Saudi Journal of Biological Sciences 28 (2021) 5841–5848

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Mechanism of rice bran lipase inhibition through fermentation activity of probiotic bacteria

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ARTICLE INFO

Article history:
Received 24 April 2021
Revised 4 June 2021
Accepted 13 June 2021
Available online 18 June 2021

Keywords:
Lactobacillus
Rice bran oil stabilization
Rancidity
Antilipase activity

ABSTRACT

Rice bran oil is known as wonder oil and it is the most important vegetable oil in Asia. Rice bran oil is extracted from bran that is the outer hard layer of rice. It is an emerging category in edible oil with a lot of nutritional properties and health benefits. Rice bran oil is heart-friendly, boosts up immunity, and prevents from other diseases occurring commonly in Pakistan. The current study aimed to stabilize rice bran oil through different probiotic isolates and to assess the nutritional content of rice bran oil after stabilization. The study was aimed to inactivate naturally occurring lipases that can hydrolyze oil into glycerol and free fatty acid which is a serious problem that gives it a rancid taste and smell. Antilipase activity was used to inactivate naturally occurring lipases that are a huge threat to the stabilization process. The fermentation process utilizes antilipase activity without affecting the nutritional value of oil. *Lactobacillus* strains were used for the stabilization of rice bran oil. Rice bran oil was extracted in the Soxhlet apparatus. The probiotic lab isolates *Lactobacillus delbrueckii* S2, *Lactobacillus casei* S5 and *Lactobacillus plantarum* S13 were applied to it to increase its shelf life and prevent oxidative rancidity. The extraction temperature of rice bran oil was maintained above 40 °C to inhibit lipase activity. Rice bran oil samples were stored at refrigeration temperature to arrest lipase activity. Probiotics maintained acidic pH to keep oil stabilization. Qualitative analysis was done to confirm rice bran oil stabilization. Determination of Free Fatty Acid (FFA) and saponification value confirmed that oxidative rancidity of rice bran oil was controlled by probiotics. FFA count was less than 10% and Saponification Value (SV) was 180. GC analysis was performed to analyze the FFA profile. Gas Chromatography results have shown 3 fatty acids. Statistical analysis has shown non-significant effect on different incubation temperatures of *Lactobacillus* isolates. Among the biological methods of stabilization, the use of probiotics is a novel concept and recommended for commercial application.
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1. Introduction

Most of the probiotic bacteria are related to the lactic acid bacteria (LAB) group. Lactic acid bacteria play an important role in food fermentation and preservation (Yang et al., 2012). LAB is the most famous group of *Lactobacillus* genus that is used as probiotics. Lactic acid bacteria are gram-positive bacteria, rod or cocci shape, nonsporulating, and anaerobic or facultative aerobic. LAB is used in food preservation due to fermentation activities. Lactic acid bacte-

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.06.042
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ria play a vital role to maintain a healthy life and preserving food. Lactic acid is produced due to the metabolism of LAB which is the main product of fermentation (Quinto et al., 2014).

The first trial of probiotics in food products was conducted in Japan (Amagase, 2008). Most probiotics that are available in the market contain *Lactobacillus* and *Bifidobacterium* species. Probiotics have nutritional advantages by conferring different beneficiary characteristics to improve human health. Health management practices include probiotics addition in the food, feed, dairy, and fermentation industry. Probiotics as a component are added into food at the appropriate stage of the fermentation process (Abatenh et al., 2018). Lactic acid bacteria have the status of GRAS (Generally Regarded as Safe) or QPS (Qualified Presumption of Safety) due to health promoting effects (Borriello et al., 2003; Mokoena, 2017). In 2013, the Italian Ministry of Health had confirmed the word 'Probiotic' and regulated the use of probiotic microorganisms in food supplements (Hill et al., 2014).

Rice bran oil is extracted from the bran layer that is the outer hard layer of rice grain and it is a food component with multiple health benefits. Rice bran has oil content that varies from 10 to 23% (Zarei et al., 2017). Rice bran contains 10% of grain by weight. Each year, 63–76 million tons rice bran is produced in the world (Dubey et al., 2019). A total of 15 to 20% of edible oil consumed in the United States come from Rice bran (Kahlon, 2009). Rice bran oil is called a miracle product or wonder oil due to its enormous health benefits. Major fatty acids are palmitic, oleic, and linoleic acid (Garba et al., 2017).

Stabilized rice bran oil is used as cooking oil and has no negative impact on the organoleptic properties of other foods. It is better in lipid profile and shelf life as compared to other vegetable oil. Rice bran oil gives a better taste and flavor to food and is stable at high temperatures so used in snack industries and restaurants. Rice bran oil is also reported to be used in bread preparation in the baking industry. Rice bran oil also used in baking industry, muffins, mayonnaise, and salad dressing. It is used in the production of nail polishes, hair conditioners, beauty soaps, sunscreen lotions, body masks and lipsticks. It protects the skin from free radicals (Ghatak and Panchal, 2011). It improves skin moisture and maintains the normal pH of human skin. Skin becomes soft and can be repairable through tocotrienol and squalene present in rice bran oil. Due to the existence of γ-oryzanol hair growth is stimulated and it prevents aging (Wang, 2019). γ-oryzanol is a protective agent to prevent lipid peroxidation that is caused by UV light (Garba et al., 2017). Rice bran oil competes with all other cooking oils in its properties. It has high non-fat content, so it exerts hypocholesteraemia activity. It has a nutritional benefit of balance fatty acids that encounter AHA (American Heart Association) recommendation. It enhances cholesterol reduction (Orthoefer, 2005).

Rice bran oil is destabilized by inherent lipase enzyme in bran. Rice bran oil is stabilized by different techniques that inactivate the lipase enzyme. Stabilized rice bran oil has a shelf life of 6 months. When oil is extracted it needs stabilization to inhibit the enzyme activity and inactivate oxidation of lipids. Stabilization is carried out to prevent rancidity. Stabilization is necessary to avoid the decay of fat and bran bioactive compounds. The current research was aimed to stabilize the rice bran oil through a novel strategy. It was focused to utilize beneficial probiotic Lactobacilli strains to inactivate rice bran oil lipase enzyme through fermentation activity.

2. Materials and methods

2.1. Sample collection

Rice grains were collected from farmers in Narowal (32° 5′ 58.1136′′ N and 74° 52′ 29.0388′′ E) rice growing areas of Punjab, Pakistan and milled for obtaining rice bran. Samples were brought to the Food Microbiology Lab, The University of Haripur, Pakistan. Already prepared rice bran in powdered form stored at room temperature was utilized for the experiment. Identified probiotic lab isolates *Lactobacillus delbrueckii* S2, *Lactobacillus casei* S5 and *Lactobacillus plantarum* S13 obtained from fermented food were stored in a preserved form in the Eppendorf tubes at freezing temperature. Further, extraction of rice bran oil was done in the Soxhlet apparatus and revival of *Lactobacillus* strains was carried out for their microbiological examination.

2.2. Description of Lactobacillus isolates

Twenty five stored strains of Lactobacilli were refreshed and revived onto MRS media for fermentation activity (Table 1). Media plates after samples inoculation were incubated at 37 °C for 24 to 48 h under aerobic conditions. Colonies were further sub-cultured to get pure culture.

2.3. Lipase inhibitory assay

Thirteen *Lactobacillus* strains were selected out of 25 after the fermentation process. Two ml supernatant, 2 ml olive oil, 0.061 tris HCl buffer were mixed in a tube and 3.5 ml of lipase enzyme was added to start the enzyme reaction. After incubation at 37℃ for 30 min, its absorbance was measured at 490 nm. The selected strain's absorbance was checked at 490 nm using a BK-UV-1000 Bio base. The percentage of lipase inhibitory activity was calculated (Bendicho et al., 2001) as follows

$$\text{Lipaseinhibitoryactivity}(\%) = (\text{Ac} - \text{As} \times \text{Ac}) \times 100$$

Whereas; Ac = Absorbance of control and
As = Absorbance of sample

2.4. Rice bran oil extraction

Rice bran oil extraction was carried out in the Soxhlet extractor (Model Behr Labor- Technik. Germany – 2013). It is laboratory

Table 1
Description of Lactobacilli isolates used for stabilization of rice bran oil obtained from fermented foods.

S.No	Source of isolation	Strain Identity	Sample code
1	Homemade pickle	*Lactobacillus plantarum*	S1
2	Homemade curd	*Lactobacillus delbrueckii*	S2
3	National pickle	*Lactobacillus brevis*	S3
4	Vendor pickle	*Lactobacillus delbrueckii*	S4
5	Homemade Onion pickle	*Lactobacillus casei*	S5
6	Mitchels pickle	*Lactobacillus salivarius*	S6
7	Shikarpuri pickle	*Lactobacillus acidophilus*	S7
8	Homemade pickle	*Lactobacillus salivarius*	S8
9	Homemade Curd	*Lactobacillus acidophilus*	S9
10	Vendor curd	*Lactobacillus delbrueckii*	S10
11	Nestle yogurt	*Lactobacillus delbrueckii*	S10
12	Haleeb yogurt	*Lactobacillus brevis*	S11
13	Turnip pickle	*Lactobacillus plantarum*	S12
14	Prime yogurt	*Lactobacillus plantarum*	S13
15	Mango pickle	*Lactobacillus casei*	S14
16	Mixed vegetable pickle	*Lactobacillus brevis*	S15
17	Homemade garlic pickle	*Lactobacillus plantarum*	S16
18	Homemade butter	*Lactobacillus delbrueckii*	S17
19	Vendor butter	*Lactobacillus casei*	S18
20	Spinach pickle	*Lactobacillus acidophilus*	S19
20	Lemon pickle	*Lactobacillus brevis*	S20
21	Yogurt	*Lactobacillus plantarum*	S21
22	Cow milk	*Lactobacillus casei*	S22
23	Buffalo milk	*Lactobacillus delbrueckii*	S23
24	Sheep milk	*Lactobacillus acidophilus*	S24
25	Camel milk	*Lactobacillus acidophilus*	S25

apparatus consists of the round bottom flask, siphon tube, distillation path, cooling water inlet and outlet, condenser, thimble, and heating source. Ethanolic extraction power was determined by the Soxhlet apparatus (Baumler et al., 2016).

2.5. Preparation of sample

The Rice bran sample was present in powdered form and it was dried in the oven to remove its moisture. In this way, the entry of organic solvent was facilitated for rice bran oil extraction. A 25 g of dried rice bran sample was weighed and placed in its thimble. The thimble was placed in the thimble chamber of the Soxhlet apparatus.

2.6. Selection of organic solvent

Ethanol organic solvent was selected for extraction purposes. The boiling point of ethanol is 78 °C. Ethanol was selected due to its advantages over another organic solvent. It is nontoxic. It is chemically stable. Its density is lower than water. It is thermally stable at high temperatures due to its volatility. To determine the quality of extracted oil it is essential to use ethanol as solvent (Baumler et al., 2016).

2.7. Selection of temperature

The temperature for extraction of rice bran oil was adjusted above 40 °C (Banat et al., 2013).

2.8. Soxhlet working

Round bottom flask of 250 ml of Soxhlet apparatus was taken out and it was filled with ethanol up to 100 ml. It was heated with a heating source. One cycle of extraction was completed in 2 h. Ethanol was recycled and it was only one batch of solvent so there is no need for filtration of extract. Rice bran oil extract was collected after few days. Rice bran oil was collected in a falcon tube. The Falcon tube was properly labeled and the rice bran oil sample was allowed to cool down. It was stored at room temperature. The yield of extract was calculated according to the method of Jensen (2007).

$$\text{Theyieldofoilextraction} = \text{Weightofoilextracted}/\text{Weightofricebranused} \times 100$$

2.9. Solvent evaporation

Solvent evaporation was done through a rotary evaporator according to the method of Baumler et al. (2016).

2.10. Evaporation of solvent through a rotary evaporator

Rotary evaporation was done by using model Rotary evaporator (Heizabad Hei- VAP (EU) S/N; 200,112,784 0717) to evaporate ethanol at 40 °C. Ethanol solvent was evaporated in all rice bran oil samples through this method. Rotatory evaporation was done at 50 °C by maintaining low pressure to evaporate ethanol (Baumler et al., 2016).

2.11. Lactobacillus strains applied on rice bran oil samples

All revived *Lactobacillus* strains were applied on rice bran oil to evaluate their effect on the stabilization of rice bran oil (Table 1 and 3).

2.12. Three different growth temperature for Lactobacillus strains

Lactobacillus strains were grown at three different incubation temperatures as shown in Table 3. *Lactobacillus* strains were incubated at different temperatures to analyze their effect on the stabilization of rice bran oil samples.

2.13. Stabilization of rice bran oil with probiotic

Two different methods were applied to rice bran oil for stabilization purposes;

2.13.1. Bacterial suspension was applied on rice bran oil extract

For the preparation of bacterial suspension autoclaved distilled water was used. Distilled water was autoclaved at 121 °C for 15 to 20 min at a pressure of 15 psi. It was cooled down. A sterile falcon tube was taken and filled it with autoclaved distilled water up to 12 ml. A bacterial colony was picked with a sterilized loop and mixed in a falcon tube to make a bacterial suspension. Then this bacterial suspension was applied to rice bran oil. In this way, suspension of different bacterial strains was made and applied on each rice bran oil sample. These samples were stored in a refrigerator for 6 months and the effect of probiotics for rice bran oil stabilization was analyzed. The storage period was from Feb. 2020 to Aug. 2020 and then taken out and given the experimental temperature.

2.13.2. Bacterial suspensions sprinkled on the rice bran sample before rice bran oil extraction

Selected probiotic bacterial strains were sprinkled over twenty five grams rice bran in a beaker. The sample was incubated in a shaker incubator for 3 days at different temperatures. A total of 9 strains were selected for this type of stabilization method. Three strains were applied separately, and 6 strains were applied in the consortium. Later, rice bran oil extract was extracted in the Soxhlet apparatus. Rice bran oil was collected in a sterilized falcon tube. After rotary evaporation, samples were stored in the refrigerator for 1 week. Further, quality analysis of rice bran oil was done to evaluate stabilization results.

2.14. Quality analysis tests

2.14.1. Determination of free fatty acid

A beaker was taken and 0.5 ml of rice bran oil sample was put in it. A 5 ml neutral 95% ethanol was added to it. It was kept on a hot plate till bubbles appeared. It was allowed to cool down after removal hot plate. 2–3 drops of phenolphthalein indicator were added to it. It was titrated with alkali to that point until the pink color appears for 15 s. Free fatty acids were calculated as the percent of oleic acid by using Akhter et al. (2015) method.

Free fatty acids per kg of fat = (mL NaOH) × (N) × 28.2 / Weight of sample used

2.14.2. Determination of saponification number

Take 2 ml stabilized rice bran oil and put in a 250 ml flask. Pour 25 ml alcoholic potassium hydroxide with the help of a pipette into it. Flask was heated on boiling water for 1 h, shaking frequently. It was cooled down and a 1 ml phenolphthalein indicator was added to the flask. Excess KOH was titrated with HCL (Amarasinghe et al., 2009). Saponification number was calculated by the following formula;

$$\text{Saponificationnumber} : (\text{mlKOH} \times \text{NKOH}) - (\text{mlHCL} \times \text{NHCL}) \times 56.1/\text{Weightofsample}$$

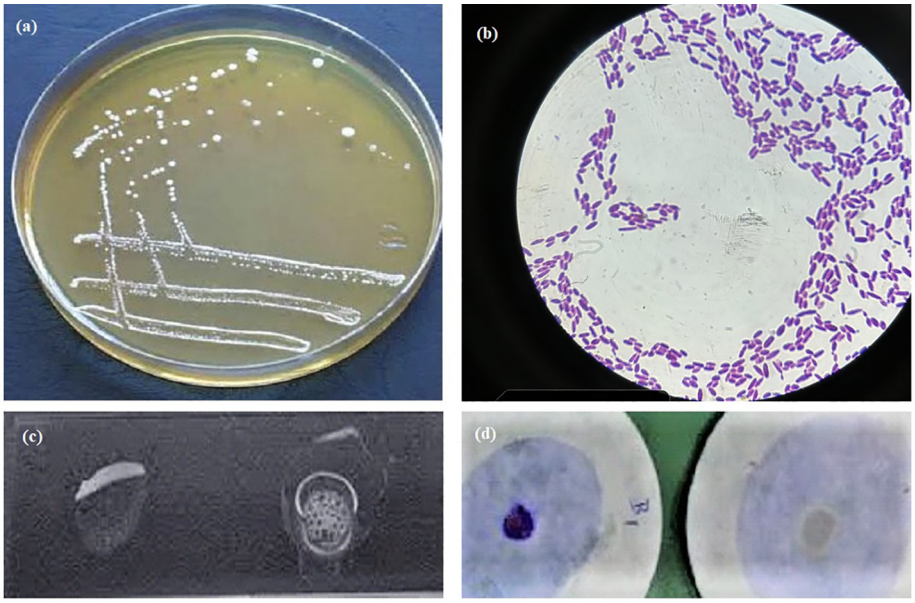


Fig. 1. (a) *Lactobacillus delbrueckii* colonies growth on MRSA (S2); (b) Microscopic view of rod shape *Lactobacillus casei* (S3); (c) catalase negative test for *Lactobacillus* strains (S2); and (d) oxidase negative test for *Lactobacillu* strains.

Table 2
Morphological characteristics for the identification of *Lactobacillus* strains.

S. No	Colony Color	Colony Shape	Gram Staining	Arrangement
1	White	Sticky/Smooth/round convex/shiny	Gram-positive	Rod shape

2.15. GC analysis

Rice bran oil samples, stabilized, and control was sent to the oil quality laboratory, National Agricultural Research Centre, Islamabad, Pakistan and analyzed. Gas chromatography (GC Model: GC–FID 7890A Agilent©, USA) was done to analyze the FFA profile using Fused Silica Capillary Column (FSCC) Cat. Log No. FC0557216A: 30 m × 0.25 mm with Film thickness 0.25 μm. Detector FID temperature was maintained 300 °C. Inlet (Injector) temperature was maintained at 250 °C. GC analysis was done using Kail et al. (2012) method.

2.16. Statistic analysis

Data obtained were analyzed for analysis of variance (ANOVA) by two-factor factorial in completely randomized design and mean comparisons ($p \leq 0.05$) was carried out by Least Significant Difference (LSD) test using M−Stat−C Statistical software (Steel et al., 1997).

3. Results

3.1. Revival and characterization of Lactobacillus strains

The probiotic bacterial isolates were refreshed from preservation vials and their morphological characteristics were noted to confirm pure culture (Fig. 1a). These strains were further verified by different biochemical tests, as a member of the genus *Lactobacillus*. *Lactobacillus* formed small round, creamy yellow color colonies on MRSA agar with convex, smooth, and shiny appearance (Table 2). Microscopic results revealed that all bacterial strains were Gram-positive and rod shape (Fig. 1b). Different biochemical analyses showed that all strains were anaerobic, nonmotile, catalase-negative, oxidase-negative, and able to ferment sugars (Fig. 1c and 1d). All these morphological and biochemical characteristics confirm the already lab identified *Lactobacillus* isolates.

3.2. Antilipase inhibitory activity shown by Lactobacillus strains

A total of 25 *Lactobacilli* preserved strains were refreshed and 11 strains were randomly selected to check antilipase inhibitory activity. These 11 strains were selected based on their fermentation activity. Only 3 strains had shown the highest antilipase inhibition activity (Fig. 2). These included *Lactobacillus delbrueckii* (S2), *Lactobacillus casei* (S5) and *Lactobacillus plantarum* (S13). It was detected that S13 can inhibit lipase enzyme up to 76.80%. Minimum activity (2.60%) was observed for *Lactobacillus* S18 (Fig. 2).

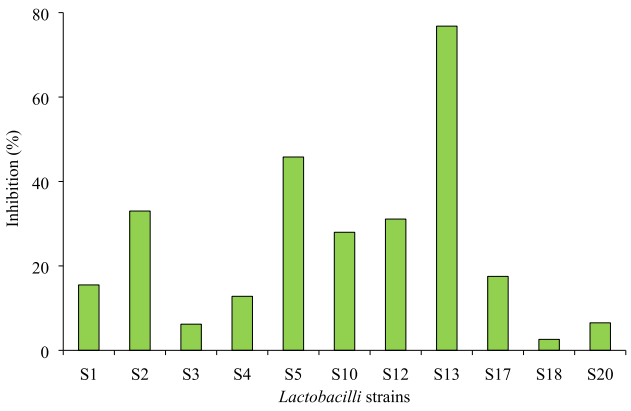


Fig. 2. Lipase inhibitory assay through different *Lactobacilli* strains.

Table 3
Description of applying probiotic on Rice bran oil.

S. No	Probiotic Applied	No. of Strains	Growth Temperature of Bacterial Strains	No. of Rice Bran Oil Samples
1	*Lactobacillus* strains	25	25 °C	25
2	*Lactobacillus* strains	25	37 °C	25
3	*Lactobacillus* strains	25	40 °C	25

The storage period for samples = 6 months (Feb 2020-Aug 2020).

Table 4
Determination of free fatty acid value.

S. No	Strains Codes	Free fatty acid count		
		25 °C	37 °C	40 °C
1	S1	5.64	7.34	6.66
2	S2	4.64	3.45	5.64
3	S3	2.82	2.89	4.82
4	S4	1.69	2.82	5.69
5	S5	2.25	2.20	2.25
6	S6	1.12	4.57	7.12
7	S7	0.56	1.17	4.56
8	S8	2.82	1.39	7.82
9	S9	0.56	3.13	4.56
10	S10	1.12	1.12	2.23
11	S11	0.56	0.56	0.56
12	S12	4.51	4.51	4.56
13	S13	1.69	1.69	1.90
14	S14	2.67	2.67	4.67
15	S15	0.57	0.57	2.57
16	S16	0.56	0.56	3.56
17	S17	0.56	0.56	2.56
18	S18	0.76	0.76	4.76
19	S19	1.69	1.69	1.89
20	S20	1.12	1.12	1.62
21	S21	1.12	1.12	2.12
22	S22	0.56	0.56	2.56
23	S23	2.25	2.25	2.25
24	S24	1.13	1.13	3.13

3.3. Probiotics application on rice bran oil

The above three probiotics (showing maximum antilipase inhibitory activity) were applied on rice bran oil (Table 3). The bacterial suspension was applied to rice bran oil to control and its oxidative rancidity was arrested. Appropriate conditions were given at this step to inhibit lipase activity for sake of rice bran oil stabilization. Samples were stored in the refrigerator initially.

3.4. Determination of free fatty acid value

Titration analysis showed that free fatty acid count was below 10%. The rice bran oil stabilized through probiotic lab isolate S13 has maximum free fatty acid value (9.4). Free fatty acid values for rice bran oil stabilized through 3 strains were lower than that of control (11). The result indicates that oxidative rancidity of rice bran oil was in the acceptable range for human consumption (Table 4).

Table 5
Determination of saponification tests values.

Designation	Saponification No
Sample 1 (S2)	145
Sample 2 (S5)	153
Sample 3 (S13)	169
Control	200

3.5. Determination of saponification tests values

The stabilized rice bran oil indicated that the saponification number ranged from 140 to 188 (Table 5). It was observed through statistical analysis that there is no effect of temperature difference on FFA and values are non-significant ($p \leq 0.05$) at 25, 37 and 40 °C (Table 6).

3.6. GC analysis

Gas chromatography results show a free fatty profile that includes three fatty acids i.e. palmitic fatty acid, oleate fatty acid and linoleate fatty acid (Table 7). Gas chromatography is determined the fatty acid composition of stabilized rice bran oil. The results of the *Lactobacillus delbrueckii* (S2) are depicted in Fig. 3. Three major acids were identified and shown in the chromatograph. Oleate was found in the highest quantity (69%) followed by palmitic acid (15%). Linoleate was found in lower quantities (14%). Results indicate that the presence of palmitic fatty acid is 15%. The palmitic acid value is within the range of stabilized rice bran oil value. *Lactobacillus delbrueckii* strain stabilizes only one fatty acid.

Lactobacillus casei strain stabilizes only one fatty acid (Fig. 4). Oleate was found in higher quantity (56%) followed by linoleate (28%) and palmitic have the lowest quantity (14%). The rice bran oil sample which was treated with *Lactobacillus plantarum* indicates the presence of palmitic fatty acid (14.87%). The palmitic acid value is within the range of stabilized rice bran oil value. Oleate was found in higher quantity (65%) followed by linoleate (17%) and palmitic have the lowest quantity (16%) (Fig. 5). Results indicate that the presence of palmitic fatty acid is 16%. Linoleate fatty acid value is also within the range of stabilized rice bran oil value i.e. 31%.

Table 6
Statistical analysis on free fatty acid count during temperature variation.

		Sum of Squares	Df	Mean Square	F	Sig.
Temp 40	Between Groups	26.977	9	2.997	0.924	0.525
	Within Groups	64.845	20	3.242		
	Total	91.822	29			
Temp 37	Between Groups	7.046	9	0.783	0.191	0.993
	Within Groups	81.890	20	4.095		
	Total	88.936	29			
Temp 25	Between Groups	6.680	9	0.742	0.244	0.983
	Within Groups	60.786	20	3.039		
	Total	67.465	29			

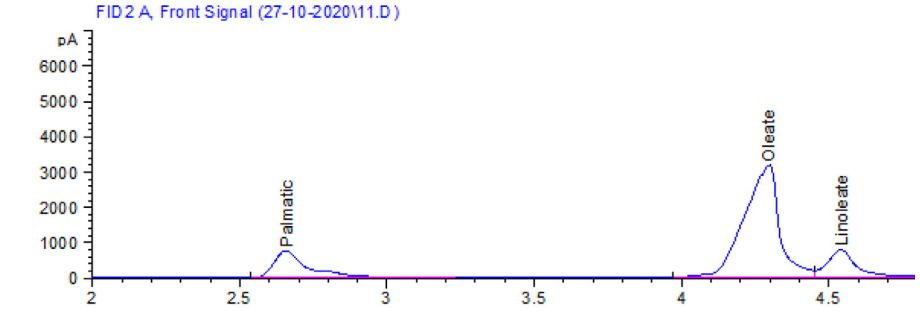
Table 7
Stabilized Rice bran oil fatty acid composition.

S. No	Fatty Acid Name	Composition
1	Palmitic	13 – 20.3%
2	Oleate	34 – 43.9%
3	Linoleate	31 – 35.7%

4. Discussion

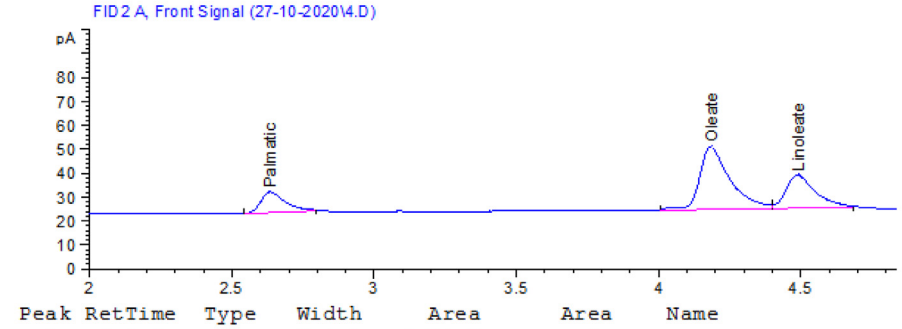
The present study is a novel approach to ensure rice bran oil stabilization through microbial activity. Rice bran oil was stabilized by applying Lactobacilli probiotics strains on it. Identified and preserved strains of *Lactobacillus* genus were applied. Among 11 Lactobacilli isolates 3 were found the most efficient in inhibiting lipase enzyme in *in vitro* studies. These strains were originated from fermented food and dairy products. A non-significant difference was noted for depicting effect of temperature difference between the three Lactobacilli isolates. It is pertinent that these strains can metabolically stabilize the rice bran oil at 25, 37 and 45 °C. The temperature range of 25 to 45 °C is quite lower than the temperature (120 °C) required for thermal stabilization of rice bran oil as determined by Orthoefer (2005). Present study revealed that probiotic strains can lower the Lipase inhibition temperature. This finding vary from the one conducted by Akhter et al. (2015) who used different chemicals to stabilize rice bran oil. The shelf life of rice bran oil was checked for 3 to 6 months and it remained stabilized till the six months duration. Results indicate that oxidative rancidity reduced during the storage period. Lavanya et al. (2019) reported that low temperature maintains stabilization of rice bran oil. Refrigeration temperature reduces the hydrolysis of free fatty acids. Lipase activity was arrested at low temperature. Zaghlol et al. (2018) identified the effect of microwave and dry heat stabilization techniques on the nutritional composition of rice bran and it was noticed that both microwave and dry heating methods help in the stabilization of rice bran and also reduce the amount of free fatty acids and peroxide value.

GC–MS results declare that lipase enzyme activity was successfully arrested to stabilize rice bran oil when different types of heat treatment were given. Heat treatment included microwave heating, ohmic heating, and dry heating (Ahmed et al., 2007). Current study has confirmed that *Lactobacillus* strains can successfully inhibit the lipase activity and ensure the stabilization of rice bran oil. Free Fatty Acids (FFA) count for probiotic stabilized oil was less than 10%. It is acceptable for human consumption. The most suitable method to increase shelf life of rice bran oil was extrusion.



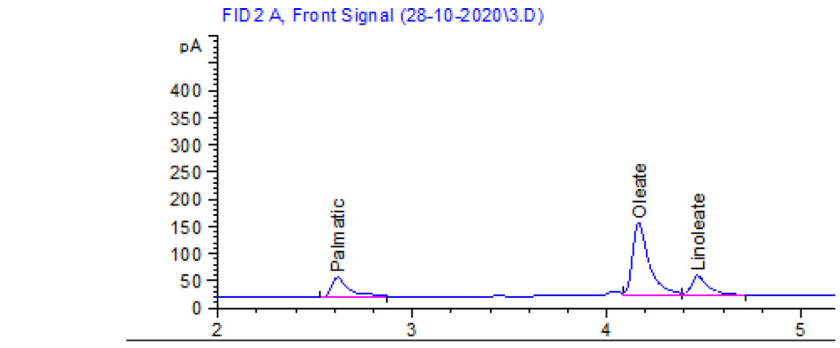
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
2	2.659	BB	0.1070	5945.91504	15.76535	Palmatic
5	4.296	BV	0.1097	2.63326e4	69.81973	Oleate
6	4.541	VV	0.0987	5436.59912	14.41492	Linoleate
Totals :				3.77151e4		

Fig. 3. GC analysis of rice bran oil stabilized through S2.



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
2	2.635	BB	0.0863	51.79399	14.87732	Palmatic
5	4.186	BB	0.1020	197.36337	56.69069	Oleate
6	4.490	BB	0.0922	98.98335	28.43200	Linoleate
Totals :				348.14072		

Fig. 4. GC analysis of rice bran oil stabilized through S5.



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
2	2.616	BB	0.0861	207.04999	16.96048	Palmatic
5	4.166	VV	0.0841	797.14319	65.29790	Oleate
6	4.469	VB	0.0883	216.58595	17.74162	Linoleate
Totals :				1220.77913		

Fig 5. GC analysis of rice bran oil stabilized through S13.

Extrusion includes high temperature and high pressure to inactivate lipase enzymes. The extrusion method of stabilization has more benefits as compared to other methods of stabilization due to better stability, simple processing, and short processing time (Rafe et al., 2017). Mourad et al., (2009) utilized biological method of oil stabilization through enzymatic treatment. Protease enzyme was used for lipase degradation. Rice bran was mixed with water and a constant temperature was maintained for irreversible lipase inactivation.

The convincing results of probiotic stabilization of rice bran oil compel for the preservation of rice bran nutrients through fermentation. It was the novel biological approach of rice bran oil stabilization. Papain was applied successfully on rice bran for its stabilization in one of previous study. Rice bran was stored for 15 days and the FFA count was calculated. The result of the FFA count was less than 10% (Mangal et al., 2014). Statistical analysis has shown non-significant impact of storage temperatures and probiotic treatment on the FFA of bran oil. Microbial strains maintain acidic pH and lipase activation require slightly basic pH so in this way, probiotics remain successful for rice bran oil stabilization. These 3 probiotics were S2 (*Lactobacillus delbrueckii*), S5 (*Lactobacillus casei*) and S13 (*Lactobacillus plantarum*). These promising strains exhibited the highest antilipase inhibition activity. Probiotics may have stabilized the rice bran oil due to their metabolic acidity and organic acids production. These three Lactobacilli are reported for their role in stabilizing rice bran oil for the first time.

Successful stabilization is evident in the present findings of acceptable FFA count and saponification number of bran oil. GC–MS and liquid chromatography were also used to investigate the quality of rice extract in previous studies (Hartono et al., 2017; Sawada et al., 2021). In the present study, GC analysis was done for the FFA profile. FFA profile has shown presence of 3 fatty acids. These three fatty acids are palmitic, oleate and linoleate fatty acid. Palmitic acid is in stabilized range in each rice bran oil sample and it is 15%. Linoleate fatty acid is also stable range in one rice bran oil that was stabilized by S13 strain and it is 31%.

5. Conclusions

It is revealed from the present study that stabilization of rice bran oil was maintained through fermentation activity of probiotics. *Lactobacillus* strains were selected to inhibit lipase activity during the storage period of rice bran oil. *Lactobacillus* strains exhibited a useful impact on rice bran oil samples and increased its shelf life. In the present study, strains analyzed for Lipase inhibitory activity show that S2 (*Lactobacillus delbrueckii*), S5 (*Lactobacillus casei*) and S13 (*Lactobacillus plantarum*) have the potential to be recommended for commercial application to ensure stabilization of rice bran oil.

Author contributions

H.N., S.S. and S.A. conducted the experimental work; A.N. and Y. B. collected and analyzed data; S.A. and A.Q. wrote the paper; W.A., M.L., S.S. and M.M.K. gave suggestions and equally contributed to manuscript writing; S.A.A. and S.A. funding acquisition; S.A. and A.Q. critically advised the study, and contributed to improving the quality of the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to acknowledge the support of Prince Sultan University for paying the Article Processing Charges of this publication.

Funding

Higher Education Commission, Islamabad, Pakistan for financial support (HEC-NRPU#10655) during this research work under National Research Program for Universities.

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