ISOLATION AND CHARACTERIZATION OF ORYZANOL FROM CRUDE RICE BRAN OIL

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Abstract— The isolation of oryzanol from crude rice bran oil (RBO) which have extracted with 4 kind of solvents (n-hexane, iso-propanol, methanol dan ethanol) is done to decide the best solvent was achieved by a two-step crystallization process. In the first crystallization, oryzanol was concentrated in the liquid phase along with FFA,MG, squalene, tocols, and phytosterols, whereas the solid phase contained mainly TG and steryl esters. Oryzanol-rich product obtained from the first crystallization was subjected to the second crystallization where the oryzanol-rich product was kept at -20 ^{O}C for 24 h. N-Hexane was added as anti-solvent to the oryzanol-rich product and kept at 1±5 ^{O}C for another 48 h. From the research, we could conclude that the suitable solvent to extract CRBO from rice bran was ethanol, which could give the best result with oryzanol purity 1,258%, yield of CRBO 5,89% and low %FFA, 27,47%. Many factors such as the process of Dewaxing and Degumming, the solvent to CRBO and DDRBO ratio on the first crystallization, the storage temperature on the first crystallization, and the effect of storage to oryzanol rich product from first crystallization process could give impact to purity and recovery of oryzanol. So the best result could be reached in 80/1 ratio of DDRBO with first crystallization temperature at -30 ^{O}C after storage, with oryzanol purity in oryzanol rich product (LP1) 2,109% and recovery 82,109%. But unfortunately, oryzanol crystal could not be formed in that temperature of this research.

Key Word: Rice Bran, Crude Rice Bran Oil, Dewaxed Degummed RBO, dewaxing and degumming, first and second crystallization, oryzanol rich product

I. INTRODUCTION

Rice production in Indonesia has a substantial productivity. Every years, almost rice production have to be increased. According to Badan Pusat Statistik Indonesia reported that rice production in 2012 estimated at 68.96 million tons of *Gabah Kering Giling (GKG)*. In other case, this event has increase of 3.20 million tonnes (4.87 per cent) compared in 2011. With that condition, increasing of rice production can have an impact that to be good for Indonesian.

On the other hand, with a large amount of rice production cause a large increase of waste product from co-miling process. This process produces by-products such as groats, broken rice, and rice bran. Groats and broken rice can be milled into flour as material of various cakes and other foods. Chaff can be used for fuel and compost. And then rice bran only used for animal feed and rarely used as a source of human food.

Rice bran can be extracted to produce *Crude Rice Bran Oil (CRBO)*. CRBO is one type of oil that has a high nutrient content and a variety of fatty acids, biologically active compounds and antioxidant compounds such as oryzanol, tocopherols, tocotrienols, phytosterols, polyphenols and squalene.[1]

The color of rice bran oil is generally brown. The brown color because of the pigment content contained in rice bran. Rice bran oil is also difficult to be purified, because the components present in rice bran oil, one of them is lipase, which can involve high free fatty acids and unsaponifiable compounds. Therefore, rice bran oil can not be used as an edible oil.[2] From that explanation, we can conclude that the benefits of rice bran oil is still limited. Therefore, our effort is needed to increase the value of the rice bran oil. On the other hand, we know that in the rice bran oil contains antioxidant compounds, one of them is oryzanol. Oryzanol is a combination of at least 10 components of ferulic acid esters and triterpene alcohols. [3]

The benefits of oryzanol content are an antioxidant that only found in rice bran oil, very powerful oxidation preventive, reducing agent for absorption of cholesterol and liver cholesterol, inhibit the effective time of menopause and prevent free radicals better than vitamin E. [4] The content of oryzanol in rice bran oil amount can reach 10 to 20 times more than the total content of tocopherols and tocotrienols.[5]

Various methods and ways have been carried out in a scientific journal that has been published. One of them is the isolation of oryzanol using preparative HPLC,done by Lai [6] in 2005. In this method gave results oryzanol up to 90-98% purity by the percent recovery of up to 90%. On the other hand, the weakness of this method were the small of productivity (10 mg / injection) and only suitable to be applied on a laboratory scale. Before that, in 1998, P.K. Das [7] isolated oryzanol through calcium ion. From this method showed that the purity and recovery are quite high, between 76-96%. The weakness of this method was many step which was needed to isolate and many solvents used in this process. In the same year, Saska and Rossiter [8] did isolation of oryzanol using silica with a combination of multi-stage crystallization. The results obtained oryzanol purity can reach up to 90-95% with 90% recovery. But the weakness of this method was the high cost of silica replacement. In 2008, Siti Zullaikah [9] did isolation of oryzanol with 2 step of crystallization method. In this method, done with a low temperature in the first step of crystallization until reached -60 ^{O}C and the oryzanol content were quite high. Oryzanol purity and recovery of the Liquid Phase 1 (LP1) was 13.68% and 63,34%. On the other hand,at temperature -22 ^{O}C in the first step of crystallization, oryzanol was also obtained at Liquid Phase 1 (LP1) with a purity and recovery of 12.59% and 47.17%, and at the end of the second step of crystallization oryzanol crystals obtained with a purity and recovery of 93-95% and 59%. However, the results given from this method performed at a very low temperature of first crystallization process, so it

was difficult to apply in a simple cooling device. Therefore, in this work, the author make an improvement of the methods that have been performed by Siti Zullaikah. Two-stage crystallization methods are re-developed using the middle low temperature at the first crystallization. Temperature used is in the range of -20^{O}C to -30^{O}C. With this temperature, we expected that at the end of the second stage of crystallization would also isolate oryzanol from rice bran oil.

II. METODHS

II.1 Extraction of Rice Bran Oil

The research was started with the extraction of rice bran. Rice bran obtained from Bali, stored in the refrigerator before used. It aims to prevent the increase of FFA (Free Fatty Acid) which can affect the content of oryzanol in rice bran.[10]. Crude RBO was extracted from rice bran (100 g), and wrapped with filter paper. Next put the rice bran that has been wrapped into sokhlet.

Then 500 ml of 4 types solvent (N-Hexane, Isopropanol, Methanol and Ethanol), put into a round bottom flask 500 ml. The selection of the type solvent aims to compare the effect of solvent on yield and concentration of oryzanol in the oil obtained, which can be influenced by polarity of solvent. Therefore, the solvent used have different polarity properties,from non-polar to polar solvent.

The extraction process was done in 4 hour at a constant temperature of 85 ^{O} C for each type of solvent. Selection of extraction time is based on the previous experiments, which stated that the total yield of oil extracted is greatest when the extraction process lasts for 4-6 hours. [11]

Results of the extraction process is a mixture of solvent and Crude Rice Bran Oil (CRBO), then a distillation process was done to separate the solvent and CRBO.

II.2 Dewaxing and Degumming of CRBO

Dewaxing and Degumming process of CRBO aims to eliminate *fat-soluble impurities.* Impurities that can be eliminated or reduced were Free Fatty Acid (FFA), phosphatides, metal ions, waxes, gums, oxidation products, color bodies, moisture, volatiles, and solid impurities.[12]

The initial step in this process was 50 g of CRBO was dissolve with 300 ml of acetone into a 500 ml glass vessel stopper, and then heated at 60 ^{O} C for 1 hour with stirring to increase the solubility.[9]

Then if the solution has been reached room temperature, we kept it Sin a freezer at a temperature of 5^{O}C for 24 hours to crystallize wax and gum, until formed 2 layers solution. If it has already formed 2 layers of the filtrate and solid, it was separated by filtration using a vacuum jet ejectors. Next we put it back into the freezer at a temperature of -5 ^{O} C for 24 hours, until the re-formed two layers, the filtrate and solid.

Next we separated the filtrate and solid precipitate with filtration process using vacuum jet ejector and distillation process was done to separate the solvent and filtrate using a simple distillation method, so that we can got Dewaxed Degummed RBO (DDRBO) as a residue of Dewaxing and Degumming CRBO process and a solvent as distillate, then we kept DDRBO in the refrigerator at a temperature of -5^{O}C.

II.3 First Step Crystallization

In the first step crystallization, CRBO and DDRBO was mixed with methanol/acetone (7:3), with appropriate comparison predetermined variables (solvent / RBO: 40/1, 60/1, and 80/1) to the 500 ml glass vessel stopper, then we stirred it with a magnetic stirrer for 1 hour at room temperature 24+32^{O}C until completely mixed and then stored it in the freezer at -20 ^{O} C for 24 hours. The next step is a filtration process to separate the filtrate (LP1) and oryzanol poor solid phase (SP1) with the vacuum jet ejectors,then distillation process was done. Then we analyzed oryzanol purity and recovery for each solvent mixture ratio of mehanol / acetone (7:3) with CRBO and DDRBO. Then we kept the oryzanol rich product (LP1) at temperature 10 ^{O} C for 24 hours to allow the growth of oryzanol crystal. After that we analyzed oryzanol purity and recovery.

II.4 *Second Step Crystallization*

Second Step Crystallization was done by mixing oryzanol rich product from the process first crystallization with 20 ml n-hexane as washing solvent, then we kept it in the freezer at a temperature of 1 + 5 ^{O} C for 48 hours until it formed 2 layers, filtrate and solid. Then filtrate and the solid was separated by vacuum filtration through jet ejector. Next we mixed the solid with 30 ml N-Hexane as washing solvent, then separate the filtrate (LP2) with solid (SP2) which was formed through the filtration process with jet ejector vacuum to obtain a white crystalline oryzanol, then kept the oryzanol crystall in freezer at temperature -20 ^{O} C.

II.5 Oryzanol Content Analysis

Oryzanol concentration in the sample can be measured using a spectrophotometer spectrophotometer (V-550 UV-vis spectrophotometer) and cuvette (quartz cell). For oryzanol content analysis, first we created a calibration curve by measuring the sample absorbance oryzanol (Wako ɣ-oryzanol standard 97 ppm) to find the wavelength (ʎ) used. From samples measurements, showed that the largest absorbance reached at wavelengths **ʎ = 311 nm.** The next step was prepare a standard solution with a concentration range of 0-97 ppm for analysis using UV-Vis spectrophotometer. Calibration curve obtained in Figure 1 below:

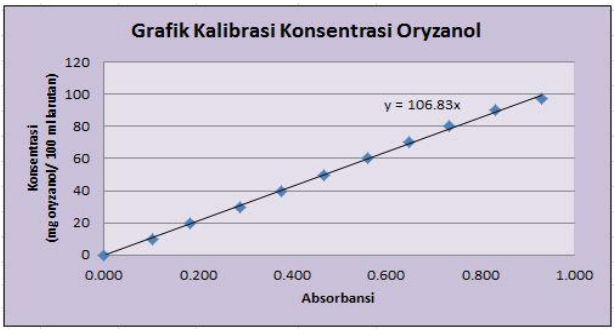

Figure 1. Calibration curve of Standard Sample ɣ-Oryzanol

II.6 Yield Analysis of Crude Rice Bran Oil (CRBO)

Calculation of CRBO yield was performed to compare the number CRBO obtained for each type of solvent in the extraction of rice bran, which will be used as

one of the basic considerations to made the choice of solvent.

The equation is:

$$\%\textbf{yield} = \frac{\textbf{mass of product (gr)}}{\textbf{mass of rice bran (gr)}} \; \boldsymbol{x}\; \mathbf{100}\% \quad \textbf{(1)}$$

[13]

II.7 Analysis of Free Fatty Acid (%FFA)

Equipment is used in this step include burette and 500 ml erlenmeyer. Mechanism analysis and computation is based on the FFA content of the journal AOCS [14]. Then the sample size and reagent based on the of the journal I.H Rukunudin [15]. The first step is measured 0.7 g CRBO then put into erlenmeyer. Then dissolved the sample in 7.5 ml CRBO into ethanol while heated above heater and stirred using a stirrer. Calculate the volume of NaOH used for titration and% FFA can be calculated by the equation:

$$\%\textbf{FFA} = \frac{\textbf{volume of alkaly (mL) x normalitas of alkaly x 28,2}}{\textbf{weight of sample (g)}} \textbf{ (2)}$$

[14]

II.8 Analysis of Oryzanol Recovery

Calculations can be done if it was no chemical reaction during the process takes place, such as the product purification process.[15]

Equations for the calculation of recovery :

$$\textbf{\% Recovery} = \frac{weight\ of\ finish\ material}{weight\ of\ starting\ material} \text{ x } 100\ \% \textbf{ (3)}$$

[15]

III. RESULT AND DISCUSSION

III.1 Determination of Solvents for Extraction Type CRBO with 4 Variable Solvent

Analysis results obtained through UV-Vis Spectrophotometer absorbance measured from each oil, oryzanol concentration that would be obtained for each of the oils extracted from various solvents.

Table 1

Experimental results First Variable (Extraction CRBO) with 4 Variable Solvent

Run	Larutan	%*Yield*	%*FFA*	% Oryzanol
1.	N-Hexane	8.845	32.47	0,449
	Iso-Propanol	6.53	19.98	0,746
	Methanol	6.65	23.70	1,130
	Ethanol	5.89	27.47	1,258
2.	N-Hexane	7.64	38.60	0,585
	Iso-Propanol	5.18	27.45	0,656
	Methanol	5.91	26.83	0,961
	Ethanol	6.95	25.59	1,196

From Table 1 above, we was found that for yield, the greatest value is the CRBO obtained from the solvent n-hexane, which is 8.845% for RUN I and 7.64% for RUN II. It was happened because the chemical ability of oil is non-polar, so it is very soluble in the non polar solvent such as n-hexane, according to the literature mentioned that polar compounds will be soluble in polar solvents and non-polar compounds will be soluble in non-polar solvents. [16]

Then, if we review from oryzanol content in the oil from the four types of solvents, we found that the greatest value for the concentration of oryzanol in the oil was from ethanol, which was 1.258% from RUN I and 1.196% from RUN II. From literature we knew that ethanol and methanol have a higher polarity value of the two other solvents, n-hexane and iso-propanol. This is indicated by both the dielectric constant of the solvent (ethanol and methanol) are 24.3 and 33 is greater than n-hexane and iso-propanol is 2.02 and 18. [17]

Selection of solvent polarity based on the chemical property of oryzanol is slightly polar,so it can be a decision that the methanol and ethanol can be used. However, the concentration of oryzanol in the oil from the solvent ethanol is higher than the concentration of oryzanol in the oil from the solvent methanol. This is appropriate if the terms of the FFA content, according to the content of FFA in oil from the solvent ethanol is lower than in the FFA content of the oil from the solvent methanol.

In the extraction method using sokhlet, operating temperature when using ethanol solvent is higher than the other three types of solvents. With increasing temperature, caused the viscosity of the solvent decrease and diffusivity of the solvent increased, so that the rate of extraction increased. On the other hand, oryzanol itself is a combination of triterpene alcohol and ferulic acid is slightly polar components making it easy to dissolve in polar solvents. [3]

Therefore, in this study ethanol was selected for the solvent extraction process which is suitable for isolate oryzanol from rice bran.

III.2 Dewaxing and Degumming Process of CRBO (Crude Rice Bran Oil)

At dewaxing and degumming process, first we analyzed the oil extracted using ethanol solvent using spectrophotometry to determine the concentration of oryzanol contained. The purpose of dewaxing and degumming process was to remove the content of wax and gum during the extraction process. Wax and gum can interfere the growth of crystalline oryzanol during the first crystallization. The color of wax and gum from the process was blackish brown. Wax and gum were insoluble in several solvents, such as acetone. Therefore, removal or separation of wax and gum using acetone solvent to settle it. [19]

Then DDRBO is analyzed using spectrophotometry. Results of spectrophotometric analysis showed increasing concentrations of oryzanol before dewaxing and degumming process, shown in Table 2 below.

Table 2

Results Measurement and Calculation of Concentration Absorbance CRBO and Dewaxed Degummed RBO

Minyak	Konsentrasi Oryzanol (%)
CRBO	0,855
Dewaxed Degummed RBO	1,158

III.3 Comparison of the Solvent Ratio (Methanol / Aceton) and Total CRBO and DDRBO (solvent / RBO or DDRBO: 40/1, 60/1 and 80/1) in the First Crystallization Process

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III.3 Comparison of the influence of solvent (methanol / acethon) and Total CRBO and Dewaxed certain degummed RBO (solvent / RBO: 40/1, 60/1 and 80/1) in the First Crystallization Process

In the first stage of step Crystallization, used 2 types of oil, which were CRBO and DDRBO to study the increasing of oryzanol concentration from both types of oil. So at the end of the first step Crystallization, we could get oryzanol poor solid phase (SP1) and the filtrate,which have separated from the solvent with distillation, so it could form oryzanol rich product (LP1), for each CRBO and DDRBO. Then from the analysis through UV-Vis Spectrophotometer, we got result showed in Figure 2 and Figure 3 below.

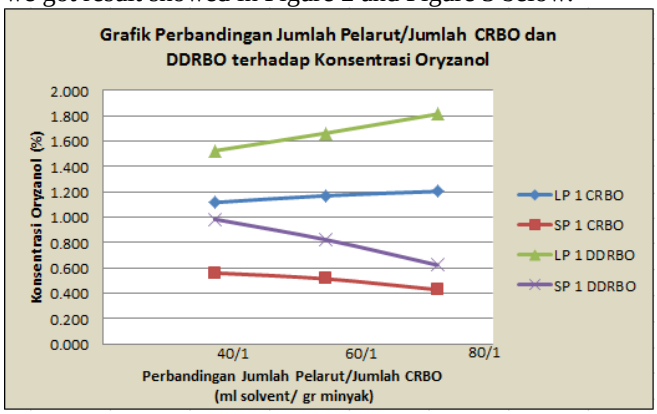

Figure 2. Comparison of Solvent Ratio to the Concentrations of Oryzanol in CRBO and DDRBO

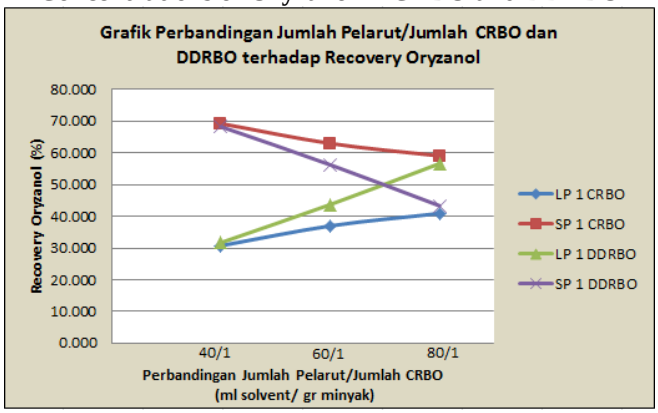

Figure 3. Comparison of Solvent Ratio to the Recovery of Oryzanol in CRBO and DDRBO

From Figure 2 and Figure 3 above, for CRBO and DDRBO with variable of solvent / CRBO and DDRBO ratio (40/1, 60/1 and 80/1) shows that for oryzanol rich product (LP1),so if the ratio of solvent / CRBO and DDRBO was great, concentration and recovery oryzanol would be be greater.

While for the poor oryzanol solid phase (SP1), it showed that the greater the ratio of solvent / CRBO and DDRBO,concentration and recovery oryzanol getting smaller.

This could occur because the larger the ratio of solvent / CRBO or DDRBO, the content of triglyceride (TG) were dissolved in oryzanol rich product (LP1) became smaller, so that the concentration of oryzanol in oryzanol rich product (LP1) could be increased. Basically the nature of triglyceride itself was a bit of a non-polar from oryzanol, so it was generally soluble in non-polar solution. But in the poor oryzanol solid phase (SP1), so if the ratio of solvent /CRBO or DDRBO was great, the content of triglyceride (TG) in the oryzanol poor dissolved solid phase (SP1) became greater, so that the concentration of oryzanol in oryzanol rich product (LP1) decreases. [9]

Next we tried to study the effect of temperature rise to increasing of oryzanol concentration. Therefore we did again the first step crystallization solvent with mixture ratio of mehanol/acetone (7:3), for comparison of CRBO and DDRBO with solvent/RBO and DDRBO was 80/1, and stored in a freezer at a temperature of -30^{O}C for 24 hours . At the end of the first stage of step Crystallization poor oryzanol obtained solid phase (SP1) and after distillation process, filtrate would have been separated from the solvent, thus forming oryzanol rich product (LP1). The results as shown in Table 3 and Table 4 as follows:

Table 3

Calculation results for CRBO Concentration and Recovery of First Step Crystallization with temperature -30 ^{O}C

Fase	Perbandingan 80/1	
	Konsentrasi Oryzanol (%)	Recovery (%)
LP1	1,229	43,75
SP1	0,547	56,25

Table 4

Calculation results for DDRBO Concentration and Recovery of First Step Crystallization with temperature -30 ^{O}C

Fase	Perbandingan 80/1	
	Konsentrasi Oryzanol (%)	Recovery (%)
LP1	1,840	58,856
SP1	0,656	41,144

So based on the above data, when compared between LP1 and SP1 CRBO or DDRBO with variable ratio and the number of solvent/CRBO or DDRBO number of 80/1, at the end of the first stage of Crystallization step at -20^{O}C and -30^{O}C, the oryzanol concentration and recovery was increased.

Increasing of concentration and recovery oryzanol in RBO LP1 and DDRBO at -20^{O}C and -30^{O}C happened because the lower the crystallization temperature, the more oryzanol dissolved in the liquid phase (LP1), so the concentration and recovery oryzanol in the solid phase (SP1) on RBO and DDRBO could be reduced. [9]

In the present study, researchers tried to raise the temperature. In terms of temperature which was originally used in the first crystallization process by Siti Zullaikah [9] at -22^{O}C have been obtained oryzanol with purity and recovery of 8.47% and 71.95% on LP1, but the highest levels of oryzanol obtained at lower temperatures 60^{O}C, with purity of oryzanol 13.68% on LP1, while the recovery dropped to 63.34%. While from the results obtained in this

study, for crystallization temperature at -20^{O}C with solvent ratio 80/1,oryzanol on CRBO with purity and recovery of 1.204% and 40.917% on LP1, and for oryzanol content on DDRBO with solvent ratio 80/1 obtained the purity and recovery of 1.814% and 56.642% on LP1. And oryzanol content in LP1 CRBO with solvent ratio 80/1 with purity and recovery of 1.229%% and 43.75%, respectively, was obtained when the temperature is lowered by up to -30^{O}C, while oryzanol content in LP1 DRBO with solvent ratio 80/1 with purity and recovery of 1.840% and 58.856%. With the results shown, if we decreased the temperatures from-20^{O}C to-30^{O}C, it would made impact on oryzanol levels and recovery, although the slight increased in temperature could give far enough results.

At the initial hypothesis that the raising of the first crystallization temperature at -60^{O}C to -30^{O}C then -20^{O}C, we hoped that the purity and recovery of oryzanol would be increased. The results obtained the same result, but the increase is still far from previous studies, and unfortunately oryzanol crystal formation is not happened in this study.

Therefore, the purpose of improvement here still did not work because when compared to previous research, the purity and recovery of oryzanol could still be higher by the raising temperature.

III.4 Effect of Storage Oryzanol Rich Product (LP1) in the First Step Crystallization Temperatures at 10^{O}C For 24 Hours

After the first step crystallization, we got products such as oryzanol rich product (LP1) for CRBO and DDRBO, next we kept it in the temperature that has been determined (refrigerator temperature 10^{O}C) for 24 hours to allow the growth of oryzanol crystal . And subsequently measured levels of oryzanol recovery process to determine the effect of such storage.

From the experiment obtained oryzanol rich product (LP1) of CRBO and DDRBO from first step crystallization which can be seen in Table 7 as follows.

Table 7

Results of Purity and Recovery for First Step Crystallization results CRBO at -20 ^{O} C after storage at 10 ^{O} C For 24 Hours

(ml) Solvent (Methanol/Acetone) (gr) CRBO	LP1	
	Konsentrasi Oryzanol (%)	Recovery (%)
40/1	1,192	39,50
60/1	1,318	54,17
80/1	1,534	79,50

From Table 7 above, for oryzanol rich product (LP1) CRBO with variable proportions of solvent / number CRBO (40/1, 60/1 and 80/1) shows that for oryzanol rich product (LP1) the greater the ratio of solvent / number CRBO oryzanol concentration and greater recovery, ie the ratio of the amount of solvent / CRBO number 80/1, with oryzanol concentration of 1.534% and recovery of 79.50%, and an increase compared with oryzanol rich product (LP1) CRBO before storage at 10 ^{O} C for 24 hours.

While the effect of oryzanol rich storage product (LP1) for the first step Crystallization DDRBO results can be seen in Table 8 as follows.

Table 8

Concentration Calculation Results and Recovery for First Step Crystallization results DDRBO -20 ^{O} C after storage at 10^{O}C for 24 Hours

(ml) Solvent (Methanol/Acetone) (gr) DDRBO	LP1	
	Konsentrasi Oryzanol (%)	Recovery (%)
40/1	1,735	49,815
60/1	1,830	58,057
80/1	2,098	81,181

From Table 8 above, for oryzanol rich product (LP1) DDRBO with variable proportions of solvent / number DDRBO (40/1, 60/1 and 80/1), it is seen that the greater the ratio of solvent / concentration and recovery DDRBO number oryzanol greater, ie the ratio of the amount of solvent / DDRBO number 80/1, oryzanol concentration of 2.098% and the recovery was 81.181%, and an increase compared with oryzanol rich product (LP1) DDRBO before stored at 10^{O} C for 24 hours.

While to compare the concentration and recovery oryzanol comparison between LP1 stage results first step ^{O} Crystallization temperature of -30 C to CRBO and DDRBO with variable proportions of solvent / CRBO number 80/1 before and after storage at 10 ^{O} C, the results are as follows:

Table 9

Concentration Calculation Results and Recovery for First Step Crystallization results CRBO -30 ^{O} C after storage at 10 ^{O} C For 24 Hours

(ml) Solvent (Methanol/Acetone) (gr) CRBO	LP1	
	Konsentrasi Oryzanol (%)	Recovery (%)
80/1	1,551	81,424

So based on the above data, when compared between LP1 stage results first step Crystallization temperature of -30 ^{O} C for CRBO with variable proportions of solvent / number CRBO 80/1 before and after storage at 10^{O}C, then obtained an increase in the concentration and recovery oryzanol compared with LP1 CRBO before stored at 10^{O}C for 24 hours.

Then for the concentration and recovery oryzanol Dewaxed degummed RBO with variable proportions of solvent / CRBO number 80/1 after storage at 10 ^{O} C, the results are as shown in Table 10 as follows:

Table 10

Concentration Calculation Results and Recovery for First Step Crystallization results DDRBO -30 ^{O} C after storage at 10 ^{O} C For 24 Hours

(ml) Solvent (Methanol/Acetone) (gr) DDRBO	LP1	
	Konsentrasi Oryzanol (%)	Recovery (%)
80/1	2,109	82,109

So based on the above data, when compared between LP1 stage results first step Crystallization temperature of -30^{O}C for DDRBO with variable proportions of solvent / number DDRBO 80/1 before and after storage at 10^{O}C, then obtained an increase in the concentration and recovery oryzanol compared with LP1 DDRBO before stored at 10^{O}C for 24 hours.

After the Second Step Crystallization whole process, it did not work oryzanol crystals formed. Filtration results obtained from the filtrate (LP2) with solid precipitate (SP2) is not close to the characteristics of the crystal near

oryzanol which is not characteristic of a white crystalline oryzanol.

Basically crystal formation occurs in a supersaturated state or condition through 2 stages, ie nucleation and crystal's growth.

Supersaturated condition of a solution affects the solubility oryzanol in solution. If the solubility is still large (unsaturated conditions), the crystals can not be formed or shaped small crystals formed. In this case, the temperature is a factor that affects the saturated solution. The lower the temperature, the faster the solution reaches saturation conditions (saturated conditions). And if the condition of a solution has been reached over saturated by (super-saturated conditions) then the crystals formed therein. [19]

What happens is not yet formed crystalline oryzanol (crystallization has not occurred), in the sense that there is a solution that has not yet reached a state of saturation through. Nucleation process that occurs can not be entered on the crystal growth phase as the temperature is still not able to make it past the saturation point solution. In the sense that the temperature used should be lower than the temperature of-200C used in this study, in order to achieve supersaturated condition that oryzanol crystals can be formed.

IV. CONCLUSION

1. Suitable solvent for the extraction of rice bran oil is ethanol solvent because it provides the largest oryzanol concentration up to 1.258%.
2. Factors affecting purity and recovery of oryzanol include:
   - Proses dewaxing and degumming dengan menghilangkan sejumlah wax dan gum dapat memberikan pengaruh terhadap kadar oryzanol yang terkandung pada RBO.
   - Dewaxing and degumming process which decreased the content of wax and gum could give effect to the oryzanol content in RBO.
   - Comparison of the solvent (methanol/ acetone) to RBO and DDRBO ratio at first crystallization. In this case, the higher the use of solvents, the higher result are also obtained. Comparison of selected is 80:1.
   - At lower temperature (-30^{0}C) could give impact to increasing the purity of oryzanol, and higher than temperature -20^{0}C.
3. Oryzanol crystall could not be formed at temperature -20^{0}C.

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