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The effect of single screw conveyor stabilization on free fatty acids, α-tocoferol, and γ- oryzanol content of rice bran

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Abstract

The benefit of rice bran to human health is already wellknown. It is a rich source of fiber, vitamin B1, tocoferol and γ-oryzanol. Unfortunately, freshly milled rice bran has a short shelf life because of its high triaclyglycerol content, which tends to decompose into free fatty acids (FFA). The last are responsible for off flavor development during rice bran storage. In this research, hot screw conveyor was used to stabilize fresh rice bran. The effects of several stabilization parameters, those are conveyor temperature and conveyor rotation speed to the content of FFA, α-tocopherol, and γ-oryzanol of rice bran stored at 37°C for two weeks were observed. The results showed that after two weeks of storage, an increase of total FFA in unstabilized rice bran (39.91%) was significantly higher as compared to the stabilized one (6.75%). Overall, it was concluded that the best treatment for rice bran stabilization was obtained at 120°C with a screw speed of 15 Hz. The treatment was chosen because it gave minimum loss of tocopherol and oryzanol (4.74% and 22.28%) and FFA content less than 10%.

Introduction

Rice bran is a by-product of rice milling to produce white rice, it is a thin layer covering the rice seeds after husk removal (Tao *et al*., 1993; Prakash, 1996) which is nearly 8% of milled rice (Sereewatthanawut *et al.*, 2008). The production amount of rice bran is about 50-60 million tons per year (Devi and Arumughan, 2007), which is mostly utilized as an animal feed ingredient, fertilizer, and fuel (Pan *et al.*, 2005; Zullaikah *et al*., 2005).

Rice bran is a good source of protein, fiber, and carbohydrates (Qureshi *et al.*, 2002). Rice bran also contains tocopherols and tocotrienols (Xu *et al*., 2001; Schramm *et al.*, 2007). Many studies have reported the potential of rice bran as a functional food because of its tocopherols and oryzanol content (Iqbal *et al.*, 2005). Oryzanol is the only antioxidant found in rice bran and it was reported as more powerful than tocopherol (Adom and Liu, 2002). The γ-oryzanol belongs to phytosterol family which is a mixture of ferulic acid esters of sterol and triterpene alcohols. This compound has been reported to decrease cholesterol in humans (Gerhardt and Gallo,1998), and to reduce the risk of coronary heart disease (Cicero and Gerosa, 2005).

Rice bran oil contains significant amount of mono-, di-, and triglycerides, free fatty acids, glycolipids, and phospholipids (McCaskill and Zhang, 1999). Therefore, rice bran must be stabilized immediately upon production to protect it from lipase activity, which can reduce quality of the rice bran by stimulating off flavor formation (Tao *et al*., 1993; Lakkakula *et al*., 2006).

Several heating methods to inactivate lipase in rice bran have been reported. Randall *et al.* (1985) reported that heat treatment is practical and unexpensive method to deactivate lipase in fresh rice bran immediately after milling. Heating temperature below 100°C does not increase storage capacity while heat treatment above 140°C is not recommended because it damages the rice bran quality.

Microwave heating was used by Tao *et al.* (1993) while ohmic heating was applied by Lakkakula *et al.* (2006). Microwave heated rice bran showed that FFA content only slightly increased during 4 weeks of storage at 25°C (Tao, 1993). The uses of extruder and conveyor to stabilize rice bran have also been reported. Randall *et al.* (1985) using a temperature of 125-135°C for 1-3 seconds with a single screw extruder. In our previous study (unpublished data), twin screw extruder was used for rice bran stabilization at the optimum conditions T1 (input temperature) = 130°C, T2 (middle temperature) = 160°C, T3 (output temperature) = 230°C, screw speed and feeding speed of 12 Hz. The method has successfully inactivated rice bran lipase which was shown by only 1.48% increase of free fatty acids

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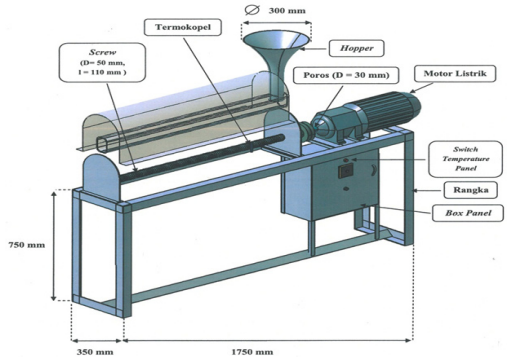

Figure 1. Single screw convenyor

content after 15 days of storage. However, more energy is needed in the operation of twin screw extruder, so the method is less suitable for small rice milling units. In this study, we developed rice bran stabilization method by using a single screw conveyor (Figure 1). The principle of this instrument is similar to twin screw extruder that is using screw rotation to push the material out through a die. Variables controlled by this instrument to stabilize rice bran are temperature and the speed of screw rotation. The instrument does not need steam input like that in double screw extruder, the size is relatively small and compact, and is very easy to install and to operate. Thus, it can easily be coupled to small-medium rice milling unit. The effect of stabilization treatment by using the conveyor on the content of free fatty acids, nutrients and bioactive components (α-tocoferol and γ-oryzanol) of rice bran was also studied. As the final output, we expected to find an optimum stabilization condition which can be applied when one wants to combine the instrument with small-medium rice milling unit. The proposed method should be able to preserve bioactive compounds in rice bran as well.

Materials and Methods

Materials

The IR-64 rice bran used in this study was obtained from paddy field in Kebumen, Central Java, Indonesia. Rice bran was milled with a method similar to obtain brown rice. Brown rice was polished for 2 minutes to obtain fresh rice bran. Rice bran was further sieved (100 mesh), wrapped with almunium foil and kept at freezer (-18°C) before the analysis.

The chemicals used in this research were HPLC grade acetonitrile, n-hexane, methanol, dichloromethane, and acetic acid glacial (Merck, Germany) and ascorbic acid (JT. Baker, USA). The standard compounds γ-oryzanol (Wako, Tokyo Chemical Industry, Japan) and α-tocoferol (Sigma, Japan) were generously provided by Dr. Takuya Koseki from Laboratory of Nutrition, Faculty of Agriculture, Tohoku University, Japan. The instruments used in this research were RP-HPLC (Agillent 1200 series, USA) with a UV-Vis detector (Agilent, USA), and a single screw conveyor (F-Technopark, Indonesia).

Methods

Analysis of free fatty acids was performed after 2 weeks of storage in an incubator with the temperature of 37°C. The temperature was chosen because it is the optimum temperature for lipase activity. Free fatty acid of fresh rice bran and stabilized rice bran were analyzed at week 0, 1, and 2. Analysis of α-tocopherol and γ-oryzanol were also performed for both fresh and stabilized rice bran. Fresh rice bran was used as a control. Three conveyor temperatures (100°C, 120°C, and 140°C) and two screw rotation speeds (15 Hz and 25 Hz) were applied. The samples were coded according to the given treatments (A100 = screw speed 15 Hz, 100°C, A120 = screw speed 15 Hz, 120°C, A140 = screw speed 15 Hz, 140°C, B100 = screw speed 25 Hz, 100°C, B120 = screw speed 25 Hz, 120°C, B140 = screw speed 25 Hz, 140°C). The determination of the best stabilization conditions was based on the decrease of rice bran free fatty acids (FFA) of each treatment combination. Additionally, the effect of stabilization on the γ-oryzanol and α-tocopherol content of rice bran was also observed.

Analysis of FFA and α-tocopherol

Rice bran oil extraction

Rice bran was macerated by using n-hexane with the ratio of rice bran: n-hexane 1:3 and shaked for 24 hours, followed by filtration. Hexane residu was evaporated using a rotary vacuum with temperature ranges between 54-60°C (Damayanthi, 2002) and the oil was ready for FFA and α-tocopherol analysis.

Free fatty acids (AOAC 940.28)

Rice bran oil (1.0 g) was put in the 250 ml erlenmeyer, then 50 ml of 95% ethanol was added and the mixtures was then heated and boiled in a water bath (±10 minutes). The solution was titrated with 0.1 N NaOH by using phenolphthalein indicator. Titration was done until a stable pink color appeared for 30 seconds. The free fatty acids content was calculated by using the following formula:

$$\text{FFA (\%)} = \frac{\text{ml KOH x N KOH x 282}}{\text{10x W sample (mg)}} \text{ x 100}$$

Analisis α-tocoferol (AOAC 971.30)

A total of 0.5 g rice bran oil, 0.15 g of ascorbic acid and 2 ml of ethanol were added to 25 ml test tube. Saponification was performed by adding 0.5

mL of 70% (w/v) potassium hydroxide to the test tube. The solution was heated in a waterbath at 80°C for 15 min. Water was added 10 ml to the solution to stopped saponification. After that, sample was extracted with hexane addition of 3 x 10 ml. Hexane extracts was pooled, washed with distilled water until neutral and evaporated under nitrogen flow.

Samples were redissolved in 5.0 ml of mobile phase and filtered with 0.45 µm Whatman paper. As many as 20 µl sample was injected to the coloumn. The wavelength was set at λ = 280 nm. The mobile phase was methanol : isopropanol (98:2/v:v). The flow rate was controlled at 1.0 ml min^{-1}. The standard was prepared by dissolving 25 mg of α-tocoferol with mobile phase to volume of 50 ml. Identification oryzanol was done by comparing the with the standard compound.

γ- oryzanol analysis (Xu and Godber, 2000)

Rice bran (1.0 g) was suspended in 5 mL of distilled water in a 25-mL test tube. Ascorbic acid (0.2 g) was added to the test tube. The solution was vortexed and incubated in a waterbath at 60°C for 30 minutes. A total of 5 ml solvent isopropanol: hexane (50:50) was added in the tube and vortexed for 30 seconds. After homogeneous, the solution was centrifuged at 200 g for 15 minutes at 30°C. The organic layer was collected. The remaining residue is mixed with 5 ml of solvent and centifugated again. The organic layer obtained was collected and washed with distilled water. Washing process is repeated twice. The extract was obtained by evaporating the solvent under nitrogen flow.

Standard γ-oryzanol (25.0 mg) was dissolved with mobile phase at 50.0 ml flask and the standard series between 12.5-250 ppm was made. The bran oil and standard compound were dissolved with mobile phase and filtered using PTFE membranes 0:45 µm. A total of 20 µl sample solution was injected in the HPLC column. The mobile phase consisted of methanol, acetonitrile, dichloromethane, and acetic acid glacial (50:44:3:3). Flow rate was controlled at 1.0 mL/min. Analytes were detected at 330 nm. Identification of γ-oryzanol was done by comparing with the standard compound. The concentration of γ-oryzanol was obtained by summing all individual components.

Statistical analysis

Completely randomized design was used in this experiment with a combination of temperatures and screw speeds as treatments. Analysis of variance (ANOVA) was conducted by using SPSS version 20.0. Significant differences were identified by Duncan's Multiple Range test ($p < 0.05$).

Results and Discussion

Analysis of free fatty acids

Free fatty acids content is widely used as an indicator of rice bran stability (Randall, 1985; Tao *et al*., 1993; Lakkakula *et al*., 2004; Budijanto, 2010). The results of free fatty acids analysis can be seen in Table 1. Free fatty acids in stabilized rice bran increased only between 3.34-0.89% in the first week and 2.71% -6.75% in the second week. While free fatty acids of fresh bran increased to 25.45% in the first week and 39.91% in the second week of the initial storage. The results of rice bran fatty acid content analysis at week-2 showed that the stabilized rice bran was still acceptable to be used in food products. Free fatty acids content in the rice bran at more than 10% is no longer suitable for human consumption (Tao *et al*., 1993; Lakkakula *et al*., 2004). Free fatty acid can be oxidized to produce aldehydes and ketones which are responsible for off odor formation. In this study stabilized rice bran fatty acid content is at the level of less than 10% (temperature treatment 120°C and 140°C).

The comparation of an increase of free fatty acid content between single screw conveyor and twin screw extruder for rice bran stabilization obtained from our previous study is displayed in Table 2. It can be seen that both twin screw extruder and single screw conveyors are potential to be used for rice bran stabilization. It is shown that FFA of unstabilized rice bran decreased 10 times when compared with the control. The increase of FFA in rice bran stabilized with a single screw conveyor at a temperature of 120-140°C was twice of those of twin screws extruder at 130°C. This is likely to occur due to the thermal distribution in rice bran with twin srew extruder is better than with a single screw conveyor. The shear of the twin screw extruder may cause heat and increase the temperature in the extruder. However, as we previously discussed, the twin screw extruder is not suitable for small rice milling unit because of high energy demand.

Analysis of α-tocopherol

The results of rice bran α-tocopherol analysis from each treatment including the control (fresh rice bran) can be seen in Table 1. The α-tocopherol content was found to be between 192.81-236.86 µg g^{-1} oil. The content of α-tocopherols was similar to those reported by Schramm *et al*. (2007) in Cypress rice bran varieties which were between 170.26-218.21 µg g^{-1} rice bran oil. Anwar *et al*. (2005) reported that

Table 1. The results of the FFA, α-tocopherol, and γ-oryzanol content in rice bran during 2 weeks storage at 37°C

Sample	FFA content (%)	Increased FFA (%)	γ-oryzanol content (µg g^{-1})	Decreased γ-oryzanol (%)	α-tocoferol content (µg g^{-1})	Decreased α-tocoferol (%)
Control	39.91±0.71	44.53	2408.03±5.01		242.14±6.27	
A100	6.75±1.19	12.11	2025.79 ±63.52	18.87	227.99±6.85	5.48
A120	3.88±0.31	8.53	1871.35±12.07	22.29	230.66±4.691	4.74
A140	3.09±0.22	8.3	1571.81±22.81	34.73	227.8±7.02	5.92
B100	4.53±0.24	10.22	1807.24±7.13	24.95	236.86±0.88	2.18
B120	2.71±0.10	7.83	1793.41±64.88	25.52	227.48±1.79	6.05
B140	3.13±0.29	7.6	1766.81±74.99	26.63	192.81±2.22	20.37

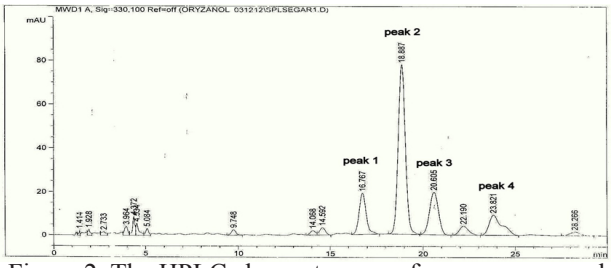

Figure 2. The HPLC chromatogram of gamma-oryzanol analysis in fresh rice bran

Table 2. The comparison of increased FFA (%) in rice bran during 2 weeks storage at 37°C with single screw conveyor and twin screw extruder

Treatment	FFA increase (%)
Kontrol	39.91±0.71
Single screw (100°C, 15 Hz)	6.75±1.19
Single screw (100°C, 25 Hz)	4.53±0.24
Single screw (120°C, 15 Hz)	3.88±0.31
Single screw (120°C, 25 Hz)	2.71±0.10
Single screw (140°C, 15 Hz)	3.09±0.22
Single screw (140°C, 25 Hz)	3.13±0.29
Twin screw (130°C, screw speed 15Hz, and feed speed 15Hz)	1.42±1.21

α-tocopherol content of rice bran from Pakistan was between 175.12-304.2 g g^{-1} oil. The highest value was obtained from the α-tocopherol content of B100 treatment (236.86 µg g^{-1} oil) while the lowest was the treatment of B140 (192.81 µg g^{-1}).

Significant differences were seen in B140 and A140 treatments. The B140 α-tocopherol content was 20:37% decreased while A140 was 5.92% decreased. According to Miller (2004), higher screw speed causes high friction which resulted in higher temperature during process. Additionally, screw rotation causes aeration in the conveyor which may allow the oxidation to occur. These two situations may lead to a reduce content of tocopherol although other mechanism needs to be analyzed further.

γ- oryzanol analisis

From HPLC analysis it was found that there were four main peaks at retention time of 17-25 minutes (Figure 2). This is similar to that have been reported previously (Pascual *et al*., 2011; Chen and Bergman, 2005; Xu and Godber, 1999). The identification of each peak was carried out by Cho *et al.* (2012) who identified γ-oryzanol in the form of cycloartenyl ferulic (peak 1), 24-methylenecycloartanyl ferulate (peak 2), campesteryl ferulate (peak 3), and sitosteryl ferulate (peak 4).

The results of γ-oryzanol analysis can be seen in Table 1. The average γ-oryzanol content of the fresh bran was 2408, 50 ± 03 µg g^{-1}. This is similar with the result of Qureshi *et al.* (2002) who reported that γ-oryzanol content of the fresh rice bran was around 2200-3000 µg g^{-1}. The highest γ-oryzanol content was obtained in the treatment of A100 (2025.79 ± 63.53 µg g^{-1}). It decreased as much as 15.87% from control. The lowest oryzanol content was obtained from A140 treatment (1571.81 ± 22.81 µg g^{-1}). This is probably because A140 experienced longer exposure with higher temperature (140°C) at screw speed 15 Hz than those of A100.

In rice bran, γ-oryzanol serves as natural antioxidant for the plant itself but as previously mentioned it also offer much health benefit for human. Its antioxidant property was reported as higher than the four forms of vitamin E (Xu *et al*., 2001). Lerma-Garcia *et al.* (2009) reviewed some biomedical properties of γ-oryzanol from rice bran. It was reported to lower blood cholesterol level and to improve blood lipid profile of rats and hamsters fed with mixture of oryzanol, to reduce inflammatory reactions in mice, and to protect diabetic mice against oxidative damage. In a recent review from Henderson *et al.* (2012), chemopreventive effect of dietary rice bran *in-vivo* or *in-vitro* was summarized. Rice bran was reported to possess promising anticancer activity against several cancer types, such as breast, lung, liver, and colorectal cancer. Apoptosis induction, cell proliferation inhibition, and cell cicle alteration were suggested as rice bran anticancer mode of actions. Gamma-oryzanol is among phytochemicals reported as a responsible compound for rice bran chemopreventice activity. The abovementioned reports on rice bran health benefit showed the potential of rice bran as functional food ingredients to be incorporated to various types of food products. The results of this study facilitate such applications since it extends rice bran shelf life while preserving phytochemicals important for its functional properties.

Conclusion

In this research it was shown that rice bran stabilization by using screw conveyor was able to

significantly suppress an increase of FFA content of stabilized rice as compared to control. Particularly, A120 was the best treatment for preventing rancidity (FFA content <10%) with a minimum decrease in α-tocopherol and γ-oryzanol content. It can be concluded that single screw conveyor is potential to be used in combination with small-medium rice milling unit to produce rice bran with better self-life while the nutrition content can be preserved.

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References

Adom, K.K. and Liu, R.H. 2002. Antioxidant activity of grains. Journal of Agricultural and Food Chemistry 50: 6182-6187.

Anwar, F., Anwer, T. and Ahmood, Z. 2005. Methodical characterization of rice (*Oryza sativa*) bran oil from Pakistan. Journal Grasas Aceites 56 (2): 125-134.

AOAC. 1998. Official Methods of Analysis of AOAC International. Virginia: USA AOAC International

AOAC. 1999. Official Methods of Analysis of AOAC International. Virginia: USA AOAC International

Chen, M.H. and Bergman C.J. 2005. A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. Journal of Food Composition and Analysis 18: 139–151.

Cho, J.Y., Lee, H.J., Kim, G.A., Kim, G.D., Lee, Y.S., Shin, S.C, Park, K.H. and Moon, J.H. 2012. Quantitative analyses of individual γ-oryzanol (steryl ferulates) in conventional and organic brown rice (*Oryza sativa* L.). Journal of Cereal Science 55 (3): 337–343.

Cicero, A.F.G and Derosa, G. 2005. Rice bran and its main components: potential role in the management of coronary risk factors. Nutraceutical Research 3(1): 29-46.

Gerhardt, A.L. and Gallo, N.B. 1998. Full-fat rice bran and oat bran similarly reduce hypercholesterolemia in humans. Journal of Nutrition 128 (5): 885-889.

Henderson, A.J., Ollila, C.A., Kumar, A., Borresen, E.C., Raina, K., Agarwal., R. and Ryan, E.P. 2012. Chemopreventive properties of dietary rice bran: Current status and future prospects. Advances in Nutrition 3: 643-653.

Iqbal, S., Bhanger, M. I. and Anwar, F. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Journal of Food Chemistry 93: 265-272.

Lakkakula, N.L., Lima, M. and Walker, T. 2004. Rice bran stabilization and rice bran oil extraction using ohmic heating. Journal Bioresource Technology 92: 157–161.

Lerma-Garcia, M.J., Herrero-Martinez, J.M., Simo-Alfonso, E.F., Mendonca, C.R.B. and Ramis-Ramos, G. 2009. Composition, industrial processing and applications of rice-bran γ-oryzanol. Food Chemistry 115 (2): 389-404.

McCaskill, D.R. and Zhang, F. 1999. Use of rice bran oil in foods. Journal of Food Technology 53: 50-53.

Pan, Z., Cathcart, A. and Wang, D. 2005. Thermal and chemical treatments to improve adhesive property of rice bran. Journal Industrial Crops and Products 22: 233-240.

Pascual, C.S, I., Massaretto, I.L., Kawassaki, F., Barros, R.M.C, Noldin, J.A. and Marquez, U.M.L. 2013. Effects of parboiling, storage and cooking on the levels of tocopherols, tocotrienols, and γ-oryzanol in brown rice (*Oryza sativa* L.). Food Research International 50 (2): 676 – 681.

Prakash, J. 1996. Rice bran: properties and food uses. Critical Reviews in Food Science and Nutrition 36: 537-552.

Qureshi, A.A., Sami, S.A. and Khan, F.A. 2002. Effect of stabillized rice bran, its soluble and fiber fraction on bloods glucose and serum lipid parameter in humans with diabetic mellitus type I and II. Journal of Nutritional Biochemistry 13: 175-187.

Randall, J.M., Sayre, R.N., Schultz, W.G., Fong, R.Y., Mossman, A.P., Tribelhorn, R.E. and Saunders R.M. 1985. Rice bran stabilization by extrusion cooking for extraction of edible oil. Journal of Food Science 50 (2): 361-364.

Devi, R.R. and Arumughan, C. 2008. Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. Journal of Bioresource Technology 98: 3037-3043.

Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M. and Shotipruk, A. 2008. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. Journal of Bioresource Technology 99: 555-561.

Schramm, R., Abadie, A., Hua, N., Xu, Z. and Lima, M. 2007. Fractionation of the rice bran layer and quantification of vitamin E, oryzanol, protein, and rice bran saccharide. Journal of Biological Engineering 9(1): 1-9.

Tao, J., Rao, T. and Liuzzo, Z. 1993. Microwave heating for rice bran stabilization. Journal of Microwave Power and Electromagnetic Energy 28 (3):156-164.

Xu, Z. and Godber J.S. 2000. Comparison of supercritical fluid and solvent extraction methods in extracting γ-oryzanol from rice bran. Journal of the American Oil Chemists Society 77(5):547-551.

Xu, Z., Hua, N. and Godber, J.S. 2001. Antioxidant activity of tocopherols, tocotrienols, and γ- oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis (2-methyl propionamidine) dihydrochloride. Journal of Agricultural and Food Chemistry 49: 2077-2081.

Zullaikah, S., Lai, C.C., Vali, S.R. and Ju, Y.H. 2005. A two-step acid-catalyzed process for the production of biodiesel from rice bran oil. Journal of Bioresource Technology 96:1889-1896.