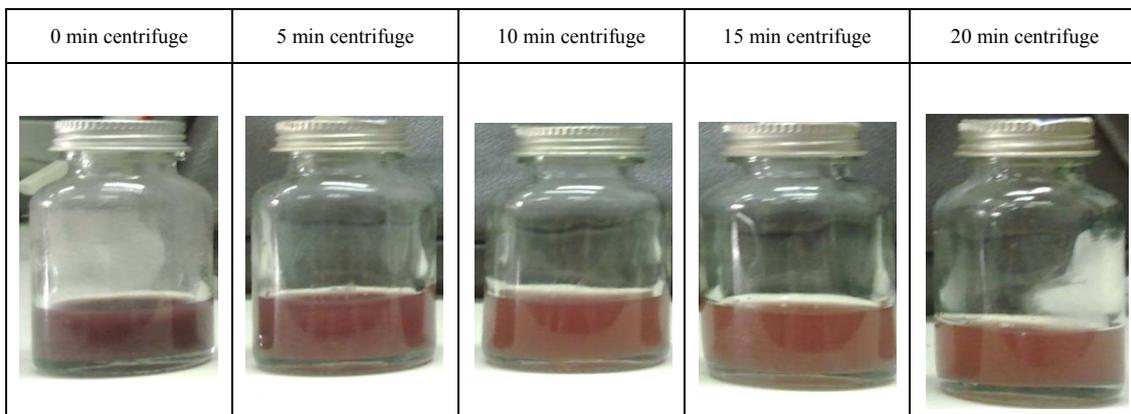


Figure 1. Photographs Of Fermented Purple Rice Supplemented With Probiotic

TABLE I. SURVIVAL OF PROBIOTIC IN HEALTH BEVERAGE FROM FERMENTED PURPLE RICE SUPPLEMENTED WITH PROBIOTIC (HBFP)

Storage time at refrigerator (days)	Survival of probiotic (log cfu/ml)					Average
	Time of centrifuge (min)					
	0	5	10	15	20	
0	8.73 ± 2.24	8.69 ± 1.93	8.65 ± 1.98	8.58 ± 2.89	8.51 ± 2.06	8.63 ± 0.68 ^{ab}
7	9.43 ± 2.78	9.30 ± 1.11	9.32 ± 3.25	9.20 ± 3.84	8.99 ± 2.44	9.25 ± 0.55 ^a
14	9.15 ± 1.76	9.26 ± 0.86	8.76 ± 2.73	8.86 ± 2.99	8.38 ± 1.02	8.65 ± 0.86 ^a
21	7.97 ± 1.33	7.91 ± 2.08	7.81 ± 1.96	7.80 ± 1.86	7.77 ± 1.06	7.85 ± 0.38 ^{bc}
28	7.72 ± 1.24	7.63 ± 2.07	7.54 ± 2.54	7.71 ± 3.56	7.68 ± 2.77	7.66 ± 0.53 ^c
35	6.36 ± 0.75	6.75 ± 3.04	6.67 ± 3.00	6.46 ± 1.39	6.34 ± 3.39	6.51 ± 0.70 ^d

Values are averages of triplicate determinations. Values with different letters in the column average significantly differ ($p < 0.05$)

TABLE II. ANTIOXIDANT ACTIVITY FROM HEALTH BEVERAGE FROM FERMENTED PURPLE RICE SUPPLEMENTED WITH PROBIOTIC (HBFP) ON A, A-DIPHENYL-B-PICRYLHYDRAZYL (DPPH) RADICAL

Storage time at refrigerator (days)	% Inhibition				
	Time of centrifuge (min)				
	0	5	10	15	20
0	70.69 ± 0.69	60.74 ± 0.71	41.87 ± 0.78	27.10 ± 0.78	18.22 ± 0.67
7	71.95 ± 0.88	60.65 ± 0.46	42.11 ± 0.38	27.62 ± 1.28	18.40 ± 0.24
14	73.35 ± 0.32	62.73 ± 0.12	43.30 ± 0.54	28.35 ± 0.63	18.88 ± 0.52
21	69.51 ± 0.32	59.59 ± 0.46	41.58 ± 0.53	26.32 ± 0.93	17.10 ± 0.67
28	71.40 ± 0.21	61.17 ± 0.26	42.18 ± 0.31	27.36 ± 0.73	17.86 ± 0.26
35	70.03 ± 0.16	60.19 ± 0.29	41.38 ± 0.21	26.04 ± 0.30	16.13 ± 0.81
Average	71.15 ± 1.38 ^a	60.84 ± 1.07 ^b	42.07 ± 0.76 ^c	27.13 ± 1.06 ^d	17.76 ± 1.05 ^e

Values are averages of triplicate determinations. Values with different letters in the row average significantly differ ($p < 0.05$).

IV. CONCLUSIONS

Centrifugation seemed to lower antioxidant activity of HBFP but did not affect survival of *L. acidophilus* FBRL-B07. Storage decreased survival of *L. acidophilus* FBRL-B07 but did not adversely affect the antioxidant activities. Future studies should to be carried out to study other parameters affecting HBFP quality. 5 min centrifugation should be used to obtain the highest antioxidant activity in HBFP. After 35 day of storage at refrigerator (7±1°C), HBFP still had more than 6 log cfu/ml of lived probiotic.

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REFERENCES

- [1] C. G. Vinderola, N. Bailo, and J.A. Reinheimer, "Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage," *Food Research International*, vol. 33, pp. 97-102, 2000.
- [2] A. Chiang, H. Wu, H. Yeh, C. Chu, H. Lin, and W. Lee, "Antioxidant effects of black rice extract through the induction of Superoxide dismutase and Catalase activity," *Food Chemistry*, vol. 41, pp. 797-803, 2006.
- [3] X. Xia, W. Ling, J. Ma, M. Xia, M. Hou, Q. Wang, and H. Zhu, "An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in Apolipoprotein E- deficient mice," *Journal of Nutrition*, vol. 136, pp. 2220-2225, 2006.
- [4] H. Guo, W. Ling, Q. Wang, Y. Hu, M. Xia, X. Feng, and X. Xia, "Effect of Anthocyanin-Rich Extract from Black Rice (*Oryza sativa* L. indica) on Hyperlipidemia and Insulin Resistance in Fructose-Fed Rats," *Plant Foods for Human Nutrition*, vol. 62, pp. 1-6, 2007.
- [5] P. Boonsit, D. Karladee, and P. Pongpiachan, "Gamma oryzanol content in purple rice Thailand local genotype," *Agricultural Science*, vol. 337, pp. 191-194, 2006.
- [6] R. Yawadio, and N. Morita, "Color enhancing effect of carboxylic acids on anthocyanins," *Food Chemistry*, vol. 105, pp. 421-427, 2007.

- [7] S. Mandal, A.K. Puniya, and K. Singh, "Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298," vol. 16, pp. 1190-1195, 2006.
- [8] F. Que, L. C. Mao, and C. G. Zhu, "Antioxidant properties of Chinese yellow wine, its concentrate and volatiles," *LWT-Food Science and Technology*, vol. 39, pp. 111-117, 2006.
- [9] X. Xia, W. Ling, J. Ma, M. Xia, M. Hou, Q. Wang, and H. Zhu, "An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in Apolipoprotein E- deficient mice," *Journal of Nutrition*, vol. 136, pp. 2220-2225, 2006.
- [10] J.H. Lee, S.K. Lee, K.H. Park, I.K. Hwang, and G.E. Ji, "Fermentation of rice using amyolytic *Bifidobacterium*," *International Journal of Food Microbiology*, vol. 50, p.p. 155-161, 1999
- [11] C. G. Vinderola, N. Bailo, and J.A. Reinheimer, "Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage," *Food Research International*, vol. 33, pp. 97-102, 2000.
- [12] Y.R. Lee, K.S. Woo, K.J. Kim, J. Som, and H. Jeong, "Antioxidant Activities of Ethanol Extracts from Germinated Specialty Rough Rice," *Food Science and Biotechnology*, vol. 16, p.p. 765-770, 2007.
- [13] G. Yen, Y. Chang, S. Su, "Antioxidant activity and active compounds of rice koji fermented with *Aspergillus candidus*," *Food Chemistry*, vol. 83, pp. 49-54, 2003.
- [14] A. Moongngarm, and N. Saetung, "Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice," *Food Chemistry*, vol. 122, p.p. 782-788, 2010.
- [15] K. Shimada, K. Fujikawa, K. Yahara, and T. Nakamura, "Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion," *Journal of Agricultural and Food Chemistry*, vol. 40, pp. 945-948, 1992.
- [16] C. Isabel, PB. Ferreira, V. Miguel, and B. Lillian, "Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stripe activity," *Food Chemistry*, vol. 100, pp. 1511-1516, 2007