***Interprof. J. Health Sci.* 2019,*17(1)*: *09-14*.**
ISSN (online) 2672-9423 ISSN (print) 2672-9628

SCREENING OF GAMMA-ORYANOL CONTENT FROM THAI RICE BRAN WATER EXTRACTS FOR DEVELOPMENT OF A COLD DRINK PRODUCT

Pattamaporn Jaroennon*, Yaiprae Chatree and Sakunta Manakla

Department of Nutrition and Dietetics, Faculty of Science and Technology,
Valaya Alongkorn Rajabhat University under the Royal Patronage

*Corresponding author : E-mail : pattamaporn@vru.ac.th
Received 17 March 2019; Revised 2 June 2019; Accepted 25 June 2019

Abstract: This study aimed to quantify gamma oryzanol in rice bran water extracts and develop a cold drink product containing rice bran with the highest content of gamma oryzanol. Thai rice bran of four varieties including Riceberry, Black glutinous, Mali Nil and Sung Yod were extracted with distilled water. Black glutinous rice was found to have the highest yield of 33 w/w. Water extracts from the four varieties (200 µg/ml) were quantified for gamma oryzanol. Riceberry extract contained the highest amount of gamma oryzanol (3.38 g/100 g dry weight), followed by Black glutinous extract (2.92 g/100 gDW), Mali Nil extract (2.15 g/100 gDW) and Sung Yod extract (1.36 g/100 gDW). Therefore, Riceberry rice bran was selected to be developed into cold drink products. The study on sensory characteristics of 50 subjects using the Hedonic-9 scale test was performed. The study was divided into two phases. In the first preliminary phase, grain recipes were found to show positive feedback, while rice bran formulations were less popular than grain formulations. Hence, the cold drink formulation containing both grain and rice bran was developed in the second phase. The cold drink formulations were prepared using different weight ratios. The result showed that the most preferred score was formula with 2 g of rice bran mixed with 1 g of grain (5.28 ± 1.95).

Keywords: Rice bran, Gamma oryzanol, Cold drinks, Sensory characteristic

INTRODUCTION

The age of rapid information sharing has become a catalyst for increasing health awareness that encourages people to be selective with diet and take better care of their bodies. As the population is more informed about healthier lifestyle, the consumption of supplemental natural products for health benefit has also received an insurgence in popularity. Especially in Thailand which has hot weather resulting in the drinking of a cold drink products is a very popular. One of the natural products that is becoming more abundant in the market is products from rice (*Oryza sativa* Linn.) bran. Rice bran is easily soured as it is the by-product from the milling process of rice paddy (Moongngarm, Daomukda & Khumpika, 2012). More importantly, rice bran is rich in nutrition and antioxidants (Chen, McClung & Bergman, 2017; Sukrasno, Tuty & Fidrianny, 2016). It contains a high level of several phytochemicals including α-tocopherol, tocotrienol and γ-oryzanol (Ghasemzadeh, Karbalaii, Jaafar & Rahmat, 2018). Gramma-oryzanol is a complex mixture of ferulate that is esterified with triterpene alcohol (Imsanguan, Roaysubtawee, Borirak, Ponggamphai & Douglas, 2008). The major components are 24-methylene cycloartanyl ferulate, campestanyl ferulate and cycloarteny ferulte (Lilitchan, Tangprawat, Aryusuk, Krisnangkura, Chokmoh & Krisnangkura, 2008; Trinovita, Sutriyo, Saputri & Munim, 2017). Several studies showed that γ-oryzanol can be used to reduce blood cholesterol levels, treat nerve imbalance, treat

inflammatory, increase HDL cholesterol level, inhibit dietary cholesterol absorption and inhibit platelet aggregation (Jung et al., 2017; Pungseeklao, Opanasopit & Khuwijitjaru, 2016). Although a variety of colors exist in Thai rice such as white, black, purple and red, many studies found that black and purple rice contain higher γ-oryzanol than white rice (Daiponmak, Senakun & Siriamornpun, 2014; Saenjum, Chaiyasut, Chansakaow, Suttajit & Sirithunyalig, 2012). The few well-known purple or red varieties such as Riceberry, Black glutinous, Mali Nil and Sung Yod are popular for consumption, but a study on γ-oryzanol and develop a cold drink are still lacking. Therefore, this study aimed to investigate the γ-orizanol content from rice bran water extract of four varieties and develop a cold drink product from rice bran.

MATERIALS AND METHODS

Screening of gamma-oryzanol content from four varieties of rice bran

Rice materials of four varieties were collected from difference sources, Riceberry (Sisaket province), Black glutinous (Sisaket province), Mali Nil (Surin province) and Sung Yod (Phatthalung province). Rice grains were identified at Pathumthani Rice research center, Pathumthani, Thailand. The rice paddy was milled to obtain rice bran and grain. All rice brans were dried in hot air oven at 60 °C for 48 hour. The dried samples were ground with electronic mill and passed through a sieve. Extraction method was performed by maceration. One gram of each sample was individually extracted for eight hours with 100 mL of distilled water and filtered. The extraction was repeated twice and dried by freeze drying.

The method to determine total γ-oryzanol content was modified by Sing et al. (2015). The calibration curve of γ-oryzanol standard was prepared at concentrations of 5-40 µg/ml in isopropanol. Rice samples were prepared at the concentration of 200 µg/ml in distillated water. The absorbance of the standard and sample were measured at 314 nm using UV-Vis spectrometer. The standard calibration curve was $y = 0.0235x + 0.0057$ ($R^2 = 0.999$). The absorbance of samples were calculated from a linear equation of calibration curve. The result were expressed as gram per 100 gram dry weight (g/100 g DW).

Development of cold drink products

The selected rice bran was dried in hot air oven at 60 °C for 12 hour and the grain were heated for 5 min. Rice bran and grain was used in two phases. The first preliminary phase, two formulations were prepared in tea bags which were only rice bran and only grain (Table 1).

All formulations were dipped in 200 ml of boiling water for 5 min. After cooling, all drink products were added with 0.0067 g of sucralose. Before the sensory test, the drink products were refrigerated at 5 °C for 12 h. The secondary phase included three formulations in tea bags which are shown in table 1. All formulations were dipped in 200 ml of boiling water for 5 min. After cooling, all drink products were added with 0.0067 g of sucralose. Before the sensory test, the drink products were refrigerated at 5 °C for 12 h.

Table 1. Formulation of cold drink products from selected rice bran

Phase	Formulation	Rice bran(g)	grain(g)
preliminary	1	3	-
	2	-	3
secondary	1	1	2
	2	1.5	1.5
	3	2	1

The study on sensory characteristics was achieved by performing a sensory test with 50 subjects who were without rice allergy. Products of both the first preliminary phase (2 formulations) and second phase (3 formulations) were given a code of three random numbers. Six sensory characteristics were evaluated by the sample's appearance, color, odor, taste, flavor and overall preference. The 9-tailed test (Hedonic-9 scale test) was used to determine the degree of consumer acceptance for a product, 9 being most preferred to 1 being least preferred
(Dimple Singh-Ackbarali & Rohanie Maharaj, 2014).

Data analysis

Statistical data were analyzed using ANOVA. The data were shown as average and standard deviation ($p \leq 0.05$).

RESULTS AND DISCUSSION

Screening of gamma-oryzanol content from 4 varieties of rice bran

Physical characteristics of the four rice varieties were identified by size, length: width, shape, color and weigh. The vouched specimens were compared with Thai rice quality criteria and the result indicated that all rice were of the Thai rice standard criteria. Percentage of rice extracts were calculated and the result is presented in Table 2.

Gamma oryzanol contents of rice bran extracts ranged from 1.36 – 3.38 g/100 g DW (Table 2). The highest γ-oryzanol content was found in the extract of Riceberry rice bran (3.38 g/100 g DW), followed that of Black glutinous rice bran (2.92 g/100 gDW) and Mali Nil rice bran (2.15 g/100 gDW). The extract of Sung Yod rice bran contained the lowest amount of γ-oryzanol (1.36 g/100 g DW). Based on the results, γ-oryzanol content in purple rice bran was found to be higher than that of red rice bran (Table 2). This study showed that darkened color of rice bran results in a high quantity of γ-oryzanol. The result were supported by Daiponmak et al. (2014) reporting that color of rice effect to active phytochemical compound. Among the four varieties of rice bran extracts, Riceberry showed the highest gamma oryzanol content and therefore was selected to be developed as a cold drink product.

Table 2. Color, percentage yields of extracts and γ-oryzanol contents of different rice varieties

Rice varieties	Color	Percentage of extracts (w/w)	Gamma oryzanol contents (g/100 g DW)
Riceberry	purple	30	3.38
Black glutinous	purple	33	2.92
Mali Nil	purple	20	2.15
Sung Yod	red	28	1.36

Development of cold drink products

Fifty subjects were students of Valaya Alongkorn Rajabhat University under the Royal Patronage who consented to join the test. The subjects for the sensory test in 2 phases were

the same group of 11 males and 39 females. In the first phase, two formulations were developed. Based on the six characteristics of the two formulations, grain was significantly more preferred than rice bran ($p \leq 0.05$) (Table 3). Since rice bran was less popular, the cold drink formulation containing both grain and rice bran was developed in the second phase to appeal to consumers.

Table 3. Preliminary study on sensory characteristics of cold drink products from Riceberry rice variety

characteristics	Formulation of cold drink products	
	1	2
	Rice bran (3 g)	grain (3 g)
appearance	4.80 ± 1.65^{b}	5.98 ± 1.44^{a}
color	4.76 ± 1.64^{b}	6.02 ± 1.58^{a}
odor	4.72 ± 1.51^{b}	5.84 ± 1.46^{a}
taste	5.10 ± 1.76^{b}	6.10 ± 1.69^{a}
flavor	4.98 ± 1.56^{b}	6.08 ± 1.54^{a}
overall preference	5.08 ± 1.74^{b}	6.36 ± 1.35^{a}

$^{a-b}$ different upper case latters in the same rows indicate values that are significantly different in statistics ($p \leq 005$)

In the second phase, the development of cold drink products was done based on different weight ratios to produce three formulations. The highest score in appearance was the formulation with 1 g of rice bran mixed with 2 g of grain (4.58 ± 1.40) whereas the formulation with 2 g of rice bran mixed with 1 g of grain (4.40 ± 2.19) showed the lowest score. The formulation with 1 g of rice bran mixed with 2 g of grain (4.48 ± 1.27) the highest score in color but was not significantly different when compared with other formulations. The highest scores in odor and flavor belonged to the formulation with 2 g of rice bran mixed with 1 g of grain which were 5.26 ± 2.02 and 5.26 ± 1.84, respectively. However, the odor and flavor scores of all formulations had a no significant difference. Among the three formulations, the formulation with 2 g of rice bran mixed with 1 g of grain still exhibited the highest taste score (5.36 ± 1.99) and was significantly different. Overall, the formulation with 2 g of rice bran mixed with 1 g of grain received the highest preference score in odor, flavor and taste characteristics (5.28 ± 1.95). It indicated that the high degree of consumer acceptance depend on odor, flavor and taste properties. Therefore, the formulation with 2 g of rice bran mixed with 1 g of grain was a suitable formulation of a cold drink product that was high in γ-orizanol content and acceptance of taste.

Table 4. Secondary study on sensory characteristics of cold drink products from Riceberry rice variety

characteristics	Formulation of cold drink products		
	1	2	3
	Rice bran : grain 1 : 2 (g)	Rice bran : grain 1.5 : 1.5 (g)	Rice bran : grain 2 : 1 (g)
appearance	4.58 ± 1.40^{a}	4.34 ± 1.73^{a}	4.40 ± 2.19^{b}
color	4.48 ± 1.27^{b}	4.28 ± 1.74^{b}	4.42 ± 2.29^{b}

characteristics	Formulation of cold drink products		
	1	**2**	**3**
	Rice bran : grain	**Rice bran : grain**	**Rice bran : grain**
	1 : 2 (g)	**1.5 : 1.5 (g)**	**2 : 1 (g)**
odor	4.86 ± 1.41^{a}	4.66 ± 1.59^{a}	5.26 ± 2.02^{a}
taste	4.58 ± 1.75^{b}	4.88 ± 1.90^{b}	5.36 ± 1.99^{a}
flavor	4.60 ± 1.64^{a}	4.76 ± 1.85^{a}	5.26 ± 1.84^{a}
overall preference	4.88 ± 1.69^{a}	4.98 ± 1.96^{a}	5.28 ± 1.95^{a}

$^{a-b}$ different upper case latters in the same rows indicate values that are significantly different in statistics ($p \leq 005$)

CONCLUSION

This study quantified γ-oryzanol in four varieties of rice bran water extracts and showed that Riceberry was contained the highest content of γ-oryzanol, followed by Black glutinous, Mali Nil and Sung Yod. There is a correlation between darkened color of rice bran and quantity of γ-oryzanol. Riceberry rice bran was selected to be developed into cold drink products due to the high γ-oryzanol content. Sensory characteristics of 50 subjects using the Hedonic-9 scale test found that only grain formulation were more popular than that of rice bran. Therefore, the cold drink formulation containing both grain and rice bran was developed in the second phase. The cold drink formulation was prepared on different weight ratios. The suitable formulation contained 2 g of rice bran mixed with 1 g of grain was reported to be most satisfactory.

ACKNOWLEDGMENTS

The authors would like to thank Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathumthani, Thailand for the encouraging laboratory room. We also thank Pathumthani Rice research center, Pathumthani, Thailand for rice identification.

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