

## RICE BRAN STABILIZATION AND $\gamma$ -ORYZANOL CONTENT OF TWO LOCAL PADDY VARIETIES "IR 64" AND "CISADANE MUNCUL"

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### ABSTRACT

*An autoclaving procedure was developed to produce stable rice bran from two local paddy varieties IR 64 and Cisadane Muncul. The stable rice bran showed no significant increase in free fatty acid content for 144 hours at 37°C. In the optimum wet heating process, rice bran was heated at 121°C and held for 3 minutes before cooling for both varieties. Stable rice bran contained 3.8 % moisture and the content of total tocopherol was not much changed by heating process, i.e., 209.8 mg/100 g rice bran oil and 279.8 for control (without heating process) of IR 64 variety and 227.4 mg/100 g rice bran oil and 248.8 for control of Cisadane Muncul variety, respectively. The content of  $\gamma$ -oryzanol varied and was depended on the degree of milling ranged from 39.1 to 147.7 mg/100 g rice bran oil for both of IR 64 and Cisadane Muncul rice varieties. The content of soluble dietary fiber varied and was depended on the degree of milling which ranged from 3.56 to 8.76 % for both of IR 64 and Cisadane Muncul rice varieties. The content of insoluble dietary fiber varied and was depended on the degree of milling which ranged from 15.00 to 25.38 % for both of IR 64 and Cisadane Muncul rice varieties.*

**Keywords :** rice bran, autoclave, stabilization,  $\gamma$ -oryzanol, total tocopherol, dietary fiber

### INTRODUCTION

New products are the lifeblood of any industry. This is particularly true for the food industry. Long term financial success is directly related to how effectively and efficiently a company can recognize and meet consumer needs through new products. As the food industry has become more competitive the importance of new products has also grown (Hoban, 1998).

Consumer trends to 2020 and beyond for food industry products have been influenced by the changing of lifestyle and science (Sloan, 1998). There is no doubt that health will remain on the front burner way beyond the year 2020. Concerns over disease prevention, anti-aging, obesity, immunity and to some extent treatment will get primary attention. The most important health claims five years from now in USA will be "reduce the risk of heart disease" and "reduce the risk of cancer". In addition, future healthy food trends will be affected by the rising incidents of serious diseases.

At the same time, however, science will continue to unravel the health-promoting and curative powers of food components and natural substances. Americans belief in food as medicine will continue to grow. Already, 52% Americans believe that foods can replace the use of drugs and 33 % regularly use foods for treatment (Hoban, 1998). Nearly three quarters (70%) of them have heard of naturally occurring food substances that can help prevent disease, even cancer. Scientific knowledge of the beneficial role of various food ingredients for the prevention and treatment of specific disease is rapidly accumulating.

Rice bran has been promoted as healthy material for human. The use of rice bran as human food source is limited, it is sometimes used as supplement in bread, cookies and cake. The utilization of rice bran is mostly being used for feed due to its low price, because it is a by-product of rice milling. It has been predicted that production of rice bran in Indonesia was over 4

million MTs per years (Tangenjaya, 1991). The fact that rice bran contains bioactive compounds which have the functional and nutraceutical properties that provide several unique food applications. The important compound in rice bran especially are antioxidants namely  $\gamma$ -oryzanol, tocopherol, tocotrienol and dietary fiber.

The degenerative diseases, such as atherosclerosis, cardiovascular disease (CVD), hypertension, neoplasm or cancer, have a tendency as a major cause of death in Indonesia (Sumantri, 1994). Therefore it is hoped that the role of active substances from plant foods will be significantly increased in the future in order to decrease the prevalence of such degenerative diseases.

$\gamma$ -oryzanol from rice bran has been suggested to possess the capability of lowering blood cholesterol (Raghuram, et al., 1989; Seetharamaiah and Chandrasekhara, 1989) and to have antioxidant functionality (Duve and White, 1991). The 10 components of  $\gamma$ -oryzanol has been identified successfully at the end of 1999. They were identified as  $\gamma$ -stigmasteryl ferulate, stigmasteryl ferulate, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate,  $\Delta^7$ -campestenyl ferulate, campestenyl ferulate,  $\Delta^7$ -sitostenyl ferulate, sitostenyl ferulate, campestenyl ferulate and sitostenyl ferulate. Three of these, cycloartenyl ferulate, 24-methylenecyclo-artanyl ferulate and campestenyl ferulate, were major components of  $\gamma$ -oryzanol (Xu and Godber, 1999). Soluble dietary fiber from rice bran has been suggested to possess important role in prevention of hypercholesterolemic (Kahlon and Chow, 1997; Kritchevsky, 1997). Among some cereals bran, only from oat and rice have been shown to possess the capability of reducing blood cholesterol in experimental animal and human.

Rice bran contains antioxidant namely  $\gamma$ -oryzanol, tocopherol and tocotrienol at the level of 2-5 % of the rice bran oil

(Stark and Madar, 1994),  $\alpha$  only  $\gamma$ -oryzanol varies within the range of 1.1 – 2.6 % (Seetharamaiah and Prabhakar, 1986). There is limited information about the content of  $\gamma$ -oryzanol at local paddy varieties and distribution of  $\gamma$ -oryzanol content within degree of milling. It is important to look for information about them because the chemical composition of rice bran depends on a variety of factors associated with the rice grain itself and the milling process (Luh, et al., 1991).

The limitation of using rice bran in food is due to the rapid development of rancidity caused by lipase and lipoxidase activities after milling. Rice bran stabilization methods have been reviewed by Sayre et al. (Randall et al., 1985). Heat inactivation of the lipases appears to be the only method with commercial potential. Wet heating is much more effective in permanently denaturing lipases, and pressurized heating (autoclave) will reduce the heating time and so will reduce the destruction of bioactive compound in rice bran. The stabilization process can make rice bran become a valuable food source for human. After the stable rice bran was provided, we can further process it as health food or drink for human.

Another nutraceutical properties of  $\gamma$ -oryzanol in rice bran as antioxidant might give a new expectation to prevent cancer disease. The abundant of free radical in our body and/or low of the antioxidant level may initiate the cancer to develop. Because the quantity of antioxidant in our body is important to prevent the degenerative diseases, it is important to have enough supply of antioxidant from our daily diet. Considering the functional and nutraceutical properties of rice bran, this research was designed to study the rice bran stabilization by autoclave method and to study the distribution of  $\gamma$ -oryzanol at various degree of milling.

## MATERIALS AND METHODS

### Materials and Equipments

Two local varieties of paddy that are IR 64 and Cisadane Muncul were obtained from Breeder Seed Laboratory at Sukamandi. To obtain rice bran, the paddy was milled in the laboratory. The solvent and standard for identification and quantification of tocopherol were obtained from Sigma Chemical while oryzanol standard was obtained from Rice Growers' Co. Australia. TLC plates were bought from a chemical store in Bogor and other chemical materials for analysis were obtained from the same source.

### Stabilization of rice bran

Rice bran was autoclaved 121°C for 0 to 5 minutes. Immediately after cooling and drying (oven, at 105°C for 1 hour), the bran was packed in plastic bag, sealed and stored in refrigerator until analysed. Lipase activity was determined by measuring the increase of FFA content (titration method) of oil extracted before and after bran samples were incubated in sealed container for 144 hours at 37°C. Nutrient destruction was

determined by measuring the total vitamin E (tocopherol) using spectronic 20 method (Wong et al., 1988).

### Proximate, dietary fiber and $\gamma$ -oryzanol contents: effect of paddy varieties and degree of rice milling.

Two groups of paddy samples of different varieties were milled at 5 degrees of milling i.e. 7, 10, 13, 16 and 19% (weight of bran/weight of brown rice). The rice bran was stabilized as above. All samples were analyzed for proximate, dietary fiber contents (soluble and insoluble dietary fiber) using enzymatic method (Asp et al., 1983) and  $\gamma$ -oryzanol contents using TLC and Spectrophotometric method (modification of Seetharamaiah and Prabhakar, 1986).

## RESULT AND DISCUSSION

### Stabilization of Rice Bran

Raw rice bran used in this study had 7.5 % of degree of milling (w/w, rice bran/brown rice bran). The increase of FFA content from the initial levels of bran and after storing for 144 hours at 35°C is shown in Figure 1 as a function of autoclaving time at 121°C.

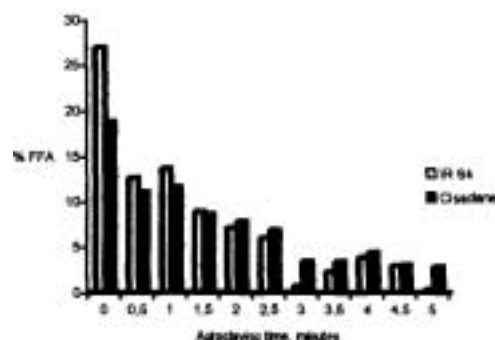


Figure 1. Free fatty acid increase in rice bran after being stored for 144 hours at 35°C as influenced by autoclaving time for 0-5 minutes at 121°C

The difference of paddy variety did not affect the increase of FFA content, however, autoclaving time influenced it significantly. Autoclaving time from 0 until 2.5 minutes reduced the increase of FFA significantly, but at 3.5 minutes the increase of FFA is not different significantly.

As shown in Table 1 the fat content of rice bran was relatively high (14-33 % d.b.). In the whole rice grain the oil is located in different layer than that of lipase enzyme. Lipase are located in the testa/cross layer of the rice grain while the oil is in the aleuron and subaleurone layers and germ (Luh et al., 1980). When the grain is milled, the substrate contacts with the enzyme and causes rapid deterioration of rice bran. In one week more than 30 % of fat was hydrolyzed to FFA (Tangenjaya, 1991).

Table 1. The effect of degree of milling on the chemical compounds of rice bran

	Degree of milling (%)									
	IR 64					Cisadane				
	7	10	13	16	19	7	10	13	16	19
Moisture	3.48	2.85	1.96	2.31	1.05	1.83	2.96	2.24	1.81	2.61
Ash	8.73	8.57	7.80	6.89	5.80	9.36	8.82	7.18	6.54	4.90
Protein	17.11	15.89	14.50	13.29	12.13	17.04	15.72	14.21	13.22	12.42
Fat	32.28	29.30	27.46	18.94	14.27	31.70	29.29	27.28	19.36	14.67
Fiber	8.61	7.55	6.60	5.81	4.52	8.58	7.64	6.65	5.79	4.62

From the data discussed above, rice bran can be stabilized at an autoclaving time of 3 minutes. Autoclaving at 121°C for less than 3 minutes did not significantly affect the lipase activity.

Moisture content of rice bran after autoclaving was 11, 11 percent and after drying in an oven (105°C, 1 hour) was 3,78 percent. Reducing the moisture content of rice bran aimed to reduce the lipase activity. Figure 1 showed that for raw rice bran (0 minute of heating) the increase in FFA content was high, i.e. 27 percent, due to high lipase activity. Randall et al. (1985) stated that the increase of FFA was 8.7 percent for raw rice bran after being incubated at 32°C for 96 hours. Juliano (1985) indicated that lipase could be inactivated in solution at 60°C for 15 minutes or in rice bran at 100°C for 15 minutes or 115°C for 5 minutes.

From this result it is concluded that for the preparation of stable rice bran it should be heated in autoclave at 121°C for 3 minutes. Other researcher suggest autoclaving at 121°C for 15 minutes because it is the standard for sterilization process. With this treatment of heating we can minimize the destruction of nutrient (tocopherol) or bioactive compound in rice bran as shown in Figure 2.

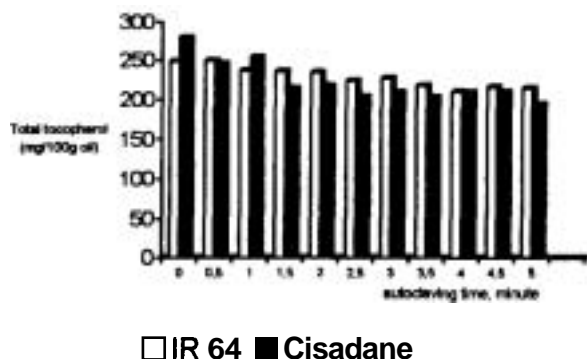


Figure 2. The effect of autoclaving on total tocopherol content of rice bran

### γ-Oryzanol Content

There were variation of oryzanol contents of rice bran with different degree of milling ranged from 39.1 to 147.7 mg/100g rice bran oil (Figure 3). The oryzanol content was lower than the result reported by Seetharamaiah and Prabhakar (1986), i.e. 1,100 – 2,600 mg/100 g rice bran oil, and Xu and Godber (1999), i.e. 980 mg/100 g rice bran oil. The difference may be due to the different analysis method used for Seetharamaiah and Prabhakar (1986) measured the sample from oil directly without purification, while Xu measured the sample after being semipurified using low-pressure silica column method. In this experiment, saponification was conducted to remove interfering the triglycerides and other hydrolyzable materials and to aid the release of lipid from sample matrix. We thought that saponification treatment probably may hydrolyze the ester bond between triterpenoids and ferulic acid components of γ-oryzanol. Diack and Saska (1994) stated that γ-oryzanol concentration was 460 mg/100g in the crude oil obtained under the saponification.

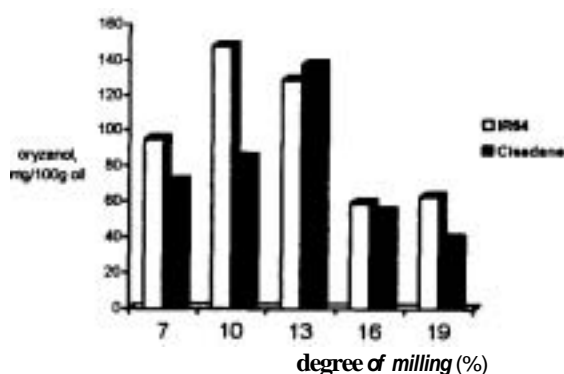


Figure 3. The effect of degree of milling on oryzanol content of stable rice bran

## Dietary Fiber

Table 2 showed the dietary fiber content of rice bran from two local paddy varieties at various degree of milling. It was shown that there was variation of soluble and insoluble dietary fiber contents between the two paddy varieties but it is not significantly **different**. The content of soluble dietary fiber increased significantly by increasing the degree of milling; on the contrary the content of insoluble dietary fiber decreased significantly by increasing the degree of milling.

Soluble dietary fiber content ranged from **3.58** to 8.76 % for both paddy varieties. The results were lower than that

reported by Kahlon and Chow (1997), i.e. 1.4 % for rice bran. Compared with other bran the content of soluble dietary fiber of rice bran is not **different**; for example oat bran contained 8.0 % soluble dietary fiber and barley 6.0 % (Kahlon and Chow, 1997). The three kind sources of bran have been reported to have **hypcholesterolemic** effects on experimental animal and human. Insoluble dietary fiber contents ranged from 15.00 to 20.06 % for both paddy varieties. Kahlon and Chow (1997) stated that rice bran contains 21.5 % insoluble dietary fiber, and 10.6 % and 11.2 % for oat and barley bran, respectively.

Table 2. The effect of degree of milling on dietary fiber content of rice bran (% d.b)

Dietary Fiber	Degree of milling (%)									
	IR 64					Cisadane				
	7	10	13	16	19	7	10	13	16	19
<b>Soluble</b>	3.68	4.80	6.50	7.92	8.67	3.56	4.80	6.58	7.72	8.76
<b>Insoluble</b>	25.38	22.17	19.76	16.85	15.00	<del>25.06</del>	22.30	19.78	16.74	15.22
<b>Total</b>	29.06	26.97	26.26	24.77	23.67	28.62	27.10	26.36	24.46	23.98

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