Analysis and Chemical Characterization of Rice Bran Oil for Dietary Consumption

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Abstract **- The main aim of this paper is to review the available data on analyzed the unsaturated fatty acid component and physical properties of Rice bran oil. The study of unsaturated fatty acid components analyzed by Gas chromatography-mass spectrometry and physical properties analyzed by specific instruments. RBO is gaining popularity among other traditionally used cooking oils because of its better cooking quality, prolonged shelf life, and well-balanced fatty acid components as well as the presence of many antioxidant components. The major fatty acid components of RBO oleic acid, measured by gas chromatography-mass spectrometry and FID of the methyl esters, was dominated by oleic acid(52.84), palmitic acid(18.75), and linoleic acid(3.07), were found while the saponification value(156 mgKOH/g) acid value(0.4), refractive index(1.4725), and specific gravity(0.922), obtained for the rice bran oil are within the range reported for the oil in literature. Nowadays, the rate of diabetes increases in the world. This oil very helpful in controlling diabetes. Rice bran oil is rich in vitamin E (both tocopherols and tocotrienols) and bioactive phytonutrients, which include phytosterols, γ-oryzanol, squalene, and triterpene alcohols. Because of its cardiac-friendly phytochemicals and antioxidant potentials, RBO has been categorized as healthy edible oil for human consumption and has attained the status of "heart-healthy oil". Rice bran oil (RBO) also called wonder oil is well known for its numerous health benefits.**

Index Terms **- Rice bran oil, GC-MS, Oryzanol, Fatty acid, Oleic acid, flashpoint.**

INTRODUCTION

During the polishing process of the rice, a unique vegetable oil rich in antioxidants produced from the outer layer of rice is what we called Rice bran oil (RBO). Studies around the globe have confirmed the cholesterol-lowering properties due to properties due to the presence of unique nutraceutical in this oil known as oryzanol and tocotrienols. The crude rice bran oil is mainly composed of glycerides (80%) while phospholipids, glycolipids, free fatty acids, and waxes are also present in less quantity. The crude RBO is mainly obtained through the solvent extraction process. To produce the edible grade vegetable oil, it is then refined and processed further either chemically or physically to meet the standards of specifications. The quality of RBO is, however, affected by the processing steps that are applied during the refining of RBO, which can affect the retention/availability of oryzanol and various other bioactive components in the commercial refined RBO. The process of refining may consist of acid degumming, centrifugation, clarification, bleaching, deodorization, and winterization (Rajam et al. 2005). Chemical refining of crude RBO yields better products in terms of color, cloud point, and other physical characteristics (Danielski et al. 2005; Rajam et al 2005).

The oryzanol content of RBO extracted from the bran of 18 different Indian paddy cultivars ranged from 1.63% to 2.72% (Krishna et al. 2001). Based on its high antioxidant potential, RBO has been categorized as valuable edible oil for human consumption (Bopitiya and Madhujith 2014). RBO contains 52.845% oleicacid, 3.076% linoleic acid. The saturated fatty acids present in RBO are 2.269% stearic acid and 18.750% palmitic acid.

In Japan, RBO is commonly known as a "heart oil", whereas, in Western countries, it has attained the status of "healthy food"(CAC 2003). It is also now becoming popular in the USA and other parts of the world because of its relatively low price and many health benefits(Liang et al. 2014). RBO has therefore great potential in the development of pharmaceutical and cosmetic products(Ammar et al. 2015). RBO relieves the menopausal symptoms, increases cognitive function, and may lower the incidence of an

allergic reaction(Mehdi etal. 2015). Mitochondrial dysfunction can lead to excessive production of reactive oxygen species (ROS) and free radicals, which are produced as a result of certain metabolic abnormalities. They cause cellular damages through the oxidation of proteins lipids and DNA and therefore result in oxidative stress and progression of various chronic diseases(Giacco and Brownlee 2010; Kaneto et al. 2010; Waly et al. 2010;Ju and Zullaikah 2013). Polyphenols play an important role in modulating the differentially regulated pathways in endothelial cells and thus can help in maintaining vascular homeostasis. The Published data underlines the significance of phytochemicals in inhibiting the pathways that activate the nuclear transcription factor-kappa B (NF-κB) that is linked to a variety of inflammatory diseases (Surh et al. 2001; Bellik et al. 2012). Polyphenols protect the endothelial cells against various stimuli by downregulating the tumor necrosis factor-alpha (TNF-α) (Suganya et al. 2016; Shih et al. 2011) reported that RBO showed preventive effects in delaying colon carcinogenesis. They observed higher hepatic antioxidant status including the glutathione (GSH) and thiobarbituric acid reactive substance levels as well as the superoxide dismutase and catalase activities, in RBO-fed rats. They concluded that this higher antioxidant status in RBO-fed rats might be responsible for delaying carcinogenesis. The inclusion of RBO in rat diets can improve their antioxygenic potential and may protect against oxidative stress (Rana et al. 2004).

The consumption of a plant sterol-based spread derived from RBO as a part of a normal diet proved effective in reducing plasma lipid levels in mildly hypercholesterolaemic individuals (Eady et al. 2011). Daily consumption of RBO-modified milk (containing 18 g RBO for 5 weeks) significantly decreased TC level and tended to decrease LDL-C level in patients with type 2 diabetes. However, no significant influence on insulin resistance was observed (Lai et al. 2012).

MATERIALS AND METHODS

The rice bran oil was purchased on VestigeMarketing Pvt Ltd Ratan Esquire, Chunni Ganj, Kanpur. The rice was harvested in the middle of July 2019 from Punjab.

PREPARATION OF ESTER

The chemical reaction that takes place during the formation of the ester is called esterification.

Esterification is the process of combining an organic acid(RCOOH) with an alcohol (ROH) to form an ester (RCOOR) and water.

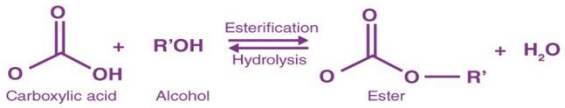


Figure 1 Chemical reaction for esterification

Take 50 mg of oil in a glass-stoppered test tube and add 1 ml of dichloromethane/benzene followed by 2 ml of 1% sodium methoxide solution. Hold the test tube at 60°C for 10 min. Cool and add 0.1 ml of glacial acetic acid followed by 5 ml of distilled water and 5 ml petroleum ether (40°C-60°C). Mix the contents. Allow the layers to separate. Take out about 2 ml of the upper layer containing the methyl ester in a small tube and concentrate it by passing nitrogen gas before injecting it to gas chromatography(AOAC Method 2000).

GAS-CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Gas chromatography-mass spectroscopy (GC-MS) a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixture [(21)]. Analysis of the fatty acid composition carried out by gas chromatography-mass spectrometry (GC-MS) using an Agilent Intuvo 9000 GC system with Mass Selective Detector (Agilent 5977B). A HP-5MS fused silica capillary column (30 mm X 0.32 mm X 0.25 µm film thickness) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 250◦C and the oven temperature program was as follows: 60◦C to 250◦C at 3◦C /min. The split ratio was 1:100, the detector temperature was 270◦C and the injection volume was 1 µL. The MS interface temperature was 270◦C, MS mode, E.I. detector voltage 1200 V, and mass range 35 to 450 Da at 1.0 scan/s.

IDENTIFICATION OF COMPONENTS

The identification of constituents Was performed based on retention index (RI), determined regarding the homologous series of n-alkanes, C_9-C_{24} under experimental conditions, co-injection with standards,

MS library search (2.0 version NIST 2002 and WILEY-7th edition May 2003), and by comparing with the MS literature data(Adams RP. USA 2007, Davies NW. et al. 1990). The relative amount of the individual components was calculated based on the GC peak area (FID response) without correction factors. The FID report is shown in Table 2 and MS spectra are shown in Figure 2.

Table 1. Fatty acid composition of Rice bran oil.

Component name	MS%	FID%
Methyl Meristate	0.57	-
Palmitelaidate	0.35	-
Palmetic acid	20.43	18.750
Linoleic acid	4.39	3.076
Oleic acid	49.9	52.845
Stearic acid	3.27	2.269
Eicrenoic acid	1.01	-
Archadic	1.47	-

SAPONIFICATION VALUE (S.V.)

The oil sample (2.0 g) was accurately weighed into a conical flask and 25ml of 0.5 N alcoholic KOH in a similar flask. Reflux condensers were fitted to both flasks and the contents were heated in a water bath for one hour, swirling the flask from time to time. The flasks were then allowed to cool a little and the condensers washed down with a little distilled water. The excess KOH was titrated with 0.46 M HCl acid using phenolphthalein indicator(Official Method of Analysis 1990).

The saponification value was calculated using the following equation:

$$S.V. = \frac{(b-a) \times F \times 28.05}{Weight\ of\ sample}$$

Where, b = titre value of blank (ml), a = titre of sample (ml), F = factor of 0.46 M HCl =1 (in this case) and 28.05 = mg of KOH equivalent to 1,l of 0.46 M HCl.

ACID VALUE (A.V.)

Ethanol was boiled on a water bath for a few minutes to remove dissolved gases and neutralized by adding a few drops of phenolphthalein and about 10 ml 0.1M potassium hydroxide (KOH) until a pale pink color was obtained. The oil sample (6.0 g) was weighed into a conical flask and 50 ml of hot previously neutralized alcohol was added. The mixture was later boiled in a water bath. The hot mixture was then titrated with 0.1N potassium hydroxide (KOH) solution until the pink color (stable for few minutes) returned(Official Method of Analysis 1990).

$$A.V. = \frac{Titre\ value\ (ml) \times N \times 56.1}{Weight\ of\ sample}$$

Where N = normality of KOH = 0.1M (in this case), 282 = molar

mass of oleic acid and 56.1 = molar mass of KOH.

REFRACTIVE INDEX (μ)

The Refractive index, also called the index of refraction, measure the bending of a ray of light when passing from one medium into another. If *i* is the angle of incidence of a ray in vacuum and *r* is the angle of refraction, the refraction index n is defined as the ratio of the sine of the angle of incidence to the sine of the angle of retraction; i.e.

n = sin*i* / sin *r*

The refractive index of the rice bran oil at 23°C temperature was determined using RUDOLPH Automatic Refractometer J47-J57 model, Made in USA. The oil drop was placed on the slide and directed towards a source of light. It was then observed through the lens after adjustment had been made to give a semi-circle on the glass prism in the refractometer. The reading was then taken.

DENSITY AND SPECIFIC GRAVITY

Density is the mass of a material divided by its volume. Some of the most commonly used units for density include: grams/cubic centimeter (g/cm^3), grams/milliliter (g/ml), kilograms/cubic meter (kg/m^3), and pounds/gallon(lbs/gal).While a material's mass does not charge as a function of temperature, its volume does. Therefore, a material's density is a function of temperature. It is necessary to always include temperature with any description of density.Density is often shown in the literature as the Greek letter rho, ρ.

Specific gravity sometimes called Relative density is a dimensionless value that is the quotient of two density values. In its most basic form,it may be expressed as:

$$SG_{t2}^{t1} = \frac{Density\ of\ material\ being\ measured\ at\ some\ temperature\ t1}{Density\ of\ some\ reference\ material\ (usually\ water)\ at\ some\ temperature\ t2}$$

Temperature t1 does not have to be equal to temperature t2, that is; t1 ≠ t2.

Therefore, it is best to always indicate the temperature in both the numerator and denominator when describing Specific Gravity. Unfortunately, this is not always done. It is common to hear or read descriptions of SG at only one temperature. That is, the SG at 20°C (60°F). If this is all the information given, one can only assume that the temperature in the numerator and denominator are the same. But assume this with some caution.

The density and specific gravity of rice bran oil at 20°C temperature was determined using the RUDOLPH Density meter DDM 2909 model, Made in USA. Take 2ml of rice bran oil in a syringe, insert into the density meter. Set temperature at 20°C.After a few minutes note the result shows on a display of density meter.

RESULT AND DISCUSSION

The results of the present study are summarized in Table 1, Table 2, and Figure 2. The major fatty acid component in RBO oil was found to be about 52% Oleic acid, 3.07% linoleic acid, 18.750% palmitic acid, 2.269% stearic acid, and little of palmitelaidale, eicrenoic acid. Atrace of arachidic acid was also found.

The saponification value was found to be 156 mgKOH/g which is less than 175 to 195 mgKOH/g reported by Ramachandran (Ramachandran HD 2001)for cured rice bran oil. A high saponification value of 156 mgKOH/g is indicative that the oil investigated in this work has the potential for uses in the industries.

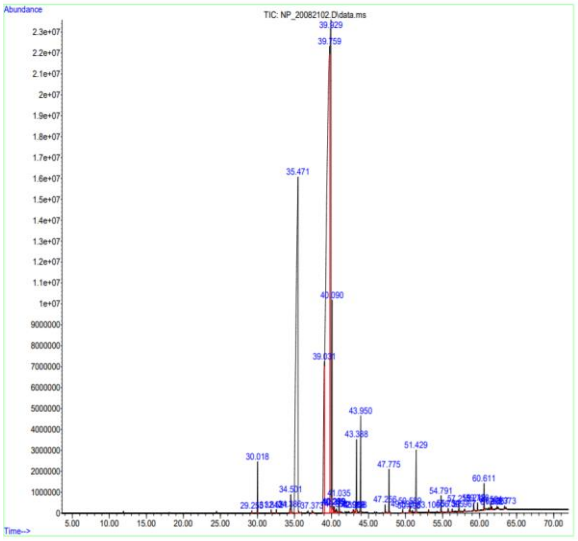
Table 2. FID report rice bran oil

Sum of corrected areas: 14151210796

Signal : NP_20082102.D\FID1A.ch

peak #	R.T. min	Start min	End min	PK TY	peak height	corr. area	corr. % max.	% of total
1	30.032	29.932	30.104	BB	276116	8666954	0.63%	0.335%
2	35.488	34.940	35.662	BB	3266078	485796225	35.48%	18.750%
3	39.043	38.802	39.074	BV	993077	79690231	5.82%	3.076%
4	39.770	39.074	39.800	VV	5308774	1369128211	100.00%	52.845%
5	39.941	39.800	39.990	VV	5977608	524900466	38.34%	20.260%
6	40.103	39.990	40.214	VB	1463710	58777155	4.29%	2.269%
7	43.401	43.161	43.461	BB	377259	15100699	1.10%	0.583%
8	43.962	43.715	44.055	BB	520592	22398241	1.64%	0.865%
9	47.787	47.655	47.878	BB	219245	7490250	0.55%	0.289%
10	51.441	51.285	51.538	BB	327871	12617402	0.92%	0.487%
11	60.624	60.521	60.715	BB	162217	6294561	0.46%	0.243%

Figure 2.Spectrum of Rice bran oil



The acid value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids in one gram of sample. It is a relative measure of rancidity as free fatty acids are normally formed during the decomposition of oil. The acid value should not be more than 0.5. The acid value of rice bran oil was found at 0.4. Some enzymes that are present in the rice bran include α-amylase, β-amylase, ascorbic acid oxidase, catalase, cytochrome oxidase, lipase, lipoxygenase, peroxidase, and many others. Particular attention should be given to lipase, lipoxygenase, and peroxidase, because of their potential in reducing the quality and shelf life of rice bran.

The Refractive index of oil is used to detect rancidity in edible oil. The index range should be between 1.4650 to 1.4750. for rice bran oil. The refractive index of rice bran oil found 1.4725. Immediate extraction and processing are considered of prime importance, as delayed extraction can lead to problems, such as color changes and deterioration of organoleptic quality and flavors.

Specific gravity is the ratio of a material to the density of water. As per the Indian standard, the specific gravity of rice bran oil should be in the range of 0.915 to 0.930. The specific gravity of rice bran oil was found 0.922.

The flashpoint of a volatile material is the lowest temperature at which it can vaporize to form an ignitable mixture in air. At the flashpoint, the vapor may cease to burn when the source of ignition is removed. It should not be less than 250°C in the case of rice bran oil. The higher the flashpoint of rice bran oil, the lower is the risk of ignition in the oil. The

flashpoint of RBO was above 250°C.Rice bran oil should be free from rancidity, sediment, suspended or other foreign matter, and separated water. The tested oil was observed to meet this requirement. The clarity of the material was judged by the absence of turbidity. Turbidity was absent in this oil.

CONCLUSION

Now a days, the rate of diabetes with the associated risk of CVD is continuously on the increase worldwide(International Diabetes Federation (IDF) 2015). Type 2 diabetes mellitus (T2DM) is a complex multifactorial condition that is caused by inappropriate dietary and lifestyle patterns and inheritance factors (Tuomilehto J, Schwarz PE 2016). T2DM is characterized by insulin resistance and is often accompanied by cardiovascular disease risk factors, including obesity, dyslipidemia, and hypertension (Hanely AJ, Williams K, et. Al 2002, Semple RK 2016). Dietary strategies are considered as the first line of defense in the prevention and management of diabetes, cardiovascular diseases, and cancers. RBO with its excellent fatty acid composition and bioactive antioxidant has demonstrated beneficial effects to improve the plasma lipid profile in rodents, rabbits, non-human primates, and humans. The vast majority of scientific data greatly augments the importance of RBO and its significant physiological action in health and diseases. Although RBO has unique physiological and biological properties, the clear-cut mechanisms of RBO and its bioactive components on health and diseases still need to be elucidated. As evidenced from several observational and animal studies, it is well documented that the RBO has an imperative role in the prevention, management, and control of chronic diseases, and therefore RBO would certainly be a valuable dietary addition as a functional food in everyday diet.

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