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Studies on the characterization and distribution of fatty acids and minor components of high-erucic acid mustard oil and low-erucic acid rapeseed oil

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Abstract

The aim of this work was to compare the positional fatty acid composition, sterols, tocopherols and oxidative stability of mustard oil (MO) and rapeseed oil (RSO). The MO contained higher levels of erucic acid (42.8%) and linolenic acid (18.2%), compared with RSO (0.4 and 8.6%) whereas the RSO contained higher levels of oleic acid (62.2%) and linoleic acid (19.5%), than that of MO (17.4 and 15.8%). The changing fatty acid compositions at different position led to different physical properties. The ω-6/ω-3 ratio of MO and RSO were 0.87 and 2.27, respectively. The balance of ω-6/ω-3 ratio is also an important determinant in decreasing the risk for coronary heart disease. MO contained higher amount of total tocopherols (38.32 mg/100g) but lower amount of total sterols (606.32 mg/100g) than that of RSO (631.98 mg/100g and 25.57 mg/100g). The oxidative stability determined by Rancimat test of MO (PF, 1.57) was higher compared with RSO (PF, 1.0).

Key words: Mustard oil, Rapeseed oil, Fatty acids, Sterols, Tocopherols, Oxidative stability

Introduction

Lipids (oils and fats), apart from providing nutrition, are an integral part of human diets throughout the world, which are products from plants and animals kingdom with great variation in the composition of fatty acids that are of importance for both the chemical and the food industries. Lipids are known to play functional roles in contributing to the palatability of processed foods which provide human nutrition. Actually, consumers tend to prefer oil which is available in their region. In Bangladesh, commonly used edible oils are soybean and mustard oil (MO). In India and Bangladesh, MO is collected from Mustard seeds by traditional way.

MO makes up about 24-40% of the mustard seeds, which is characterized by the presence of higher level of erucic acid and it has the lowest saturated fatty acids content among all the edible vegetable oils. MO has a strong smell, pungent odor (allyl isothiocyanate), hot and nutty taste. Bangladeshi consumers like such strong smell, hot nutty taste, pungent and sulfurous odor. MO contains fatty acids like erucic, oleic, linoleic and α-linolenic acid. This oil contains a little less than 60% of monounsaturated fatty acids out of which 42% erucic acid and 12% oleic acid. It contains 21% polyunsaturates (6% ω-3 α-linolenic acid and 15% ω-6 linolenic acid) and it has 12% saturated fats (USDA National Nutrient Database). It contains the pungent Allyl isothiocyanate and has about 60% monounsaturated fatty acids of which 42% erucic acid and 12% oleic acid, it has 21% polyunsaturates of which 6% is the omega-3 alpha-linolenic acid and 15% omega-6 linoleic acid and it has 12% saturated fats (USDA National Nutrient Database). MO may provide a protective effect in patients with acute myocardial infarction possibly due to the presence of α-linolenic (Singh et al., 1997). However, higher levels of erucic acid are unsuitable for human consumption as food purposes (Kramer et al., 1982). Generally, erucic acid enriched oil is useful for the polymer industry and is valuable raw material for manufacture of industrial products such as plasticizers, detergents, surfactants, polyesters and coatings whereas oils low in erucic acid are recommended for food purposes because oils high in erucic acid may cause an accumulation of

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triacylglycerol in the hearts of animals (Beare-Rogers et al., 1971). Higher consumption of erucic acid may increase the concentration of adrenal cholesterol causing fibrotic changes in myocardium, liver weight and cholesterol (Aaes-Jorgensen, 1972; Beare-Rogers et al., 1972). High levels of erucic acid is not suitable for human food in European Union and other developed countries since erucic acid showed serious pathological changes in the heart and skeletal in animals (Food Standards Australia New Zealand, 2003).

Rapeseed is the third leading source of edible oil in the world. Rapeseed oil (RSO) is the most useful of all cooking oils and it contains a significant amount of n-3 and n-6 fatty acids. RSO contains mostly of the fatty acid such as oleic, linoleic, linolenic, palmitic and strearic acid (Gunstone, et al., 1994; Hui, 1996). The oil content usually makes up about 40-60% of rapeseed. RSO consists 95% of triacylglycerols (TAG) and 5% non-triacylglycerols, known as minor components like free fatty acids, mono- and diacylglycerols, phospholipids, tocopherols, tocotrienols, flavonoids, other phenolic compounds, pigments (chlorophylls), sterols etc (Shahidi and Shukla, 1996).

Every vegetable oil has its own stability against oxidation depending on the fatty acid compositions and the content of antioxidants which it contains (Nogala-Kalucka et al., 2005; Kamal-Eldin, 2006; Przybylski and Eskin, 2006). Figure 1 shows the structure of linoleic (polyunsaturated fatty acid) and erucic acid.

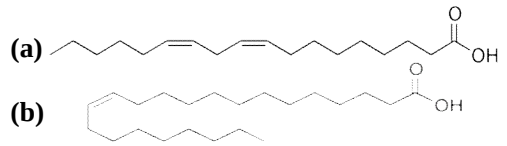

Figure 1. (a) Chemical structure of polyunsaturated fatty acid (linoleic acid); (b) erucic acid (Wikipedia, 2012).

Generally, polyunsaturated fatty acids are responsible for oxidation and off-flavours. However, antioxidant compounds present in oil which is important in the prevention and treatment of diseases such as heart disease, autism, cancer, stroke, diabetes, Alzheimer's dementia, Parkinson's disease, arthritis and muscular degeneration (Manna et al., 2002). It also contributes to lowering serum cholesterol levels in human body (Moreau, 2004) also to the oxidative stability and shelf-life of oil (Przybylski and Eskin, 2006).

The main objectives of this research work were to study the positional fatty acids composition, retention of sterols and tocopherols, and the oxidative stability of MO and RSO.

Materials and Methods

Oils and chemicals

Mustard oil (MO) was supplied from Agricultural Marketing Co. Ltd. (Dhaka, Bangladesh). Rapeseed oil (RSO) was from AAK (Aarhus Karlshamn AB, Malmö, Sweden). The heptadecanoic acid, acetic acid and pancreatic lipase from porcine pancreas (Type II) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Standard of sterols 5α-cholestane was obtained from Steraloids (Newport, RI, USA), reference samples of tocopherols were obtained from Merck (Darmstadt, Germany) and Tri-Sil reagent was from Pierce Chemical Co. (Rockford, IL, USA). The standard mixture of fatty acid methyl esters (FAME) F-07 was from Larodan Fine Chemicals AB (Malmö, Sweden). All other chemicals and solvents were from VWR (Stockholm, Sweden), unless otherwise stated.

Hydrolysis by pancreatic lipase for positional fatty acid composition

The MO and RSO (8 mg) were taken in a test tube. Eight mL of Tris-HCl buffer (pH 8.0), 2 mL of 0.05% bile salt in distilled deionized water (w/v), 0.8 mL of 2.2% $CaCl_2$ in distilled deionized water (w/v) and 20 mg of pancreatic lipase (porcine pancreatic lipase, crude type II) were mixed for hydrolysis and vortexed for 30 sec. The mixture was incubated in a water bath at 40°C for 3 min, 1 mL of diethyl ether was added. Diethyl ether was evaporated under nitrogen gas. The hydrolytic products were separated on thin-layer chromatography (TLC) plate (silica gel 60, 20x20 cm, 0.25 mm thickness, Merck, Eurolab AB, Stockholm, Sweden) by developing solvent of hexane/diethyl ether/acetic acid (50/50/1, v/v/v). The band of monoacylglycerol was scrapped off for methylation and analyzed by GC. After that, the percentage of fatty acid at sn-1,3 position was calculated by the following formula : Sn-1,3 (%) = (3 T – sn-2)/2 where T is the total fatty acid contents of MO and RSO, respectively. All analyses were conducted in triplicate and GC analysis was done as described above.

Fatty acid composition

The triacylglycerol (TAG) fraction in the MO and RSO were separated by TLC plate (silica gel 60, 20x20 cm, 0.25 mm thickness, Merck, Eurolab AB, Stockholm, Sweden) developed with hexane/diethyl ether/acetic acid (85/15/1, v/v/v). The visualized band corresponding to TAG

molecule was scraped off into a screw-capped tube and dissolved in 0.5 mL hexane. 3 mL 0.01 M NaOH in dry methanol was added into the tube. Fifty µL heptadecanoic acid (C17:0, 1 mg/mL in hexane) as an internal standard was also added in the test tube. The test tube was closed with a stopper and vortexed for proper mixing. The sample was incubated in water bath at 50°C for 30 min under continuous shaking. Two mililiters (50% $NaHSO_4$: 25% NaCl in water) solution were added and cool under running water. Three milliliters water and 1 mL hexane were added and vortexed vigorously. The hexane layer was separated and solvent was evaporated with nitrogen. Gas chromatography (GC, Chrompack CP 9001, Middelburg, The Netherlands), accompanied with auto-injection and flame-ionization detection was used for fatty acid composition analysis. A 50 m X 0.22 mm, 0.25 µm film thickness fused-silica capillary column BPX70 (SGE, Austin, TX, USA) was used for separation. Injector and detector temperatures were 240 and 280°C, respectively. Oven conditions were 160°C increased to 220°C at a rate of 2°C/min and maintained for 5 min. The carrier gas was helium and nitrogen as a make-up gas at a flow rate of 30 mL/min. Fatty acid methyl esters (FAME) were identified by comparison of their retention time with standared FAME. The peak areas were integrated by maestro version 2.4 (Chrompack Middelburg, The Netherlands) and reported as percentage of the total fatty acids (Azardmard-Damirchi and Dutta, 2008). All analyses were conducted in triplicate.

Analysis of sterols by GC

The MO and RSO (20 mg) were taken in a test tube and added 1 mL 2M KOH in ethanol (95%). The tubes was placed in a boiling water bath for 10 min with intermittent shaking and thereafter cooled under running water. Thereafter, 1 mL water, 2 mL hexane containing 20µg 5α-cholestane as internal standard and 200 µL absolute ethanol were added, shaked vigorously and centrifuged. The hexane layer was transferred to a small test tube and evaporated the solvent fully under a stream of nitrogen. For derivatization, 100 µL Tri-Sil reagents was added and dispersed by brief sonication. Then, the sample was incubated at 60ºC for 45 min and dispersed the reagent in the ultrasonic bath. The solvent was evaporated under a stream of nitrogen. The TMS- ether derivates of the sterols were dissolved in 500 µL hexane. About 1 µL sample was injected in splitless mode on GC model 6890 and software ChemStation Rev. B.02.01 (Agilent Technologies, Wilmington, DE, USA) connected with a GC PAL auto sampler (CTC Analytics AG, Zwingen, Switzerland) and a flame ionization detector. A combination of two fused-silica capillary columns, a DB-5MS (length 15 m, i.d. 0.18 mm and film thickness 0.18 µm) and a DB-35MS (length 10 m, i.di 0.2 mm, and film thickness 0.33 µm) were used which were joined together by a universal press-fit connector. The temperatures of detector and injector were 310 and 260ºC, respectively. The carrier gas was helium and nitrogen was used as make-up gas at flow rates of 0.7 and 30 mL/min, respectively. The initial oven temperature was 60ºC for 1 min and increased to 290ºC at a rate of 50 ºC per min and maintained for 5 min and then increased again by 1ºC per min to 305ºC for 15 min. All analyses were conducted in triplicates and quantification was performed using 5α-cholestane an internal standard.

Analysis of tocopherols by High-Pressure Liquid Chromatography (HPLC)

Quantitative tocopherols content were analyzed by HPLC. The HPLC system consisted of a 510 HPLC pump (Watres, Milford, USA) and Rheodyne Injector with 20 µL loop as well as a fluorescence detector Varian 9070 (Walnut Creek, CA, USA). The detector was set at the wavelength of 294 and 320 nm for excitation and emission, respectively. The column was LiChroCART 250-4 (Merck KGaA, Darmstadt, Germany) packed with Lichrosphere 100 NH_2, 5 µm particle size, and coupled to a LiChroCART 4-4 guard column (Merck KGaA, Darmstadt, Germany). Approximately, 10 mg of each sample were dissolved in 1 mL n-heptane and 20 µL were injected manually. The isocratic mobile phase was a mixture of n-heptane: tert-butyl methyl ether. tetrahydrofuran: methanol (79:20:0.98:0.02, v/v/v/v) at the flow rate of 1.0 mL/min. Each tocopherol was quantified using an external standard method and the area of each peak was calculated by an HP 3396A integrator (Hewlett-Packard, Avandale, PA, USA) (Azardmard-Damirchi and Dutta, 2008). All analyses were conducted in triplicate.

Rancimat test for oxidative stability

The induction period, measuring the increase in the volatile by-products released from the oxidizing oil of MO and RSO was determined by the rancimat method (Rancimat 743, Metrohm, Switzerland) at 100ºC with the air flow rate of 25 L/h. The conductivity was measured for estimating the concentration of the degradation products and

longer induction period showed higher oxidative stability. The protection factor (PF), which is calculated by dividing the induction period either of the MO or RSO by that of RSO (induction time, 2.2 h) was obtained as the relative activity (Schwarz and Ernst, 1996).

Results and Discussion

Total and positional fatty acid compositions

The total and positional fatty acid compositions of MO and RSO are presented in Table 1.

The MO and RSO contained total unsaturated fatty acids 94.2 and 92.3% as well as total saturated fatty acids 5.8 and 7.7%, respectively. The MO contained a slightly higher amount of total unsaturated fatty acid (94.2%) than RSO (92.3%). The major fatty acids of MO were erucic (22:1), linolenic (18:3, ω-3), oleic (18:1) and linoleic acid (18:2, ω-6) which composed of 42.8, 18.2, 17.4 and 15.8%, respectively. On the other hand, the RSO contained oleic (18:1), linoleic (18:2, ω-6) and linolenic acid (18:3, ω-3) which composed of 62.2, 19.5 and 8.6%, respectively. The MO contained higher levels of erucic acid (42.8%) and linolenic acid (18.2%), respectively as compared with RSO (0.4 and 8.6%, respectively) whereas the RSO contained higher levels of oleic acid (62.2%) and linoleic acid (19.5%), respectively than that of MO (17.4 and 15.8%, respectively). Almost similar levels of palmitic acid (16:0) ranged from 4.1 to 4.9% were found in MO and RSO.

The RSO contained higher levels of oleic acid (18:1, 54.0%) at sn-2 position than that of MO (18:1, 35.3%), whereas at sn-2 position, the MO contained higher levels of linoleic acid (18:2, 39.4%) and linolenic acid (18:3, 22.7%) than that of RSO (18:2, 32.4% and 18:3, 13.5%, respectively). The presence of higher amount of unsaturated fatty acids at sn-2 position is important nutritionally, because it is easily converted during digestion and absorbed in the body (Quinlan and Moore, 1993).

The fatty acid contents at sn-1,3 position were erucic (22:1, 63.5%), linolenic (18:3, 15.9%), oleic (18:1, 8.5%), palmitic (16:0, 5.8%) and linoleic (18:2, 4.0%), respectively for MO. In case of RSO, the fatty acid contents at sn-1,3 position were oleic (18:1, 66.3%), linoleic (18:2, 13.1%), palmitic (16:0, 7.3%) and linolenic (18:3, 6.2%), respectively. Such changes of fatty acid compositions at different position lead to different physical properties. The ω-6/ω-3 ratio (n-6/n-3 ratio) of MO and RSO were 0.87 and 2.27, respectively. Healthy ratios of ω-6:ω-3 range from 1:1 to 1:4. An individual needs more ω-3 than ω-6 (Tribole, 2007; Lands, 2005). The balance of ω-6/ω-3 ratio is also an important determinant in decreasing the risk for coronary heart disease both in the primary and secondary prevention of coronary heart disease (Simopoulos, 2002).

Table 1. Positional fatty acids composition (% ±SD) of mustard oil (MO) and rapeseed oil (RSO). All analytical results are presented as mean and standard deviation of triplicate measurement (n = 3).

Fatty acids	MO			RSO		
	sn-1,3	sn-2	Total	sn-1,3	sn-2	Total
16:0	5.8±0.1	0.8±0.05	4.1±0.1	7.3±0.12	0.1±0.05	4.9±0.1
16:1	nd[a]	nd	nd	0.3±0.0	nd	0.2±0.0
18:0	2.3±0.3	0.4±0.05	1.7±0.2	2.7±0.07	nd	1.8±0.05
18:1	8.5±0.2	35.3±0.3	17.4±0.2	66.3±0.1	54.0±0.5	62.2±0.1
18:2 (ω-6)	4.0±0.1	39.4±0.1	15.8±0.1	13.1±0.05	32.4±0.2	19.5±0.1
18:3 (ω-3)	15.9±0.2	22.7±0.2	18.2±0.15	6.2±0.02	13.5±0.2	8.6±0.05
20:0	nd	nd	nd	0.9±0.07	nd	0.6±0.05
20:1	nd	nd	nd	2.1±0.15	nd	1.4±0.1
22:0	nd	nd	nd	0.5±0.2	nd	0.3±0.05
22:1	63.5±0.4	1.4±0.1	42.8±0.3	0.6±0.15	nd	0.4±0.1
24:0	nd	nd	nd	0.2±0.2	nd	0.1±0.05
∑SFA[b]	8.1	1.2	5.8	11.6	0.1	7.7
∑UFA[c]	91.9	98.8	94.2	88.4	99.9	92.3
ω-6/ω-3 ratio			0.87			2.27
Oxidative stability[d]			1.57			1.0

a nd, not detected

b Total sum of saturated fatty acid

c Total sum of unsaturated fatty acid

d Values expressed as protection factor (PF). PF was calculated by dividing the induction period either of the MO or RSO by that of RSO (induction time, 2.2 h)

Table 2. Sterols and tocopherols content (mg/100g±SD) of mustard oil (MO) and rapeseed oil (RSO). All analytical results are presented as mean and standard deviation of triplicate measurement (n = 3).

Sterols and Tocopherols	MO	RSO
Cholesterol	2.03±0.6	7.01±0.31
Brassicasterol	72.11±0.9	59.24±1.66
Campesterol	203.60±2.31	238.86±3.39
Stigmasterol	3.27±0.25	nd
Sitosterol	307.71±4.12	323.90±5.90
Δ5-avenasterol	17.60±.22	2.97±0.31
Total	606.32	631.98
α-tocopherol	5.15±0.28	3.40±0.3
β-tocopherol	0.71±0.15	nd
γ-tocopherol	30.46±0.54	19.44±0.78
δ-tocopherol	2.0±0.29	2.73±0.2
Total	38.32	25.57

Sterols and tocopherols content of mustard oil (MO) and rapeseed oil (RSO)

The distribution of sterols and tocopherols for MO and RSO were assessed. The contents of sterols and tocopherols (mg/100g) of MO and RSO are presented in Table 2.

The contents of cholesterol, brassicasterol campesterol, stigmasterol, sitosterol and Δ5-avenasterol were 2.03, 72.11, 203.6, 3.27, 307.71 and 17.6 mg/100g, respectively) in MO. The RSO contained higher amount of sterols (631.98 mg/100g) than that of MO (606.32 mg/100g). The RSO contained cholesterol (7.01 mg/100g), brassicasterol (59.24 mg/100g), campesterol (238.86 mg/100g), sitosterol (323.90 mg/100g) and Δ5-avenasterol (2.97 mg/100g), respectively. The major sterol in RSO was sitosterol followed by campesterol, brassicasterol, cholesterol, and Δ5-avenasterol. The contents of cholesterol, campesterol and sitosterol (7.01, 238.86 and 323.9 mg/100g, respectively) in RSO were higher than that of MO (2.03, 203.6 and 307.71 mg/100g, respectively). On the other hand, the content of brassicasterol and Δ5-avenasterol (72.11 and 17.6 mg/100g, respectively) were higher in MO than that of RSO (59.24 and 2.97 mg/100g, respectively). Vegetable oils are good sources of tocopherols which are the most important natural antioxidants. The MO contained higher amount of total tocopherols content (38.32 mg/100g) than that of RSO (25.57 mg/100g). The RSO contained 25.57 mg/100g of total tocopherols which concur with previously published results by Gunstone et al. (1994). All samples contained higher amounts of γ-tocopherol than those of α- and δ-tocopherol.

Rancimat test

The oxidative stability is an important parameter in ascertaining the quality of oils and fats, as it gives a good estimation of their susceptibility to oxidative degradation (Aparicio et al., 1999). The results of rancimat test are presented in Table 2. A higher protection factor (1.57) for MO was observed compared to that of RSO (1.0). Generally, higher protection factor suggests stronger oxidative stability. The difference in protection factor of MO was mainly due to different levels of total tocopherols content.

Conclusions

Mustard oil is very popular to the Bangladeshi people due to its strong smell, hot nutty taste, pungent and sulfurous odor. This oil contains higher levels of erucic acid which is not suitable for human consumption as food purposes but it has higher oxidative stability (due to its high content of antioxidants) compared to RSO. Oxidative stability is an important criterion in ascertaining the quality of oils and fats. The balance of ω-6/ω-3 ratio (1:1 to 1:4) is also an important determinant in decreasing the risk for coronary heart disease which was determined in a good range in the case of MO (0.87). Future works are to be designed in our laboratory for the extraction of erucic acid from MO to make this valuable and nutritious vegetable oil more useful as a food product for human consumption.

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