SUMMARY
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Ricebran oil, a byproduct of rice milling industry, offers India a potentially sizeable domestic source of edible oil. Ricebran oil has gained significant position in the Indian edible oil scenario in the past one decade. The potential of ricebran oil in the country is over 0.9 million metric tonnes but current production is hardly 0.5 million metric tonnes, of which 70% is expected to be of edible grade and the balance of non-edible grade. Approximately 0.50 - 0.60 million metric tonnes of oil is sold as direct cooking medium.

Distinct features of ricebran oil make it a potential source of edible oil as well as for industrial utilisation. Ricebran oil is considered to be an important edible oil as it is rich in polyunsaturated fatty acids (PUFA) and certain minor constituents like oryzanols, tocols, sterols, etc. which have tremendous health benefits. Moreover, this oil has better keeping quality and shelf life in comparison to other cooking oils due to presence of tocols and oryzanols. In Japan ricebran oil is more popularly known as ‘heart oil’ as it keeps the cholesterol level in serum relatively low due to presence of PUFA and minor components. The oryzanol present in oil has many therapeutic benefits. The fatty acid profile of ricebran oil is good as it provides ideal PUFA to saturated fatty acids “SFA” ratio and fair amount of linoleic acid to alpha linolenic acid “LA/ALNA” ratio. The S : M : P ratio of this oil is closer to the recommended S : M : P ratio of 1:1.5:1. For exploiting the nutritional potential and other benefits of ricebran oil, this research was undertaken. The study had been broadly on three different aspects viz., studies on isolation of oryzanol, changes in its physico-chemical characteristics and thus it’s frying characteristics as compared to other oils and the hypolipidemic role of this oil in as such form and in blends with safflower oil.
Oryzanol (ferulic acid esters) is one of the most important constituent present in ricebran oil due to its therapeutic benefits and antioxidant properties. Oryzanol was first extracted from ricebran oil and was presumed to be a single component but later on it was determined to be a fraction containing ferulate esters of triterpene alcohols and plant sterols. Individual components were identified as cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campestryl ferulate, \( \beta \)-sitosteryl ferulate and cycloartanyl ferulate.

In the present study, an analytical technique was developed for identification and quantitation of oryzanol in ricebran oil by reverse phase HPLC using gradient solvent system. The mobile phase consisted of \( \text{CH}_3\text{CN} : \text{CH}_3\text{OH} : \text{CH}_2\text{Cl}_2 \) in the ratio of 45:45:5 as solvent 'A' and \( \text{H}_2\text{O} - 5\% \) as solvent 'B'. The proportion of water was increased from 0 to 5\% in the initial stages i.e. from 0 - 6 min. Thereafter, it was decreased from 5\% - 0 from 6 - 10 min. The incorporation of water in the initial mobile phase allowed for the best separation conditions for tocols and oryzanols components. The flow rate was kept at 1 ml/min., temperature 40\(^\circ\)C and wavelength 315 nm. The column used was Lichrosorb 10 RP18. The oryzanol components eluted in the decreasing order of polarity. Cycloartenyl ferulate with a terminal double bond, being most polar eluted first, then 24-methylene cycloartanyl ferulate followed by campestryl ferulate. Since the polarity of \( \beta \)-sitosteryl ferulate and cycloartanyl ferulate is almost similar they eluted as a single peak. Therefore, four good peaks of six components or \( \gamma \)-oryzanol were obtained using the developed techniques. A number of samples were run on HPLC and were found to contain 1.52\% to 2.10\% oryzanol. The percentage of individual components of oryzanol in different samples of oil varied considerably which may be due to different origin and variety of ricebran from which it was isolated. However, the percentage of 24-methylene cycloartanyl ferulate was found to be maximum ranging from 27.44\% to 39.90\% of the total oryzanol followed by campestryl ferulate ranging
from 28.52% - 36.25%, cycloartenyl ferulate ranging from 15.20% - 21.05%, and β-sitosteryl ferulate and cycloatanyl ferulate together ranging from 13.16% - 17.66%.

Isolation of oryzanol from crude rice bran oil was done in four steps viz. chromatography, rechromatography, purification and crystallisation. Chromatography and rechromatography were done on a silica gel column (60-120 mesh) containing 60 g silica gel during chromatography and 30 g during rechromatography. The columns were eluted with different ratios and volumes of hexane and diethyl ether in the increasing order of polarity. During chromatography the column was first eluted with 50 ml, 30:70 (Et₂O : hexane), then with 100 ml, 40:60 (Et₂O : hexane) and finally with 100 ml 50:50 (Et₂O : hexane). The third fraction was found to contain 18% oryzanol which was further enriched by rechromatography. During rechromatography the column was first eluted with 30 ml, 30:70 Et₂O : hexane, then with 50 ml of 35:65 Et₂O : hexane followed by 30 ml 40:60 of Et₂O : hexane and 30 ml of 50:50 Et₂O : hexane. The third fraction was found to contain 26% oryzanol. Thus 26% oryzanol rich fraction was dissolved in hexane at room temperature and hexane soluble portion was removed. The insoluble portion was found to contain 83% oryzanol. The oryzanol concentrate obtained after purification was crystallised using methanol and acetone in the ratio of 2 : 1. After crystallisation white oryzanol crystals of 98% purity were obtained. The oryzanol thus isolated resulted in a yield of 70% of the total oryzanol content present in the experimental rice bran oil. The composition of the oryzanol thus obtained was 48.50% 24-methylene cycloartanyl ferulate, 27.62% campestryl ferulate, 14.84% cycloartenyl ferulate, and 9.04% β-sitosteryl ferulate and cycloartanyl ferulate together. Oryzanol obtained by above method showed melting point of 138°C and absorption maxima at 231, 291 and 315 nm in petroleum ether.
(60° - 80°C). It was soluble in acetone, ether, benzene and methyl - ethyl - ketone slightly soluble in alcohol, n-hexane and insoluble in water.

To promote consumption of ricebran oil an edible oil it was contemplated to use the blends of this oil with other oils. Further, due to presence of nutritional compounds, the oil blend with other oils is expected to have good nutritional values. Ricebran oil (RBO) was blended with groundnut oil (GNO) and safflower oil (SAF) in the ratios of 20:80, 40:60, 60:40 and 80:20 and was subjected to deep fat frying of potato chips at 180°C for 36h. The oil samples were withdrawn after every 6h frying and were analysed for their various physico-chemical and electrical properties viz. IV, AV, PV, RI, colour, specific gravity, fatty acid composition, conjugated dienes and dielectric constant to study the deterioration of frying media during frying. In deep fat frying the oil is continuously and repeatedly used at elevated temperatures in the presence of air. Under such conditions both thermal and oxidative decomposition of oil may take place. Such unavoidable chemical reactions cause formation of both volatile and non-volatile decomposition products. It was observed that during frying the oil deteriorated and various physical and chemical changes took place but the extent of deterioration varied in different oils. The changes in physico-chemical properties were more pronounced in SAF than RBO or GNO indicating comparatively poor stability of SAF. The changes in RBO and GNO were almost of similar order indicating comparable frying properties and stability of these oils.

Ricebran oil was blended with GNO in the ratios of 20:80, 40:60, 60:40 and 80:20. The changes in the physico-chemical characteristics of these blended oils were measured during deep fat frying of potato chips for 36h at 180°C. An increase in FFA, RI, colour, specific gravity was observed. IV was found to decrease however, PV increased in the beginning but decreased after 18h of frying.
SAF, being richer in PUFA is more vulnerable to oxidation. Therefore, it was blended with RBO in the ratios of 20:80, 40:60, 60:40 and 80:20 and changes in the physico-chemical characteristics were studied. The drop in IV in RBO : SAF blends were more in comparison to the drop in IV in either RBO or GNO alone or their blends. The trends of rise in FFA and changes in PV were similar to the blends of RBO and GNO. Specific gravity increased in all the cases however, this increase was lower in case of RBO : SAF blend of 80:20 in comparison to other blends. Increase in RI and colour were also observed.

Changes in fatty acid profile of pure oils as well as blended oils during frying were studied. The oleic acid in GNO decreased from 46.5% to 39.6% which resulted in a loss of 14.8% oleic acid during frying of GNO. The loss in oleic acid was 17.9% in case of RBO frying and 19.2% in case of SAF frying. The loss in linoleic acid in GNO, RBO and SAF were 29.2%, 35.4% and 47.6% respectively. The total loss in unsaturated fatty acids was 20.9% in GNO frying, 27.3% in RBO frying and 42.0% in SAF frying.

Changes in dielectric constant and conjugated dienes were also determined. The results indicated a regular rise in dielectric constant for various oils and their blends suggesting development of higher content of polar material. The rise in dielectric constant in RBO : SAF blends was slightly more than RBO : GNO blends suggesting the latter to be more stable than former.

Diene content increased with duration of frying in all the cases. The rate of increase was more in case of RBO : SAF blends than in RBO : GNO blends suggesting the tendency for isomerisation was more in RBO : SAF blends.
All the above results indicate that during frying the stability of SAF increased on blending with RBO. The stability of GNO and RBO blends was comparable with pure GNO.

Ricebran oil is considered to be a nutritionally important edible oil. To study the hypolipidemic effect of RBO in comparison with other edible oils viz. GNO, SAF, SUN and SOYA, the experiments were done on male albino rats by feeding them control diet along with 10% (w/w) experimental oils. Two different sets of experiments were carried out, one with cholesterol free diet and the other with cholesterol rich diet to study the exogenous as well as endogenous hypolipidemic effect of experimental oils. The mean total cholesterol in rats fed with cholesterol free diet with GNO was elevated to 72 mg/dl followed by rats fed with SOYA having 69.1 mg/dl. The RBO fed rats elevated total cholesterol to only 61.9 mg/dl in serum. Triglycerides and LDL+VLDL were highest in GNO and lower in different experimental oil groups. It was found that RBO decreased the cholesterol level by 14 units in comparison with GNO, SAF lowered it by 12 units. SUN and SOYA also lowered the cholesterol level by 8 units and 4 units respectively. The trend of lipid profile in cholesterol rich diet was similar to the trend observed in cholesterol free diet but the variation in serum lipids were more in case of hypercholesterolemic diet. The only difference observed in these two experiments was in level of HDL cholesterol in RBO fed rats. The HDL cholesterol level improved with RBO containing hypercholesterolemic diet which was reverse from the trend observed in cholesterol free diet experiments indicating that RBO has cholesterol lowering effect both on exogenous and endogenous cholesterol but it lowers HDL cholesterol level in cholesterol free diet. A comparison of cholesterol level in different oils was done taking GNO as control group. In cholesterol containing diet the lowering with RBO was 26.6 units, with SAF 24.0 units, with SUN 18.0, units and with SOYA 12.9 units. Thus from the study it was clear that RBO has a greater serum cholesterol lowering
effect than SAF, GNO, SUN and SOYA which may be due to the micronutrients present in the unsaponifiable fraction.

In the clinical trials since RBO and SAF were found to be best cholesterol level reducer, blending of these two oils was done to study the combined hypolipidemic effect of these two oils. RBO was blended with SAF in the ratios of 30:70, 50:50, 70:30 and 90:10 and fed to rats alongwith cholesterol rich diet. The ratios of 90:10 and 70:30 of RBO to SAF were better in their hypolipidemic effect than RBO and SAF alone. The results suggest that maximum hypolipidemic effect was found in 70:30, RBO : SAF blend where the cholesterol level decreased to 42.3 units in comparison with GNO. The improved hypolipidemic effect of blended oils may be due to high PUFA content of SAF in combination with the micronutrients of the RBO in the unsaponifiable fraction.