Inactivation of Lipase enzyme by using Chemicals to maximize Rice Bran Shelf Life and its Edible Oil Recovery

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Abstract— Rice bran is the by-product of the rice milling industries so in view of growing need and consciousness about the nutritional and functional properties the rice bran is very important co-product. In this research article the methods were described regarding different chemicals with different concentration in controlling the activity of lipase enzyme and ultimately to maximize its oil recovery from the rice bran during 60 days of storage in a room temperature. The use of hydrochloric acid at concentration of about 30ml/Kg helps greatly in controlling the lipase enzyme activity and reduces the % of FFA while the other chemicals used in this study (Phosphoric acid, Acetic acid, Sodium metabisulphite) failed to control in the rise of free fatty acid contents. The chemicals can be applied easily by sprinkling or spraying. This operation done on small rice bran lots through manual hand mixing. This method of chemical stabilization of rice bran is really a useful method in the rice mills where there is shortage of electricity or steaming facility.

Index Terms— Stabilization, HCL, Rice Bran, Lipase Enzyme, Oil Contents, Inactivation.

1 INTRODUCTION

Rice is an important food as well as cash crop of Pakistan. It is also a major export commodity and earns about US $ 2.2 billion foreign exchange annually. There is more than 750 million metric tons production of rice paddy annually all around the world (Iqbal et al., 2005). Rice is greatly consumed all over the world and used as a staple food product in different regions of the whole world due to its nutritional value (Anwar et al., 2005).

The grain of the rice contain about 2 to 3% fat, and this fat portion is mainly concentrated in the embryo or in the germ and then in the outer layer of the seed (Sastry et al., 1974). During milling the germ and the bran powder layers detached from the endosperm and this milling finally concentrate the fat portion into the residue generally said as "Rice Bran" (Yokochi, 1977). Rice bran is massive rice milling industry discard produced during rice polishing and mainly used in animal feed. It constitutes about 7-8% of the whole paddy grain which contributes about more than 60 million tons per annum of the whole world production (Renuka and Arumughan, 2007; Iqbal et al., 2005). Rice bran has great potential to contribute to supply edible oil to the world and it ranges about 10-26% of the rice bran depending upon degree of milling, climatic conditions and variety (Anwar et al., 2005; Chatha et al., 2006; Saunders, 1985).

Research in the last decades showed that rice bran is an outstanding source of vitamins, minerals, antioxidants, proteins, fats and dietary fibers (Moldenhauer et al., 2003). Several problems of raw bran management have restricted production of edible grade rice bran oil. One of the problem is high lipase activity in the bran, which quickly hydrolyzes the fats of oil into free fatty acids (Prabhakar and venkatesh, 1986). To resolve this main problem the use of proper technique or deactivation method is very important.

To date, several studies have been conducted on stabilization techniques of rice bran and its oil (Rao et al., 2004; Tao et al., 1993; Prabhakar, 1986). Although a number of studies like microwave heating, ohmic heating, dry or moist heat treatment and little bit on pH lowering have been conducted for rice bran and its oil stabilization (Pourali et al., 2009). However, treatments with different chemicals have not been properly used for this purpose.

The objective of this research work was to investigate the effect of using different chemicals for the stabilization of rice bran on lipase enzyme, which ultimately leads to enhance the shelf life of rice bran and then maximize its oil contents.

2 MATERIALS AND METHODS

Laboratory grade Hydrochloric acid, Phosphoric acid, Acetic acid and Sodium metabisulphite were purchased from Merck, Germany and Hexane (Solvent for Extraction) from Sigma-Aldrich, Germany in this present research work. Freshly milled rice bran powder samples were collected from our local rice mills situated in areas of Muridke District Sheikhupura and Kamoke, District. Gujranwala. Stabilization of rice bran was carried out by spreading rice bran in a layer of 5 cm thick and the required amount different chemicals (Hydrochloric acid used @ a rate of 20, 30 & 35ml/Kg, Phosphoric acid used @ 0.5, 1.0 & 1.5% per Kg, Acetic acid used @ 3, 5 & 7% per Kg and Sodium metabisulphite used at 1.5, 2.0 & 2.5% per Kg) were sprinkled on bran layers with different concentrations and mixed well by hand using protective clothing. Total 12 treatments were prepared with above mentioned acids or chemicals each with 3 treatments with different concentration. After completion of the treatments, all the rice bran samples were packed in locally made polyethylene bags. Then the packed rice bran samples were stored in a room temperature

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and analyzed it for free fatty acid % and oil extraction % after every 10 days interval upto 60 days of storage (Malekian et al., 2000).

2.1 Analytical Methods: Extraction of the oil contents of the samples were conducted on a laboratory scale Soxhlet extractor (Sayre et al., 1985). Analytical grade hexane was used and hexane temperature during extraction was about 60oC. Determination of free fatty acid (FFA) was done by alkalimetric titration (AOAC, 2006). The proximate analysis of chemically stabilized rice bran for moisture, protein, fats, crude fiber and ash contents were determined by (AOAC, 1984; AACC, 1976; ISO, 1981; Randall et al., 1985).

2.2 Fatty Acid Composition of the Treated Rice Bran Samples: First from the four chemicals, we select the best treatment from each chemical on the basis of rice bran oil FFA% and then covert this crude oil into refined form. The best treatments selected for the fatty acid composition analysis are as follows:

1. Hydrochloric Acid (HCL) @30ml/Kg
2. Phosphoric Acid @1.5% W/W
3. Acetic Acid @ 7% W/W
4. Sodium Metabisulphite @ 2% W/W

All the above treatments were analyzed for fatty acid analysis by using the method of (Young-Hee et al., 2002) in which the methyl esters of fatty acids were separated with a GC-17A (Shimadzu Co., Japan) column equipped with HP-20M (25 m × 0.32 mm, 0.3 µm) at the following temperature program: initial temperature of 180oC (10 min hold) to 200oC at 4/min (2 min hold) and to 220oC at 4/min (12 min hold). Identification of fatty acid methyl esters was made by comparing their relative retention times with that of known standard samples (linoleic acid, oleic acid, palmitic acid, stearic acid, arachidic acid, linolenic acid, eicosenoic acid, myristic acid and behanic acid).

2.3 Statistical Analysis: Analysis of variance of the data was computed using the Statistica computer program. The Least Significance Difference test at 5% level of significance was used to test the differences among mean values (Steel et al., 1997).

3 Results and Discussion

3.1 Proximate analysis of rice bran: The results of analysis regarding moisture, protein, fats, crude fiber and ash contents/percentage of procured rice bran from rice processing mills and local Sheller's were shown in table 1. The rice bran used in this research work selected from local rice Sheller's having good nutritional properties regarding proximate analysis with 11.2% moisture content, 16.87% protein contents, 20.20% rice bran fats, 8.29% crude fiber and 9.21% of ash contents. All these values were significantly different from proximate analysis of rice bran procured from rice processing mills. The oil contents of the rice bran vary according to the different chemical treatments and shown in table no. 2 to 5. Acid stabilization appears to facilitate extraction of the crude oil from the rice bran. Out of these chemical treatments hydrochloric acid (HCL, table 3) gave the best results in achieving maximum oil recovery that were in the range of (16.8-13.8%) in T1, (16.6-15.1%) & (16-14.7%) in T2 and T3 in 60 days storage respectively. The treatment with concentration of 30ml/Kg of HCL gives the best optimum results in % of Crude oil recovery. While in other chemical treatments it ranges from (16.2-12.96%) in phosphoric acid, (15.94-14.2%) in acetic acid and (15.6-14.68%) in sodium metabisulphite in all three treatments respectively. Statistical analysis showed significant differences in the oil contents. The ranges of oil contents in the present work match the results of (Lee, 2002; Absar et al., 1998; Prabhakar, 1986).

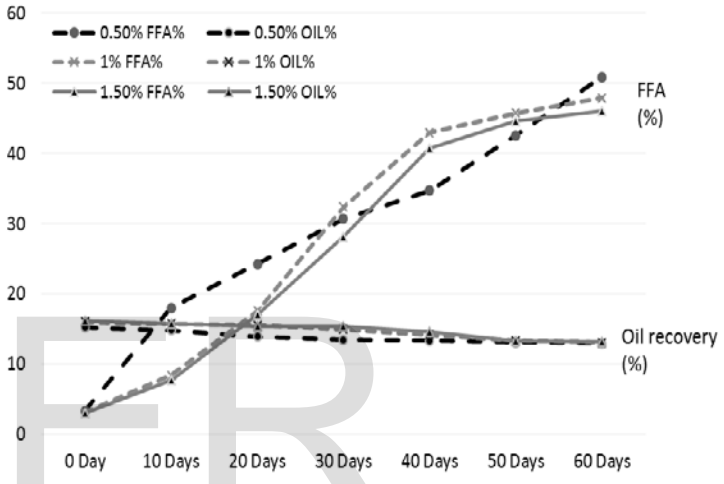

Figure 1: FFA and Oil % of the Rice Bran Treated with Phosphoric Acid during Storage

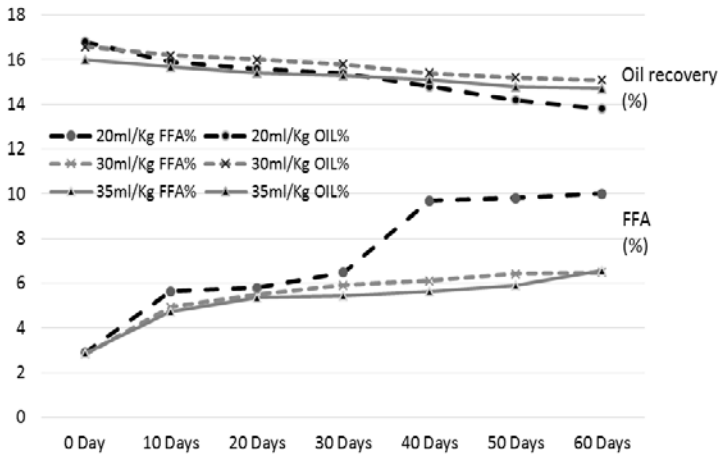

Figure 1: FFA and Oil % of the Rice Bran Treated with Hydrochloric Acid (HCL) during Storage

3.2 Free Fatty Acid (FFA) Status of Rice Bran: The free fatty acids contents (FFA %) of rice bran is shown in table 2 to 5. From all the treatments again HCL gave the good results in controlling the activity of lipases enzyme and from the three concentrations of HCL, concentration of 30ml/Kg appears to give the best results in enzyme deactivation that is in the range of (2.81-6.47%) in 60 days. While the other two concentration

of HCL also give satisfactory performance regarding enzymes deactivation that is (2.9-10.01%) in 20ml/Kg and (2.88-6.56%) in 35ml/Kg respectively in 60 days. The other chemical treatments failed in controlling lipase activity these treatments lose their control right after 10 and 20 days of storage and breaks the recommended limit of less than 10% FFA. All the results shown in the table gave significant differences in the FFA contents of rice bran and its oil contents. Prabhakar and Venkatesh, 1986 finding of FFA% depicts the results of the present study of FFA%.

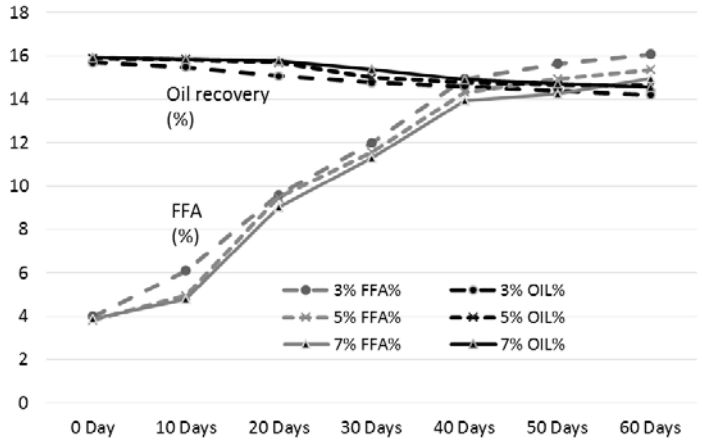

Figure 2: Free Fatty Acids and Oil % of the Rice Bran Treated with Acetic Acid during Storage

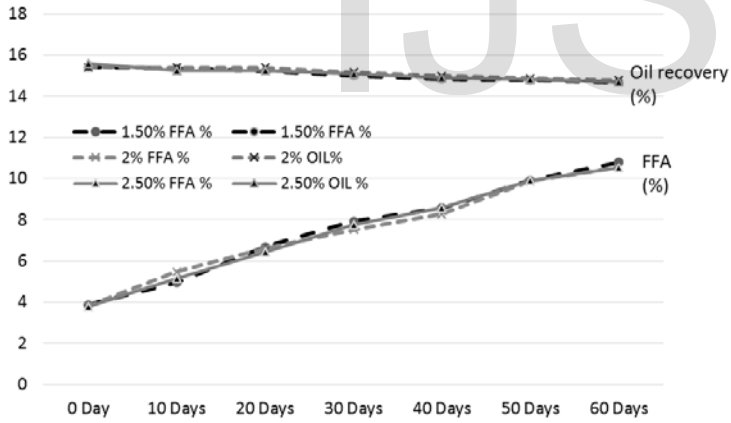

Figure 3: FFA and Oil % of the Rice Bran Treated with Sodium Metabisulphite during Storage

3.3 Fatty Acid Composition of the Treated Rice Bran Samples: Table No. 6 shows the fatty acid (FA) composition results of treated rice bran samples. The contents of total saturated fatty acids of all the treatments were in the range of 0.27-0.35 Myristic acid (C 14:0), 18.95-22.82 Palmitic acid (C 16:0), 1.43-1.55 Stearic (C 18:0), 0.62-0.76 arachidic (20:0), 0.71-1.24 Ecosanoic (20:1) and 0.43-0.76 Behanic acid (22:0) respectively. The content of total saturated fatty acids of rice bran oil appeared more in samples treated with phosphoric acid @ 1.5% W/W that is about about (32.19%) after that in acetic acid @ 7% W/W (29%), then in sodium metabisulphite @2% W/W (26%) and atlast in HCL treated sample @ 30ml/Kg (22%). While in case of unsaturated fatty acid, the percentage of unsaturated fatty acids of all the treatments were in the range of 43.10-45.00 Oleic acid (C 18:1), 23.81-31.67 Linoleic acid (C 18:2) and 0.90-1.15 Linolenic acid (C 18:3) respectively. The content of unsaturated fatty acids of rice bran oil appeared more in samples treated with Hydrochloric acid @ 30ml/Kg that is about (77%) after that in sodium metabisulphite @2% W/W (74%), acetic acid @ 7% W/W (70%) and phosphoric acid @ 1.5% W/W (68%) respectively. So the conclusion is that the sample of rice bran treated with HCL gives the best results regarding unsaturated fatty acids. The above results reveals the findings of (Rossell, 1991).Because IJSER staff will do the final formatting of your paper, some figures may have to be moved from where they appeared in the original submission. Figures and tables should be sized as they are to appear in print. Figures or tables not correctly sized will be returned to the author for reformatting.

4 Conclusion

The chemical methods for stabilization of the rice bran provides an answer to the problem of handling raw bran deterioration mainly in the developing countries with numerous small rice mills which lack adequate electricity. Out of all the chemicals used in this experiment hydrochloric acid @ 30ml/Kg gives the best results in the stabilization of rice bran from lipase enzyme during the storage of 60 days at room temperature.

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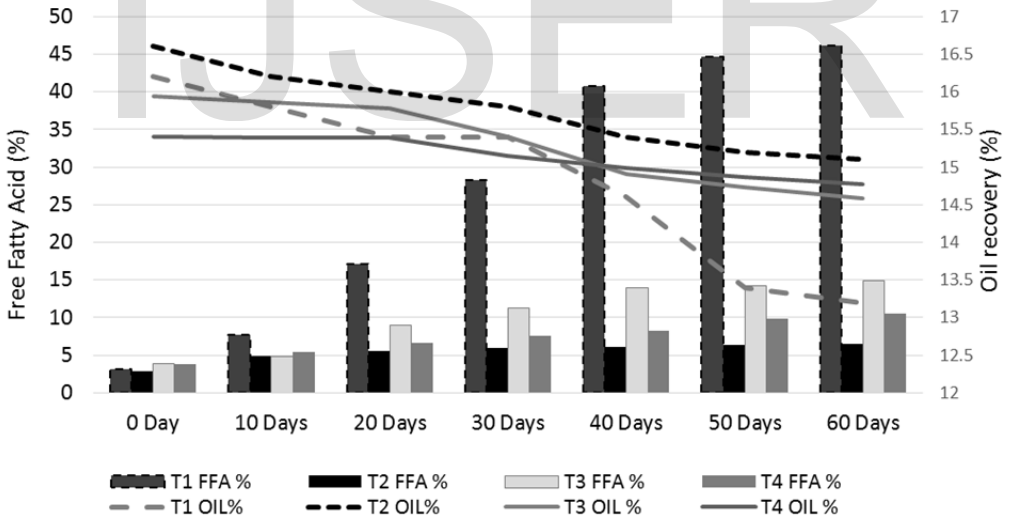

Figure 4: FFA and Oil % of the rice bran oil treated with different treatments i.e. T1 = Phosphoric acid @ 1.50%, T2 = Hydrochloric acid @ 30ml/kg, T3 = Acetic acid @ 7% and T4 = Sodium metabisulphite @ 2% during storage

TABLE 1

PROXIMATE ANALYSIS OF OILS OF RICE BRAN COLLECTED FROM DIFFERENT SOURCES

Parameters	T1 (From Local Sheller's)	T2 (From Rice Processing mills)
Moisture	11.22	11.60
Protein	16.87	16.60
Fats	20.20	19.15
Crude Fiber	8.29	7.99
Ash Contents	9.21	8.75

Values presented are averages of duplicate samples

TABLE 2

FFA AND OIL % OF THE RICE BRAN TREATED WITH PHOSPHORIC ACID DURING STORAGE

Treatments		**T1**		**T2**		**T3**	
Concentrations (%)		**0.50%**		**1%**		**1.50%**	
Parameters		**FFA%**	**OIL%**	**FFA%**	**OIL%**	**FFA%**	**OIL%**
Storage days	**0 Day**	3.38g	15.24a	3.13g	16a	3.09g	16.2a
	10 Days	18.05f	14.88b	8.46f	15.8b	7.76f	15.8b
	20 Days	24.25e	13.94c	17.62e	15.6b	17.06e	15.4c
	30 Days	30.69d	13.5d	32.4d	15c	28.2d	15.4c
	40 Days	34.72c	13.4d	43c	14.2d	40.8c	14.6d
	50 Days	42.58b	13.12e	45.8b	13.4e	44.69b	13.4e
	60 Days	50.9a	12.96f	47.9a	13.1f	46.1a	13.2f

TABLE 3

FFA AND OIL % OF THE RICE BRAN TREATED WITH HYDROCHLORIC ACID (HCL) DURING STORAGE

Treatments		**T1**		**T2**		**T3**	
Concentrations (%)		**20ml/Kg**		**30ml/Kg**		**35ml/Kg**	
Parameters		**FFA%**	**OIL%**	**FFA%**	**OIL%**	**FFA%**	**OIL%**
Storage days	**0 Day**	2.9f	16.8a	2.81f	16.6ab	2.88f	16a
	10 Days	5.64e	15.9b	4.94e	16.2a	4.72e	15.7b
	20 Days	5.8d	15.6c	5.5d	16ab	5.36d	15.4c
	30 Days	6.48c	15.4c	5.92c	15.8a	5.43d	15.3d
	40 Days	9.7b	14.8d	6.1b	15.4ab	5.64c	15.1e
	50 Days	9.8b	14.2e	6.42a	15.2b	5.92b	14.8f
	60 Days	10.01a	13.8f	6.47a	15.1b	6.56a	14.7f

TABLE 4

FREE FATTY ACIDS AND OIL % OF THE RICE BRAN TREATED WITH ACETIC ACID DURING STORAGE

Treatments		**T1**		**T2**		**T3**	
Concentrations (%)		**3%**		**5%**		**7%**	
Parameters		**FFA%**	**OIL%**	**FFA%**	**OIL%**	**FFA%**	**OIL%**
Storage days	**0 Day**	4g	15.7a	3.81g	15.91a	3.88g	15.94a
	10 Days	6.1f	15.5a	4.94f	15.85a	4.79f	15.86a
	20 Days	9.59e	15.09b	9.47e	15.71b	9.02e	15.78a
	30 Days	11.99d	14.78c	11.56d	15c	11.28d	15.4b
	40 Days	14.95c	14.6cd	14.32c	14.8d	13.96c	14.9c
	50 Days	15.65b	14.41de	14.95b	14.65e	14.24b	14.73cd
	60 Days	16.07a	14.2e	15.37a	14.62e	14.95a	14.58d

TABLE 5
FFA AND OIL % OF THE RICE BRAN TREATED WITH SODIUM METABISULPHITE DURING STORAGE

Treatments		T1		T2		T3	
Concentrations (%)		1.50%		2%		2.50%	
Parameters		FFA %	FFA %	FFA %	OIL%	FFA %	OIL %
Storage days	0 Day	3.88g	15.4a	3.81g	15.4a	3.8g	15.6a
	10 Days	4.94f	15.35a	5.47f	15.39a	5.16f	15.26ab
	20 Days	6.67e	15.23a	6.61e	15.39a	6.42e	15.26ab
	30 Days	7.93d	15b	7.52d	15.15b	7.77d	15.1ab
	40 Days	8.57c	14.84bc	8.27c	14.98bc	8.56c	14.9b
	50 Days	9.92b	14.78c	9.87b	14.86c	9.9b	14.8b
	60 Days	10.78a	14.68c	10.53a	14.77c	10.54a	14.68ab

TABLE 6
FATTY ACID COMPOSITION OF RICE BRAN SAMPLES TREATED WITH FOUR DIFFERENT CHEMICALS

Fatty Acids	HCL @ 30ml/Kg	Phosphoric Acid @ 1.5% W/W	Acetic Acid @ 7% W/W	Sodium Metabisul-phite @ 2% W/W
C 14:0	0.27	0.35	0.35	0.33
C 16:0	18.95	22.82	21.04	19.02
C 18:0	1.43	1.55	1.54	1.46
C 18:1	45.00	43.10	43.20	44.25
C 18:2	31.67	23.81	26.82	29.19
C 18:3	1.15	0.90	0.92	1.05
C 20:0	0.72	0.62	0.76	0.73
C 20:1	0.71	1.24	1.08	0.74
C 22:0	0.44	0.76	0.43	0.47
∑SAFFA	21.81	26.1	24.12	22.01
∑USFFA	78.53	69.05	72.02	75.23

C 14:0 Myristic Acid, C 16:0 Palmitic Acid, C 18:0 Stearic Acid, C 18:1 Oleic Acid, C 18:2 Linoleic Acid, C 18:3 Linolenic Acid, C 20:0 Arachidic Acid, C 20:1 Eicosanoic Acid, C 22:0 Behenic Acid.