Journal of Food Research; Vol. 4, No. 2; 2015
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Rice Bran Oil Distillate, a Choice for γ-Oryzanol: Separation and Oxidative Stability Study

Swapnil G. Jaiswal[1], Subhalaxmi Pradhan[1, 2], Madhumita Patel[1], Malaya Naik[1] & Satyanarayan Naik[1]

[1] Center for Rural Development and Technology, Indian Institute of Technology, Hauz Khas, New Delhi, India

[2] Department of Chemistry, Dhenkanal Autonomous College, Utkal University, Odisha, India

Correspondence: Satyanarayan Naik, Center for Rural Development and Technology, Indian Institute of Technology, Hauz Khas, New Delhi-110016, India. Tel: 91-11-2659-1162. E-mail: naiksn@gmail.com

Received: September 2, 2014 Accepted: December 1, 2014 Online Published: December 16, 2014

doi:10.5539/jfr.v4n2p36 URL: http://dx.doi.org/10.5539/jfr.v4n2p36

Abstract

Rice bran oil distillate is one among the secondary products produced during refining of rice bran oil. Rice bran oil distillate is a source of several micronutrients and natural antioxidants like γ-oryzanol, tocopherols etc. The aim of the present study was to separate γ-oryzanol from distillate and utilize it as a stabilizer for edible oil. In order to achieve this aim crystallization process was applied to obtain oryzanol rich concentrate. Further purification of oryzanol was achieved through column chromatography. Fractions of γ-oryzanol were quantified through HPLC which gives 0.83% yield. Separated γ-oryzanol was used to study the stability of pea nut and linseed oil using rancimat. The experiments were carried out in rancimat at varying temperature (110-130 ºC) and concentration (100-300 ppm). Stability of both the oil was found to be directly proportional to the γ-oryzanol concentration and inversely proportional to the temperature.

Keywords: γ-oryzanol, natural antioxidant, purification, oxidative stability, rancimat

1. Introduction

Fat is an essential component of our daily diet which gives us energy and helps in absorption of fat soluble vitamins like A, D, E and K. It is also required for maintaining a healthy balance between high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol (Katan et al., 1994). However the quality and quantity of fat consumed is very crucial from health point. Consumption of excess amount of modified fats like *trans* and hydrogenated fats leads to different chronic diseases such as cardiovascular disease, obesity and many other major health problems. Therefore it is important to introduce healthy oil into our daily diet. Rice bran oil (RBO) is well recognised as a healthy oil due to the natural occurrence of different antioxidants like tocopherol, tocotrienol, oryzanol and squalene as well as their ideal Saturated Fatty Acid/Monounsaturated Unsaturated Fatty Acid/Poly Unsaturated Fatty Acid ratio which is nearest to the range of WHO recommendations (Rukmini & Raghuram, 2013). Several studies have been reviewed in past on the different aspects of rice bran oil like extraction, refining, separation and purification of value added chemicals, application in different areas and health benefits (Patel & Naik 2004; Narayan et al., 2006). γ- oryzanol is a novel value added compound due to its unique antioxidant properties which stabilises food and pharmaceutical raw materials (Sapino et al., 2013).

India is the second largest rice producer after China and contributes 22% of total world rice production. In 2013-14 about 67% of paddy was processed to rice. However only 6% bran could be recovered and processed (The Solvent Extractors Association of India). Rice bran is highly sensitive to oxidation due to presence of lipase enzyme and therefore timely collection, stabilization and processing are the crucial factors for production of edible grade rice bran oil. Therefore a major chunk of the bran is used for production of commercial grade non edible oil which is utilized in various sectors like biodiesel, cosmeceuticals etc. (Ju et al., 2009; Lerma-Garcia et al., 2009). Crude rice bran oil (CRBO) produced by hexane extraction contains wax, phospholipids, free fatty acids and other pigments. Purification of this oil either by physical or chemical process results in a great loss of essential constituents like γ- oryzanol which is reduced to 2% in the refined edible grade oil (Pestana-Bauer et al., 2012).The rate of loss of γ- oryzanol in various steps of refining has been discussed by Gopala Krishna et al. (2001).

Separation and utilization of these wastes to valorise in any food pharmaceutical product build up a pressure on industries and researchers. In order to overcome from this problem present study has been designed. γ- oryzanol is an important source of natural antioxidant compound containing group of esters of the *trans*-ferulic acid. Application of such a recovered food grade natural antioxidant compound from industrial waste could prove to be the promising source in the fulfilment of the consumer demand and foster applied research in the area of natural antioxidants to increase the shelf life of food. Thermal and storage stability of ground nut oil and biscuit by addition of oryzanol has already been reported (Kumar et al., 2012; Chandrashekar et al., 2012). However, there was no report on the γ- oryzanol extraction from the waste of rice bran oil industry and its utilization as an antioxidant to increase the stability of edible oil. The aim of present study was the separation and purification of γ- oryzanol from the waste of rice bran oil distillate and to study its antioxidant potential in stability of unsaturated edible oils. Oxidative stability experiments were carried out in the rancimat and results were compared with synthetic antioxidants like BHA (Butylated hydroxyanisole), BHT (Butylated hydroxytoluene) and TBHQ (Tert-butylhydroquinone).

2. Materials and Methods

2.1 Materials

Rice bran oil distillate (waste of rice bran oil during distillation process) was obtained from rice bran oil industry situated in Delhi NCR. Reaction solvents like methanol, acetone, chloroform, sulphuric acid, hexane, ethyl acetate, acetonitrile, acetic acid and standards of synthetic antioxidants (BHA, BHT) were purchased from Merck (Darmstadt, Germany). TBHQ was from Acros organics (New Jersey, USA). γ- oryzanol standard was purchased from TCI Chemicals Pvt. Ltd. (Tokyo, Japan).

2.2 Gums, Waxes Removal and Crystallization

Some impurities which remained in the form of gums and waxes at the time of refining were removed by following the process described earlier with slight modification in terms of raw material (Zullaikah et al., 2009). After that rice bran oil distillate rich in γ- oryzanol was taken as raw material for the two steps crystallization as mentioned by Zullaikah et al., 2009. Oryzanol rich concentrate obtained was further purified through column chromatography to get pure γ- oryzanol.

2.3 Column Chromatographic Separation

To remove further triglycerides and lipid fractions, oryzanol rich concentrate was subjected to column chromatography. Partially purified oryzanol rich concentrate was adsorbed using silica gel and loaded into glass column (2.5 × 25 cm) packed with 20 g silica gel (100-200 mesh). The column was first eluted with hexane, then polarity was changed by adding ethyl acetate. Gradient program of solvent with changing polarity was used for the chromatographic separation as shown in Table 1. All the fractions of 50 mL each were eluted from the column and finally column was washed with ethyl acetate.

Table 1. Gradient program of solvent system used for γ- oryzanol separation

Sr. No.	% Ethyl acetate used	Ratio of solvent (mL)	
		Hexane	Ethyl acetate
1	0	300	-
2	5	285	15
3	10	270	30
4	20	240	60
5	30	210	90
6	50	150	150

2.4 HPLC and TLC Analyses

Oryzanol rich fractions were qualitatively monitored by TLC using 10% $CHCl_3$-CH_3OH as mobile phase. Oryzanol content was quantified by high performance liquid chromatography (Waters 600 HPLC system) equipped with photodiode array detector (Waters 2998) and ODS C18 column (4.6 × 250 mm) with 5 μm particle size. The standards were dissolved in chloroform and eluted with mobile phase, methanol, acetonitrile,

acetic acid in the ratio of 52:45:3 with a flow rate of 1 mL/min. The total run time is 45 min. The samples were also analysed by HPLC following same program. Absorbance of the chromatograms were recorded at 325 nm. Results are shown as mean ± standard deviation. Crude rice bran oil was also analysed in HPLC to know the γ-oryzanol concentration.

2.5 Extraction of Pea Nut and Linseed Oil

Pea nut and linseed were procured from the local market at Delhi. Crude oil required for the oxidative stability having no addition of antioxidant was extracted by mechanical expression process. Approximately 1 kg oilseeds were processed to obtain 40 g fresh oil for each rancimat experiments.

2.6 Oxidative Stability Study of γ- oryzanol

Antioxidant activity of γ- oryzanol in pea nut and linseed oil was evaluated by using rancimat 743 (Metrohm, AG Switzerland) which is capable over an operating range of 50-200 ºC. This method is the modified version of active oxygen method (AOM) method. To know the induction period (IP) of control sample and sample with antioxidant, all the experiments were carried out at three varying temperatures (110-130 ºC), three different concentrations of γ- oryzanol, (100-300 ppm) and air flow rate 20 L/hr. The results of oxidative stability were also compared with synthetic antioxidants (BHA, BHT and TBHQ) by following the above mentioned conditions. 5 g of linseed/peanut oil was introduced in each reaction vessel at the time of experiment. Effect of antioxidants were expressed in terms of antioxidant index calculated by the formula:

$$\textbf{\textit{Antioxidant index (AI)}} = IP_s / IP_c.$$

Where, IP_s= Induction period of sample (with antioxidant); IP_c= Induction period of control (without antioxidant)

2.7 Statistical Analyses

All data were subjected to statistical analyses. Three replicates of each treatment were examined. Values of different parameters were expressed as the mean ± standard deviation. Coefficient of variation was also calculated using statistical software package SPSS version 16.0.

3. Results and Discussion

3.1 Yield of Oryzanol Rich Concentrate During Crystallization

Crude rice bran oil is dark in colour due to presence of gums and lower amount of waxes (Rajam et al., 2005). γ-oryzanol was recovered from the rice bran oil distillate by following the process of Zullaikah et al., 2009. Nearly 1.9% of oryzanol rich concentrate was recovered after crystallizing the distillate at -60 ºC for 15 hours. The percentage of oryzanol reported here is slightly lower than the earlier report (Zullaikah et al., 2009). It may be due to the loss of γ- oryzanol during the different steps of refining prior to deodorization. The results of second step crystallization are also different from Zullaikah et al. 2009, due to its lower percentage in first crystallization. So, in order to recover more γ- oryzanol from oryzanol rich concentrate, it was subjected to column chromatography.

3.2 Yield of Oryzanol Rich Concentrate During Column Chromatographic Separation

Oryzanol rich concentrate was loaded in the silica gel column by preparing sample in the 0.01% silica. The fractions were eluted by changing polarity of solvent and simultaneous TLC analyses which gave the major spot with standard at R_f value 0.85. The purified fraction from column chromatography contain 0.83% of γ- oryzanol which is obtained from HPLC analysis (Table 2 and Figure 1). The result is comparable to the results reported for γ- oryzanol yield (0.84-1.14%) from deodorized distillate through molecular distillation (Sawadikiat et al., 2014). All the results related to the concentration of γ- oryzanol and its major individual components like cycloartenylferulate (1), *24*-methylene cycloartanylferulate (2), Campesterylferulate (3) and Sitosterylferulate (4) are summarized in Table 2. Results of γ- oryzanol from crude rice bran oil was also compared with deodorized distillate to know its concentration before and after refining. The concentration of γ- oryzanol in rice ban oil distillate was found to be less (0.83%) than that of crude rice bran oil (1.48%). Our results are in agreement with the results reported by other researchers for rice bran oil (1.1-1.7%, 1.6%) and rice bran oil distillate (0.79%, 0.8-%) (Gopala Krishna et al., 2001; Sawadikiat et al., 2014).

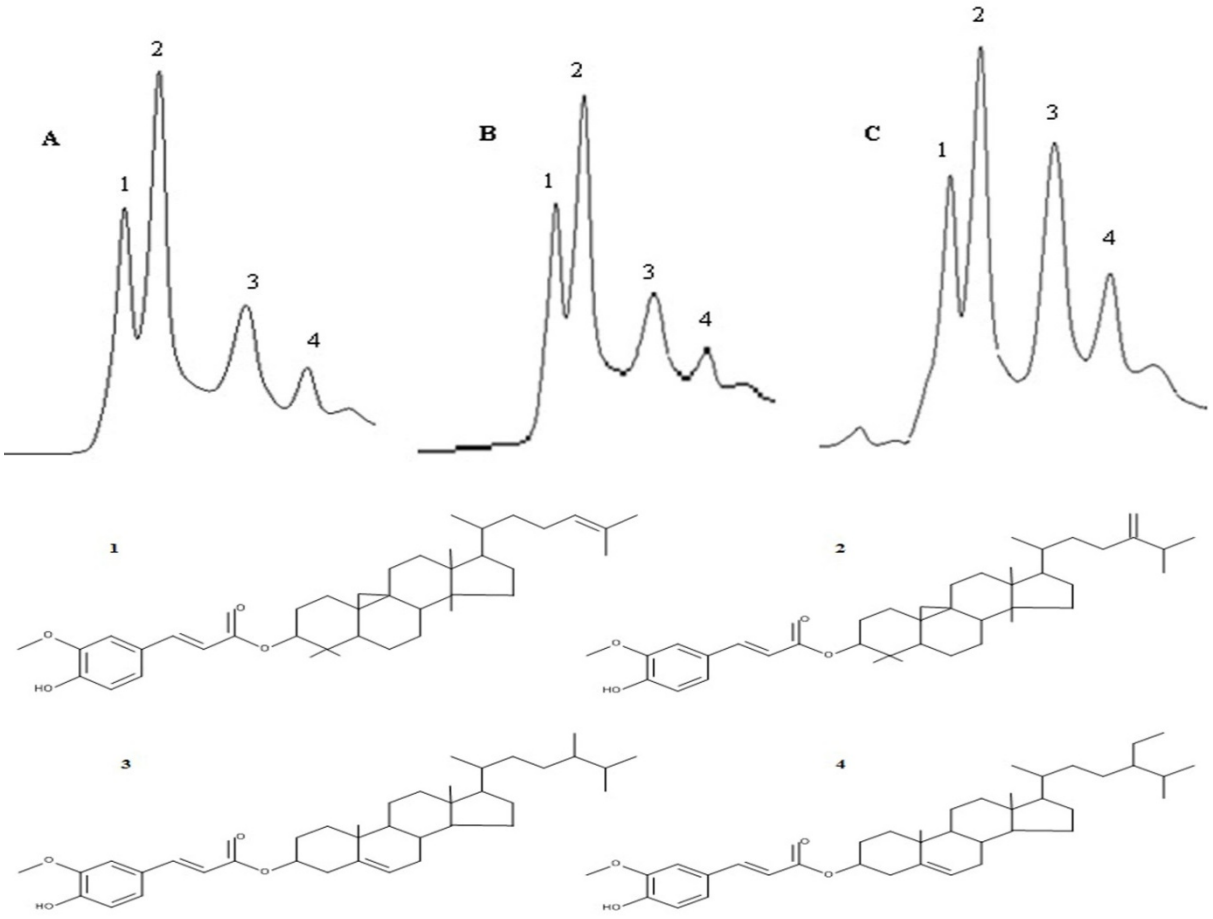


Figure 1. High performance liquid chromatographic analyses of (A) γ- oryzanol standard, (B) chromatographic fractions of rice bran oil distillate, (C) crude rice bran oil and individual components of γ- oryzanol 1. Cycloartenylferulate, 2. *24*-methylene cycloartanylferulate, 3. Campesterylferulate, 4. Sitosterylferulate

Table 2. Composition of individual component of γ- oryzanol

Sample	components	RT*	Concentration		Total concentration (g/100 g, %)
			g/100 g	%	
Oryzanol standard	1	25.94	25.30	25.30	100, 100
	2	27.32	40.43	40.43	
	3	31.00	22.21	22.21	
	4	33.89	11.83	11.83	
Rice bran oil	1	25.78	0.46	31.56	1.39, 1.48
	2	27.70	0.58	39.22	
	3	31.65	0.18	12.29	
	4	33.63	0.16	10.94	
Oryzanol fractions	1	26.02	0.23	27.78	0.81, 0.83
	2	27.58	0.32	39.43	
	3	31.83	0.19	23.42	
	4	33.23	0.06	7.45	

All values are means ± SD, *n=2* , * = Retention time (minute).

3.3 Oxidative Stability Study of Vegetable Oils

In order to know the stability of purified γ- oryzanol, rancimat test was performed with the selection of pre refined vegetable oil as a source of raw material. Peanut and Linseed oil are used for the study due to their low oxidation stability. These oils are susceptible to oxidation because of their fatty acid composition and position of double bonds in the fatty acid structures as described in the previous literature (Knothe, 2002).

The rancimat induction period (RIP) of antioxidant added (100 ppm) peanut oil at 110 ºC was follow the order, control (6.05±0.46) < γ- oryzanol (6.10±0.03) < BHA (7.63±0.08) < BHT (8.60±0.06) < TBHQ (9.90±0.02). Same trend of results were also found in the case of increasing temperature and concentration. All the mentioned values are the data of three replicates. There was no big difference observed in the induction period of γ-oryzanol at the concentration of 100 ppm but shows positive antioxidant effect. The poor performance may be increased by increasing concentration of γ- oryzanol in oil. Our results were supported by the study of Juliano et al., 2005, where dose dependant antioxidant activity of γ- oryzanol was studied to increase the stability of pea nut oil. Besides that linseed oil was found to be more prone towards oxidation as compared to pea nut oil. There is about 9% increase in stability using γ- oryzanol as an antioxidant. The increasing order of stability of externally added antioxidants in the linseed oil is as follows.

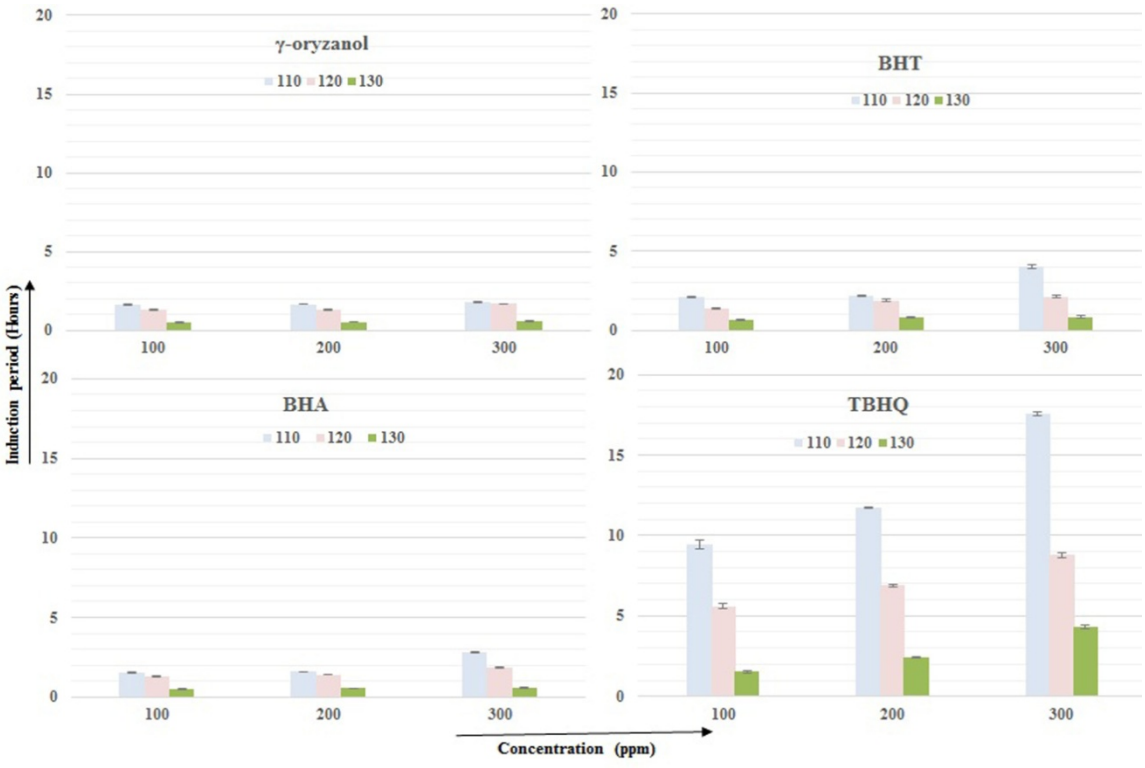
Control (1.50±0.04) < BHA (1.53±0.04) < γ- oryzanol (1.63±0.02) < BHT (2.09±0.07) < TBHQ (9.45±0.25)

Similar trend of results were again obtained at varying temperature and concentrations. From the above results for pea nut and linseed oil, it was confirmed that pea nut oil is more stable than that of linseed oil and antioxidants effect except BHA were same.

3.3.1 Effect of Varying Temperature and Concentration on Stability of Oil

The Figure 2 [A], [B] shows the effect of varying temperature (110-130 ºC) and concentration (100-300 ppm) of BHA, BHT, TBHQ and γ- oryzanol on the oxidative stability of linseed oil and pea nut oil. From the results it was clear that as the temperature increases the stability of oil decreases. The increase in temperature from 110 ºC to 130 °C results nearly 50% reduction of induction time. This type of result trend is in good agreement with the previous literature. On the other hand as the concentration of antioxidants increases, the induction period of oil increases and decreases with increase in temperature (Xin et al., 2009). The results from the figure confirms the increasing trend of induction period with increasing concentration.

A



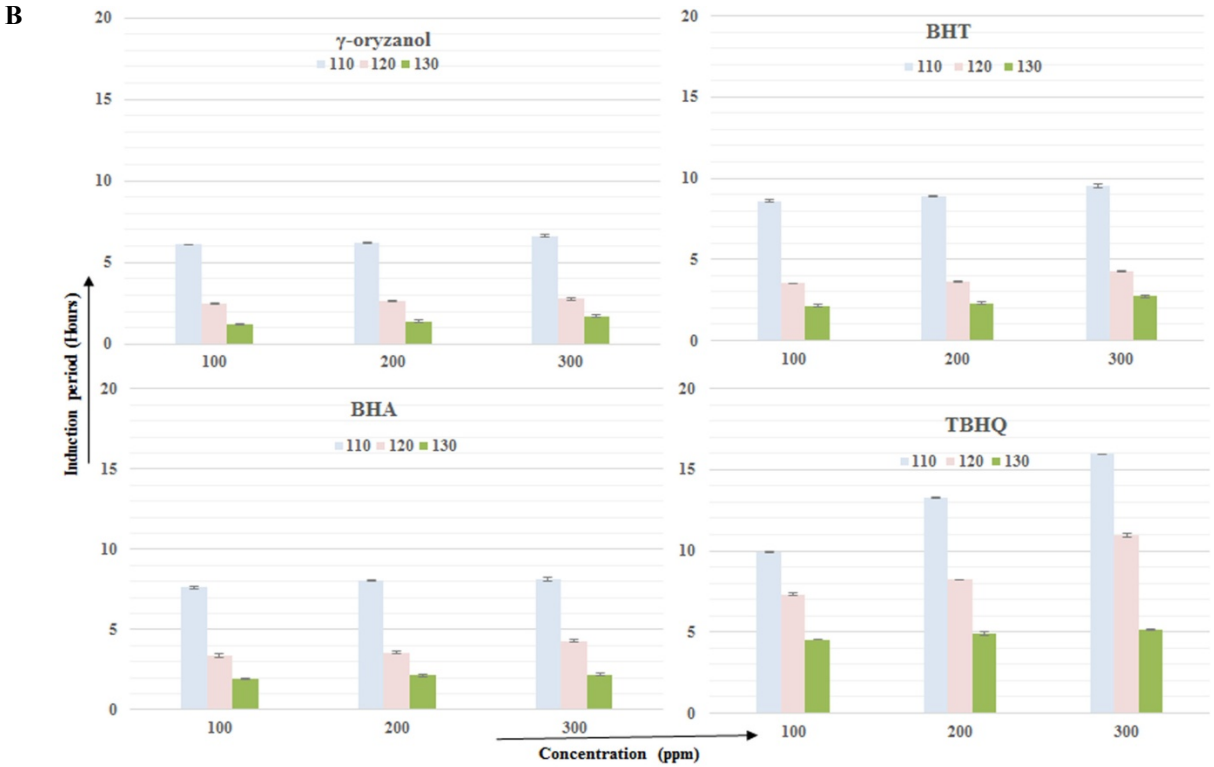


Figure 2. Effect of varying temperature (110-130 ºC) and concentration (100-300 ppm) of BHA, BHT, TBHQ and γ- oryzanol on the oxidative stability of [A] Linseed oil [B] Pea nut oil

Table 3. Effect of temperature and antioxidant concentration on antioxidant index and stability of oil

		110 ºC			120 ºC			130 ºC		
Oil	Antioxidant									
		100*	200*	300*	100*	200*	300*	100*	200*	300*
Linseed	γ-oryzanol	1.09±0.02 (1.9)	1.10±0.05 (4.37)	1.19±0.05 (3.96)	1.02±0.00 (0.44)	1.03±0.00 (0.45)	1.31±0.01 (0.72)	1.06±0.02 (2.00)	1.13±0.04 (3.90)	1.22±0.33 (2.66)
	BHA	1.02±0.01 (0.71)	1.06±0.04 (4.24)	1.87±0.04 (2.19)	1.01±0.02 (1.79)	1.11±0.03 (2.30)	1.45±0.03 (2.23)	1.06±0.04 (4.19)	1.15±0.06 (4.90)	1.26±0.03 (2.57)
	BHT	1.39±0.08 (5.71)	1.44±0.08 (5.51)	2.68±0.00 (0.14)	1.09±0.01 (1.03)	1.47±0.07 (4.86)	1.66±0.06 (3.68)	1.39±0.05 (3.85)	1.69±0.07 (4.05)	1.81±0.10 (5.39)
	TBHQ	6.30±0.35 (5.62)	7.81±0.22 (2.83)	11.71±0.44 (3.78)	4.39±0.16 -3.75	5.40±0.08 (1.40)	6.91±0.14 (2.10)	3.24±0.13 (4.01)	5.18±0.04 (0.86)	9.21±0.26 (2.77)
Pea nut	γ-oryzanol	1.01±0.08 (7.78)	1.03±0.08 (7.99)	1.10±0.09 (8.23)	1.04±0.10 (9.78)	1.09±0.11 (10.24)	1.15±0.13 (10.96)	1.02±0.10 (10.19)	1.17±0.18 (15.82)	1.42±0.23 (15.95)
	BHA	1.27±0.08 (6.57)	1.33±0.11 (8.28)	1.35±0.11 (8.26)	1.40±0.08 (5.91)	1.49±0.16 (10.49)	1.79±0.18 (9.90)	1.60±0.16 (10.13)	1.80±0.26 (14.49)	1.85±0.28 (15.23)
	BHT	1.43±0.10 (6.94)	1.48±0.10 (7.02)	1.58±0.13 (8.36)	1.46±0.11 (7.72)	1.52±0.15 (9.83)	1.79±0.17 (9.48)	1.80±0.16 (8.96)	1.93±0.26 (13.69)	2.28±0.32 (14.10)
	TBHQ	1.65±0.13 -7.64	2.20±0.17 -7.93	2.64±0.20 -7.58	3.05±0.28 -9.16	3.43±0.29 -8.58	4.58±0.43 -9.47	3.80±0.45 -11.74	4.08±0.54 -13.35	4.30±0.52 -12.02

All values are expressed as antioxidant index ± standard deviation, (n = 3) and coefficient of variation (% RSD); * = concentration in ppm.

3.3.2 Antioxidant Index

Table 3 summarizes all data related to the effect of antioxidants in oil at different temperatures and varying concentrations in terms of their mean value, standard deviation and relative standard deviation. The range of RSD for linseed and pea nut oil was from 0.14-5.71 and 5.91-15.95 respectively. From the results it can be concluded that the results in rancimat is repetitive to get more accuracy. Overall the antioxidant index of TBHQ was obtained higher in the both the oil at increasing concentration and decreasing temperature except pea nut oil where concentration of 300 ppm at 130 ºC gives the value of antioxidant index 4.30±0.52. The results for TBHQ, BHT and BHA were comparable with the results reported by Gordon and Lenka (1995) for synthetic antioxidants. The antioxidant index of γ- oryzanol was found to be lower as compared to synthetic antioxidants but it can be comparable with BHA at higher concentration and lower temperature in both the oils. No pro oxidants effect was found in the table whose antioxidant index is less than one. From rancimat results it can be concluded that the antioxidant index of γ- oryzanol can be increased by increasing concentration to achieve the antioxidant index of synthetic antioxidants. More study should be required in order to stabilize γ- oryzanol in edible oil which is the base of all lipid based food materials.

4. Conclusion

In the present study γ- oryzanol was purified from rice bran oil distillate by crystallization followed by separation through column chromatography. From HPLC analysis it is found that, rice bran oil distillate, which is a waste of oil industry contains 0.83% of γ- oryzanol. This study provides the information that rice bran oil industry waste can be used as a source of value added chemicals. Adding γ- oryzanol to pea nut and linseed oil increases the stability of these oils similar to that of synthetic antioxidants like BHA and BHT. Rice bran oil distillate contain small amount of γ- oryzanol but results of their use as an antioxidant gives the positive sign on their usage in food to increase its shelf life. γ- Oryzanol purified from oil industry waste and used as a better antioxidant to replace synthetic antioxidant will improve the economics of oil industry. Future work is required to standardise the concentration of γ- oryzanol which will be equivalent to the synthetic antioxidant TBHQ. From this study it can be concluded that rice bran oil distillate, waste of oil industries can be reutilized for purification of γ- oryzanol, which could be the important source of natural antioxidants for food, pharmaceutical and cosmeceutical industries.

Acknowledgements

Financial support from the Ministry of Food Processing Industries (MOFPI), India is greatly acknowledged.

References

Chandrashekar, P., Kumar, P. P. K., Ramesh, H. P., Lokesh, B. R., & Gopala-Krishna, A. G. (2012). Hypolipidemic effect of oryzanol concentrate and low temperature extracted crude rice bran oil in experimental male wistar rats. *J. Food Sci. Technol*.

Gopala Krishna, A. G., Khatoon, S., Shiela, P. M., Sarmandal, C. V., Indira, T. N., & Mishra, A. (2001). Effect of refining of crude rice bran oil on the retention of oryzanol in the refined oil. *J. Am. Oil Chem. Soc.,* 78(2), 127-131. http://dx.doi.org/10.1007/s11746-001-0232-0

Gordon, M. H., & Lenka, K. (1995). The effect of antioxidants on changes in oils during heating and deep fat frying. *J. Sci. Food Agric., 68*, 347-353. http://dx.doi.org/10.1002/jsfa.2740680314

Ju, Y. H., & Rayat, (2009). ACME. Biodiesel from rice bran oil. In A. Pandey (Ed.). *Handbook of plant based biofuels* (pp. 241-54). Boca Raton: CRC Press.

Juliano, C., Cossu, M., Alamanni, M. C., & Piu, L. (2005). Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils. *Int. J. Pharm., 299*, 146-154. http://dx.doi.org/10.1016/j.ijpharm.2005.05.018

Katan, M. B., Zock, P. L., & Mensink, R. P. (1994). Effects of fats and fatty acids on blood lipids in humans: An overview. *Am. J. Clin. Nutr., 60*, 1017S-1022S.

Knothe, G. (2002). Structure Indices in FA Chemistry. How Relevant Is the Iodine Value? *J. Am. Oil Chem. Soc., 79*(9), 847-854. http://dx.doi.org/10.1007/s11746-002-0569-4

Kumar, P. P. K., Manohar, R. S., Indiramma, A. R., & Gopala Krishna, A. G. (2012). Stability of oryzanol fortified biscuits on storage. *J. Food Sci. Technol.,*

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

389-404. http://dx.doi.org/10.1016/j.foodchem.2009.01.063

Narayan, A. V., Barhate, R. S., & Raghavarao, K. S. M. S. (2006). Extraction and purification of oryzanol from rice bran oil and rice bran oil soapstock. *J. Am. Oil Chem. Soc., 83*(8), 663-670. http://dx.doi.org/10.1007/s11746-006-5021-2

Patel, M., & Naik, S. N. (2004). Gamma-oryzanol from rice bran oil – A review, *J. Sci. Ind. Res., 63*, 569-578.

Pestana-Bauer, V. R., Zambiazi, R. C., Mendonca, C. R. B., Beneito-Cambra, M., & Ramis-Ramos, G. (2012). γ-oryzanol and tocopherol contents in residues of rice bran oil refining. *Food Chem., 134*, 1479-1483. http://dx.doi.org/10.1016/j.foodchem.2012.03.059

Rajam, L., Kumar, S. D. R., Sundaresan, A., & Arumughan C. (2005). A Novel Process for Physically Refining Rice Bran Oil through Simultaneous Degumming and Dewaxing, *J. Am. Oil Chem. Soc., 82*(3), 213-220. http://dx.doi.org/10.1007/s11746-005-5174-4

Rukmini, C., & Raghuram, T. C. (2013). Nutritional and biochemical aspects of the hypolipidemic action of rice bran oil: A review. *J. Am. Coll. Nutr., 10*(6), 593-601. http://dx.doi.org/10.1080/07315724.1991.10718181

Sapino, S., Carlotti, M. E., Cavalli, R., Ugazio, E., Berlier, G., Gastaldi, L., & Morel, S. (2013). Photochemical and antioxidant properties of gamma-oryzanolin beta-cyclodextrin-based nanosponges. *J. Incl. Phenom. Macrocycl. Chem., 75*, 69-76. http://dx.doi.org/10.1007/s10847-012-0147-3

Sawadikiat, P., & Hongsprabhas, P. (2014). Phytosterols and γ- oryzanol in rice bran oils and distillates from physical refining process. *Int. J. Food Sci. Technol.,*

Xin, J., Imahara, H., & Saka, S. (2009). Kinetics on the oxidation of biodiesel stabilized with antioxidant. *Fuel, 88*, 282-286. http://dx.doi.org/10.1016/j.fuel.2008.08.018

Zullaikah, S., Melwita, E., & Ju, Y. H. (2009). Isolation of oryzanol from crude rice bran oil. *Bioresour. Technol., 100*, 299-302.