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Physico-Chemical Properties and GCMS Analyses of Indigenous Rice Bran and Mustard Seed Oils and their Blends

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ABSTRACT

Blended edible oils were formulated to enrich fatty acid profile with balance fatty acid ratio. Indigenous Rice Bran Oil (RBO) and Mustard Seed Oil (MO) were mixed in the ratio of 60:40, 70:30 and 90:10 (RBO:MO). Moisture content, density, specific gravity, refractive index, viscosity, free fatty acid, acid value, iodine value, unsaponifiable matter and peroxide value of the single vegetable oils and their blends were determined. Fatty acid composition such as Saturated Fatty Acid (SFA), Mono-Unsaturated Fatty Acid (MUFA), Poly-Unsaturated Fatty Acid (PUFA) of all oils were analyzed by GCMS. GCMS analysis of rice bran oil identified 6 fatty acids with ratio SFA:MUFA:PUFA=1.43:1.46:1.0 and mustard seed oil identified 8 fatty acids with ratio SFA:MUFA:PUFA=1.72:1:2.06. All blended oils revealed 9 fatty acids with ratio SFA:MUFA:PUFA=1.24:2.1:1.0;SFA:MUFA:P-UFA=1.34:2.56:1.0andSFA:MUFA:PUFA=1.2:1.6:1.0; for 60:40, 70:30 and 90:10 (RBO: MO) respectively. Among three blendings, SFA:MUFA:PUFA=1.2:1.6:1.0 for 90:10 (RBO: MO) ratio was near World Health Organization (WHO) guidelines. According to WHO and other health agencies, nutritionally superior oil must fall in the ratio of 1:1-3:1; SFA; MUFA and PUFA respectively. The results suggest that 90:10 (RBO:MO) blended oil can be good alternative oil for edible purposes with improve nutrition as well as desirable physico-chemical properties.

Abbreviations: RBO: Rice Bran Oil; MSO: Mustard Seed Oil; SFA: Saturated Fatty Acid; MUFA: Mono-Unsaturated Fatty Acid; PUFA: Poly-Unsaturated Fatty Acid; LDL-C: Low Density Lipoprotein Cholesterol; FFA: Free Fatty Acid; AV: Acid Value

Introduction

Vegetable oil plays a variety of in human nutrition due to its different fatty acids. But high intakes of Saturated Fatty Acids (SFA) increase Low Density Lipoprotein Cholesterol (LDL-C) as well as cardiovascular diseases. On the other hand, unsaturated fatty acids have the opposite effects. To combat this fatty acid ratio problem, blending of vegetable oils is gaining popularity in the oil sector to satisfy consumer demands. Rice Bran Oil (RBO), a by-product of the rice milling industry contains unique antioxidant compound

gamma-oryzanol, high levels of phytosterols and tocols [1,2]. Due to the presence of micronutrients in RBO, the value of other edible vegetable oils can be nutritionally improved by mixing with RBO. Choudhary, et al. [3] stated that blending of non-conventional RBO with traditional oils (sunflower oil, canola oil etc.) can result in a stable cooking oil that reduces the demand for traditional oils. On the other hand, mustard oil which is rich in unsaturated fatty acids widely used for both culinary and therapeutic purposes. Oil with higher unsaturation are highly prone to oxidation [4]. Basically fatty acid composition of the oils is the main factor responsible for

oxidation and also for the formation of undesirable compounds or flavours [5,6]. However, there is global increasing demand for the production of a blending oil for edible purposes because of improved fatty acids and nutritional benefits.

Blending is one of the methods used to modify oils and fats for wide range of applications. Evidences suggest that no single oil can provide the recommended dietary fatty acid ratio. According to WHO and other health agencies, nutritionally superior oil must fall in the ratio of 1:1-3:1; SFA, MUFA and PUFA respectively [7]. The objective of the present investigation was to design an oil blend which may provide an ideal recommended fatty acid ratio regarding nutrition.

Materials and Methods

Materials

Mustard seeds were procured from local market then fresh and healthy mustard seeds were stored for oil extraction. Freshly milled rice bran, outer layer of the grain was collected from rice milling industry, Rajshahi, Bangladesh and immediately processed after milling for stabilization. Primarily sieved with 100 mesh size to separate husk and broken rice grain. All solvents and chemicals used were analytical, HPLC and GC grade. Fatty acids standards were purchased from Sigma-Aldrich.

Extraction of Oils

Rice bran oil was extracted by solvent extraction method. The extraction process was conducted in the laboratory by Soxhlet apparatus for 6 hours using n-hexane as extracted solvent. The ratio of rice bran:hexane used was 1:3 (w/v). n-hexane has been selected because the solvent has better effect over other polar solvents like alcohol, ketone, aldehyde ether and ester etc. [8]. The oil was recovered by the evaporation of the solvent under reduced pressure using a Rotary vacuum evaporator and the percentage of oil content was calculated. Mustard oil was extracted by automated oil expelling machine. The refining process of the oils was carried out using the method as described by [9] with some modification.

Preparation of Blends

Rice bran oil and mustard seed oil were taken in the ratio of $90:10,\,70:30$ and 60:40 respectively. Each blend was placed in 250 ml beakers and were mixed by using a mechanical stirrer at 180 rpm for 15 min. Then the single oils and all blended oils were stored at room temperature in air tight glass bottle for physico-chemical and GCMS analyses.

Analysis of Physicochemical Properties

Physical properties such as moisture content, refractive index, density, specific gravity and viscosity of the single and blended oils were determined by the method described by Garba, et al. [10] and AOAC [11]. Acid value, saponification value, iodine value, peroxide value, unsaponifiable matter and free fatty acid were estimated

according to the AOAC and AOCS Official Methods [12,13]. Viscosity of all oils were determined by Fungilab digital Rotational viscometer. They measure viscosity by sensing the torque required to rotate a spindle at constant speed while immersed in fluid.

Determination of Fatty Acid Composition

Preparation of Fatty Acid Methyl Ester: 200 mg of oil sample was taken in a 10 mL Pyrex test tube. 3.5 mL 0.5M Sodium methoxide was added to the test tube and heated using burner until removing the bubbles. Then, 1.5 mL n-hexane was added to the mixture and mixing by vortex mixture. Then around 5 mL deionized water was added to test tube slowly and waited for settle down. Organic upper layer was taken into the GC vial for GCMS analysis.

GCMS Condition: The gas chromatographic analyses of the oils were performed on SHIMADZU GC-2010. GC was equipped with auto-sampler (AOC- 20s), auto-injector (AOC-20i) and SH Rxi 5MS Sill capillary column with $30m\times0.25mm\times0.25~\mu m$ film. Helium was used as carrier gas at flow rate 2.0 mL/min. Initial oven temperature of GC was 1400C for 10.0 min, then increased at 70C/min to a final temperature of 250 0C for 10.0 min. The injector temperature was 250 0C and injection volume was 1.0 μL with 75:1 split ratio. Solvent cut time was 3.40 min and total run time was 35.71 min. The detector used was SHIMADZU GCMS-QP-2020 and temperature was 2550C.

Results and Discussion

Moisture is the amount of water present in edible oils. Moisture should be as low as possible. The maximum allowed moisture content in edible oils is 0.2% [14]. Previous studies have shown that fungus species such as Aspergillus niger and Mucor species survive and reproduce when the moisture content value is higher than 0.2% / [15]. Moisture content of all oils and their blends were determined presented in Table 1. All values were very low than specified limit. Density, specific gravity and refractive index of all single oils and their blends were determined presented in Table 1. Results were within the WHO/FAO permissible limits. Viscosity of oil is a measure of the oils resistance to flow. In the present study, it can be observed from Table 1 that viscosity decreased in the blended oils than single vegetable oils, this is because blending oil with different ratios decreases saturation as well as increases unsaturation fatty acids of oil. This phenomenon also verified by other researcher since oil viscosity depends on molecular structure and decreases with unsaturation of fatty acids [7]. It may due to double bonds that make bonding more rigid and rotation between c-c bonds become more strenuous.

The Free Fatty Acid (FFA) and Acid Value (AV) are most important quality parameters of edible oil. The results showed in Table 1 that the FFA value of all oils were very low and the percentages of AV for all oils were within the permissible level (0.6 mg KOH/g) [16]. All oils were of good quality because of the low

percentage of FFA and acid value. Theoretically, AV is known to be high when FFA content is high. FFA content differs from AV, which can be due to the fact that acid phosphates as well as amino acids contribute to the acid level [17]. Mean peroxide value of the oil samples are shown in Table 1. Highest level of peroxide value was observed for mustard oil (2.84 mEq/kg oil) and lowest for RBO:MO, 90:10 blended oil (1.44 mEq/kg oil). But levels of peroxide value obtained for the all oil samples were below the acceptable limit (10 mEq/kg oil) recommended by Codex Standards [18]. The low values of peroxide are indication of low levels of oxidative rancidity of the oils. A rancid taste often begins to be noticeable when the peroxide value is above 20 meq/kg [19]. The iodine value is a

measurement of the total unsaturation of vegetable oils, as well as an indicator of their susceptibility to oxidation [19]. In the present study shown in Table 1, the blends of mustard oil with rice bran oil shows increase in iodine value. Hence, the observed higher iodine value in the oils indicated that they are likely to be healthier for consumption. Studies have recommended to switch from saturated to unsaturated fats because of the risk of cardiovascular disease associated with high consumption of saturated fatty acids [20-23]. The unsaponifiable matters for all oils were within 0.35-0.62 % presented in Table 1 and the values were in good agreement with the reported result of Tan, et al. [24].

Table 1: Physicochemical properties of Rice ban oil, Mustard oil and their blends.

| Properties | Rice Bran Oil | Mustard oil | Blend (RBO:MO) | | | |
|------------------------------|---------------|--------------|----------------|--------------|--------------|--|
| | | | 60:40:00 | 70:30:00 | 90:10:00 | |
| Moisture content (%) | 0.03± 0.001 | 0.05 ± 0.002 | 0.08± 0.001 | 0.04± 0.002 | 0.01± 0.003 | |
| Density at 25°C | 0.8933±0.3 | 0.8867±0.3 | 0.8933±0.2 | 0.8933±0.3 | 0.8933±0.2 | |
| Specific gravity at 25°C | 0.9017±0.05 | 0.9021±0.01 | 0.9031±0.03 | 0.9011±0.03 | 0.9022±0.04 | |
| Refractive index at 25°C | 1.4672±0.021 | 1.4652±0.001 | 1.4652±0.002 | 1.4652±0.011 | 1.4652±0.011 | |
| Viscosity, cst | 59.06±0.03 | 61.30±0.02 | 55.36±0.04 | 53.33±0.01 | 58.37±0.01 | |
| Free Fatty Acid (as oleic %) | 0.10 ±0.004 | 0.15 ±0.006 | 0.15 ±0.005 | 0.13 ±0.004 | 0.14 ±0.005 | |
| Peroxide value (meq/kg) | 1.46±0.21 | 2.84±0.13 | 2.21±0.21 | 1.59±0.22 | 1.44±0.31 | |
| Iodine value (g/100g) | 120±0.41 | 121.3±0.32 | 124.3±0.53 | 125.3±0.33 | 122.3±0.30 | |
| Acid value (%) | 0.5±0.01 | 0.4±0.02 | 0.55±0.01 | 0.45±0.01 | 0.50±0.03 | |
| Unsaponifiable matter | 1.8±0.11 | 1.5±0.10 | 1.6±0.12 | 1.7±0.21 | 1.7±0.31 | |

Note: All the experiments were performed in triplicate and the results were expressed as mean ± SD (standard deviation).

The results of GCMS analysis of rice bran oil are displayed in Figure 1 and presented in Table 2. They revealed the presence of 6 fatty acids were classified as Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA) and Polyunsaturated Fatty Acids (PUFA) in percentages 36.704, 37.624 and 25.671 respectively. 9-octadecenoic acid methyl ester as oleic acid was the major identified monounsaturated fatty acid (37.624%). Fatty acid ratio was SFA:MUFA:PUFA=1.43:1.46:1.0 shown in Table 3. GCMS analysis of mustard seed oil are shown in Figure 2 and presented in Table 4. They identified 8 fatty acids where saturated fatty acids 34.033%, monounsaturated fatty acids 20.853% and polyunsaturated fatty acids 43.115%. Fatty acid ratio was SFA:MUFA:PUFA = 1.72:1:2.06 presented in Table 3. GCMS analysis of blended oil mixing ratio of RBO:MO:90:10 are displayed in Figure 3 and presented in Table 5. They revealed the presence of 9 fatty acids. Saturated, monounsaturated and polyunsaturated fatty acids were 31.569%, 42.297% and 26.134% respectively. Fatty acid ratio

was SFA:MUFA:PUFA = 1.2:1.6:1.0. Also Fatty acid ratio of blended oil in the mixing ratio of RBO:MO;60:40 and RBO:MO;70:30 were determined and presented in Table 3. SFA:MUFA:PUFA ratio was 1.24:2.1:1.0 for RBO:MO;60:40 blend and SFA:MUFA:PUFA ratio was 1.34:2.56:1.0 for RBO:MO;70:30 blend. Among three blending, SFA:MUFA:PUFA = 1.2:1.6:1.0 ratio for RBO:MO;90:10 blend was near World Health Organization guidelines. Results depicted that single rice bran and mustard seed oils have a higher unsaturated fatty-acids than saturated fatty acid. However, highly unsaturated oils goes oxidative degradation due to the presence of their double bonds [25] unless sufficient antioxidant is added [26-28]. Also high consumption of saturated fatty-acids associated with the risk of cardiovascular diseases [20-23]. So, mixing vegetable oil can be a cost-effective practice to modify their fatty acid profile and physico-chemical properties [7]. Present work can meet the desired requirements.

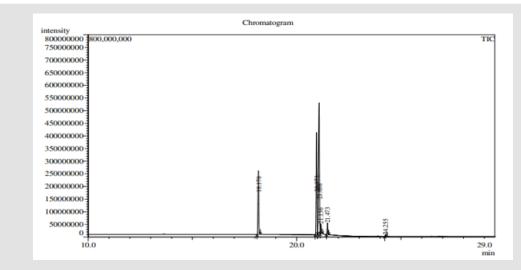


Figure 1: GCMS chromatogram of rice bran oil.

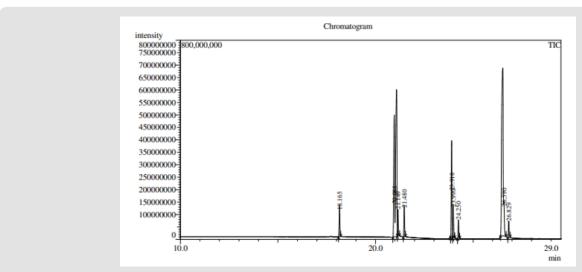


Figure 2: GCMS chromatogram of mustard oil.

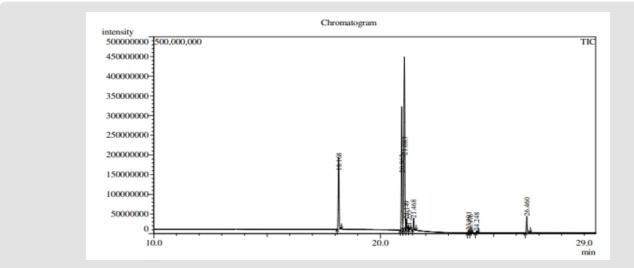


Figure 3: GCMS chromatogram of blended oil.

Table 2: Identification of the components of rice bran oil.

| Peak# | Name | R.Time | Area | Conc. | Conc.Unit |
|-------|--|--------|----------|--------|-----------|
| 1 | Hexadecanoic acid, methyl ester | 18.178 | 29307399 | 21.605 | % |
| 2 | 9.12-Octadecadienoic acid (Z,Z)-, methyl est | 20.937 | 34822715 | 21671 | % |
| 3 | 9-Ociadecenoic acid. methyl ester, (E)- | 21.056 | 40884011 | 30.14 | % |
| 4 | 9-Ociadecenoic acid (Z)-, methyl ester | 21.155 | 10152610 | 7.484 | % |
| 5 | Octadecanoic acid. methyl ester | 21.473 | 17867775 | 13.172 | % |
| 6 | Heneico.sanoic acid. methyl ester | 24.254 | 2614628 | 1.927 | % |

Table 3: Percentage of saturated, mono-unsaturated and poly-unsaturated fatty acids.

| Name of oil | Saturated fatty acid (SFA) | Mono- unsaturated fatty acid (MUFA) | Poly-unsaturated fatty acid (PUFA) | SFA:MUFA:PUFA | | | |
|--------------------------|----------------------------|-------------------------------------|---------------------------------------|---------------|--|--|--|
| Rice bran oil (RBO) | 36.704 | 37.624 | 25.671 | 1.4:1.5:1 | | | |
| Mustard oil (MO) | 36.033 | 20.853 | 43.115 | 1.7:1:2.1 | | | |
| Blended Oil Mixing Ratio | | | | | | | |
| (RBO 60%+MO 40%) | 28.437 | 48.548 | 23.015 | 1.2:2.1:1 | | | |
| (RBO 70%+MO 30%) | 27.377 | 52.234 | 20.388 | 1.3:2.6:1 | | | |
| (RBO 90%+MO 10%) | 31.569 | 42.297 | 26.134 | 1.2:1.6:1 | | | |

Table 4: Identification of the components of mustard oil.

| Peak# | Name | R.Time | Area | Conc. | Conc.Unit |
|-------|--|--------|----------|--------|-----------|
| I | Hexadecanoic acid methyl ester | 18.168 | 31968771 | 13.999 | % |
| 2 | 9,12-Omadecadienoic acid (Z,Z)-, methyl est | 20.946 | 43166088 | 18.903 | % |
| 3 | 9-Octadecenoic acid (Z)-, methyl ester | 21.149 | 21708266 | 9.506 | % |
| 4 | Octadecanoic acid. methyl ester | 21.478 | 26709374 | 11.696 | % |
| 5 | 9.12-Octadecadknoic acid, methyl ester, (E). | 23.892 | 31394211 | 13.748 | % |
| 6 | 8.11.14-Ecosatrienoic acid, methyl ester | 23.985 | 23894549 | 10.464 | % |
| 7 | Heneicosanoic acid. methyl ester | 24250 | 23607070 | 10.338 | % |
| 8 | 13-Docosanoic acid. methyl ester | 26.829 | 25911594 | 11.347 | % |

Table 5: Identification of the components of blended oil (RBO:MO;90:10).

| Peak# | Name | R.Time | Area | Conc. | Conc.Unit |
|-------|--|--------|----------|--------|-----------|
| I | Hexadecanoic acid, methyl ester | 18.163 | 24398534 | 20.33 | % |
| 2 | 9,12-Octadecadienoic acid (Z,Z)-, methyl est | 20.953 | 29844681 | 24.869 | % |
| 3 | 9-Octadecenoic acid, methyl ester, (Z)- | 21.047 | 35059914 | 29.214 | % |
| 4 | 9-Octadecenoic acid (E)-, methyl ester | 21.148 | 6142968 | 5.119 | % |
| 5 | Octadecanoic acid, methyl ester | 21.469 | 11768607 | 9.806 | % |
| 6 | 9.12-Octadecadienoic acid (E.E)-, methyl est | 23.891 | 1314795 | 1.096 | % |
| 7 | 8,11,14 Ecosatrienoic acid, methyl ester | 23.976 | 203290 | 0.169 | % |
| 8 | Heneicosanoic acid, methyl ester | 24.247 | 1719357 | 1.433 | % |
| 9 | 13-Docosenoic acid, methyl ester, (Z)- | 26.461 | 9557494 | 7.964 | % |

Conclusion

Blending mustard oil with rice bran oil in the ratio of RBO: MO, 90:10 can be encouraged to enrich fatty acid composition as well as balance fatty acid ratio of the oil, because MO is rich

in PUFA, while RBO is rich in SFA and MUFA with addition to bioactive phytochemicals. This study will help the oil producing industry to find the most economically viable oil blends for cooking purposes with the maximum fatty acids, nutrition and desirable physicochemical properties.

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