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PHYSICO-CHEMICAL AND NUTRITIONAL STUDIES OF *TERMINALIA BELERICA* ROXB. SEEED OIL AND SEED KERNEL

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Abstract

Oil was extracted from *Terminalia belerica* Roxb. seed kernel by solvent extraction process. The whole seed contained 12.28 % oil on dry basis. The physico-chemical properties of the oil were determined. Moisture, ash and crude fibre contents of the seed kernel were found to be 8. 43, 2.54, and 8.78% respectively. The refractive index, co-efficient of viscosity, specific gravity, and energy of activation of the oil were found to be 1.28, 403.6 millipoise at 30°C, 0.93 and 6.97 k.cal/mole respectively. The oil was found to be non-drying. Iodine value, acid value, peroxide value, saponification value, saponification equivalent, ester value, unsaponifiable matter, acetyl value, Reichert-Meissel value, Polenske value, free fatty acids as oleic acid and cholesterol content of the oil were recorded as 107, 3.69, 3.14, 189.24, 296.44, 185.55, 1.24%, 3.78, 0.719, 0.945, 0.87% and 26.59 mg per 100 g oil, respectively. The oil was qualitative and quantitative analysed for fatty acid composition by TLC and GLC. The results showed that the fatty acids of the oil had chain length between C_{14} to C_{22}. The oil contained 17.70% myristic acid, 21.6% palmitic acid, 45.67% oleic acid and 14.93% stearic acid. The whole kernel was analyzed for some nutrients and minerals. The kernel contained 22.57 and 8.38% total lipid and protein respectively. It also contained 0.19mg, 0.45mg, 0.79g and 1.1mg of vitamin B_1, B_2, C and A respectively per 100g of kernel. Ca, Mg, K, Na, P, Fe, Mn, Zn and Cu were found to be 0.3, 0.02, 0.2, 0.2, 0.01%; 23, 1, 12 and 12 ppm respectively in kernel and 0.12, 0.05, 1.15, 0.18, 0.45%; 204, 4, 54 and 50 ppm respectively in oil.

Key words: *T. belerica* seed oil and kernel, physico-chemical behaviour, micronutrients, fatty acid profile, GLC.

Introduction

Human beings from time immemorial are using plants. The nature endowed Bangladesh with a vast majority of indigenous medicinal plants many of which are remaining unexploited. The plant *Terminalia belerica* Roxb. is a member of the genus Combreta of the family Combretaceae (Dymock *et al*. 2005). Its vernacular name is “Boyra”, “Bohera” or “Bahera” in Bengali and “Belleric Myrobalan” in English (Kirtikar and Basu 1935). The plant is credited with many medicinal properties. Extracts of the dried fruit pulp (flesh) is used in the treatment of dyspepsia, chronic diarrhea and dysentery, intestinal parasites, fever, sore throat, cough, catarrh, bronchitis, skin diseases, edema, dropsy, piles, expectorant, laxative and various diseases (Valsaraj *et al*.1997, Nandy *et al*.1989). Dried fruit pulp of this plant is an ingredient of Indian Ayurvedic drug “Triphala” used for the treatment of intestinal related disorders, liver disorders, pancreatic cancer and many other diseases (Padam *et al*.1996).

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Inspite of having the reported usefulness of dried fruit pulp of this plant in folk medicine, no such work that described in our study, have so far been reported in the literature. This study deals with the isolation of seed oil from seed kernel, determination of its physico-chemical properties, qualitative and quantitative estimation of fatty acids in seed oil and estimation of micronutrients and other related substances in seed oil and seed kernel.

Oils and fats are either esters of fatty acids or substances capable of forming such esters. They are very wide spread in nature, being found in all vegetables and animal matter. Fats and oils have great use in medicine and in diet. Oils and lipids act as heat insulators and as reserve supplies of energy (Hawk et al.1995). Essential fatty acids in medicinal seed oils and other vegetable oils have important roles in transport and metabolism and in maintaining the function and integrity of cellular membranes. Oils of vegetable and seeds contain mainly unsaturated fatty acids and are therefore preferable more to saturated animal fats like butter, bacon fat and also hydrogenated vegetable products. Polyunsaturated fatty acids (PUFA) of omega-6 series are usually found in land plants and seed oils (Ackman *et al.*1981). Linoleic acid is called the essential fatty acid. It is essential for the complete nutrition of the human body. It cannot be synthesized in the body and must be supplied from food. Arachidonic and linolenic acids, formerly designated as essential fatty acids, can be synthesized in the body from linoleic acid. The linoleic acid has a great influence on the heart. Scientists have established a co-relation between linoleic acid and blood-pressure. The food containing linoleic acids are capable of reducing the blood-pressure (Pergamon Press, 1980). Oils having polyunsaturated fatty acids decrease blood cholesterol levels, stimulate cholesterol excretion into the intestine and inhibit biosynthesis of cholesterol in the liver. Oil containing high level of polyunsaturated fatty acid is found to inhibit the activity of HMG-CoA-reductase which is the regulatory enzyme in cholesterol biosynthesis (Dyerberg *et al.*1975, Ide *et al.*1978). The activity of such enzyme was also regulated by the degree of unsaturation of dietary oils (Ide *et al.*1978). The cholesterol lowering effect of polyunsaturated fatty acids (including eicosapentaenoic acid, 20:5, ?-3 and arachidonic acid, 20:4 ?-6) in oils have anti-aggregating effect, thus preventing coronary thrombosis and reducing the risk of heart attack (Puustinen *et al.*1985,Nelson *et al.*1972,Phillipson *et al.*1985). Our present work reports the medicinal and nutritional significance of *T. belerica* seed oil and seed kernel along with its suitability to use in edible purposes.

Minerals and vitamins are important micronutrients; the so called trace elements are essential for life. This act as co-factors of enzymes and as organizers of the molecular structure of the cell and its membrane. In excess amount they can be toxic and their insufficiency may lead to metabolic disorders (Jones *et al.*1992). The roles played by the micronutrients in biological systems are important and essential for human metabolism. Estimation of micronutrients and other related substances in seed oil and seed kernel is a remarkable part of the present work.

Hematological and histopathological effects of feeding seed oil and seed kernel for a period of 32 consecutive days at different level with formulated cereal on albino rats and microbiological investigation of seed oil and seed kernel will form a separate study.

Materials and Methods

Collection and processing of plant materials

Mature and dried fruits of *T. belerica* were collected from local market in freshly packed condition and stored in normal room temperature. The seeds were separated from the fruits by scraping the fruit pulp (pericarp) manually. The seeds were dried in the sun for about three days. The sun-dried whole seeds were powdered with a grinding machine (Hammer mill) and the powder was then dried in the oven at 40^0C for 6 hours and stored in an airtight container for experimental purpose.

Extraction of oil from *T. belerica* seed

The powdered materials (seeds) were placed in a thimble of the soxhlet extraction apparatus attached to the mouth of a round bottomed flask containing petroleum ether (B P 40-60oC) as an extractive solvent. Some boiling chips were added into the flask to avoid bumping during heating. The extraction of oil was completed in twelve cycles. The petroleum ether extract so obtained was evaporated in a rotary film evaporator (Rotavator) under reduced pressure to obtain oil. The oil obtained was purified with diethyl ether.

Physico-chemical characteristics of oil

Moisture, ash and crude fibre contents of seed kernel and drying property of the seed oil were determined by the conventional methods (Ranganna 1986). Refractive index, specific gravity, co-efficient of viscosity and energy of activation for viscous flow were measured according to the method of Kichener (1973). Chemical characteristics, *viz,* iodine value, acid value and percentage of free fatty acid (as oleic), peroxide value, saponification value and saponification equivalent, ester value, unsaponifiable matter, acetyl value, Reichert-Meissel value and Polenske value of the seed oil were determined using the conventional methods of AOAC (Official method1990). Estimation of cholesterol was done spectrophotometrically by Liebermann-Buchard test (Jayaraman 1985).

Determination of fatty acids in seed oil of *T. belerica*

Fatty acid mixture, prepared by saponification of the oil, was converted to the corresponding methyl esters in the presence of BF_3 methanol complex reagent according to the method of Metcalfe *et al*. (1961). The mixture of methyl esters was subjected to thin layer chromatography and gas liquid chromatographic analyses.

Thin layer chromatographic analysis of fatty acid methyl esters mixture

Preparation of plates, application of the sample, development and visualization of the chromatograms were performed by using the procedure of Randerath (1966). Five dry clean glass plates (20 cm ? 20 cm) of equal thickness were used. The T L C plates were developed separately with the following solvent systems (Bobbitt 1963) in an equilibrated chromatographic tank containing particular solvent system by ascending technique. After development, the plates were sprayed with 2,7-dichlorofluorescein (0.2g 2,7-dichlorofluorescein in 100 ml ethanol). The fatty acid methyl esters gave yellow spots which were visualized under UV lamp. Then the R_f values of the colour spots were calculated.

The solvent system used were: petroleum ether: diethyl ether (4:1), petroleum ether: diethyl ether (3:1), petroleum ether: diethyl ether: Acetic acid (85:15:1), petroleum ether: diethyl ether: acetic acid (80:20:1), hexane: diethyl ether (4:1).

Gas liquid chromatographic examination of the mixture of fatty acid methyl esters obtained from the seed oil

Gas-liquid chromatography (Christic 1976) is a very useful technique in oil and lipid analysis, particularly for the separation of very similar compounds within the class. Analysis of fatty acid methyl esters of *T. belerica* seed oil was carried out with a PYE UNICAM 4500 U INTEGRATOR 2220 LKB Gas Chromatograph (Phillips, England) fitted with a flame-ionization detector. One ?l of all standard fatty acid methyl ester mixture (5%) was injected to injection port. The separation was achieved on column at 120-230°C, at a rate of 4°C/min raise in temperature. The temperature of the injector, oven and detector were maintained at 220°C, 190°C and 230°C respectively. Hydrogen was used as carrier gas at a flow rate of 5ml/min. A

standard chromatogram was obtained 40 minutes after injecting the sample. Chromatograms for oil samples were also prepared by similar procedure (Christic 1976). The chromatogram for fatty acid methyl esters of the oil was compared by overlapping with the chromatogram of standard fatty acid methyl esters (Sigma Chemical Co., U.S.A.). The picks were tentatively identified by comparing their relative retention times with known picks (Park and Goins 1994) and by plotting the logarithm of the relative retention time curve against carbon number (Huq *et al*.1979). With respect to the standard peaks, four peaks were identified in the seed oil of *T. belerica*.

Nutritional analysis of the seed oil and seed kernel

The micronutrients and other related substances in seed kernel and seed oil were estimated. Vitamin C present in the seed kernel was determined by the titrimetric method of Bessey's and King 1959). Vitamin B_1, B_2 and ?-carotene of kernel were estimated by the method as described in the United States Pharmacopoeia (1965). Oil from the seed kernel of *T. belerica* was extracted by solvent extraction method. Total protein content in seed kernel was measured by micro kjeldahl's method (Jayaraman 1985). Micro-nutrients viz, Ca, Mg, K, Na, P, Fe, Mn, Zn, Cu, in seed oil and seed kernel were estimated by the method of Leif Petersen (2002). Ca, Mg, Fe, Mn, Zn, and Cu were determined by atomic absorption spectrophotometry, K and Na were measured by flame photometry and P was determined by spectrophotometry.

Results and Discussions

The moisture, ash and crude fibre contents of *T. belerica* seed kernel was found to be 8.143, 2.54 and 8.78% respectively. The refractive index of fats and oils depends to some extent on their unsaturation. The refractive index of seed oil is 1.28 at 30^{o}C. The specific gravity of the seed oil is 0.93. The Sp. Gr. of practically all fats and oils lies between 0.90 and 0.95. The viscosity of seed oil was determined to be 403.6 millipoise for 30^{o}C, 340 millipoise for 35^{o}C, 292.6 millipoise for 40^{o}C, 246.2 millipoise for 45^{o}C and 209.78 millipoise for 50^{o}C. The energy of activation for viscous flow was calculated to be 6.97 k. cal/ mole.

Iodine value of the oil of *T. belerica* was found to be 107. This result has close similarity to that of corn oil (102-114). Higher the iodine number, the more will be the degree of unsaturation in the fats or oil (Dutta 1991). The acid value and percentage of free fatty acid (as oleic) of the oil of *T. belerica* was estimated and the highest amount was found to be 3.69, and 0.87% respectively. A high acid value may indicate a higher tendency to become rancid (Karim 1997). Seed oil might be used as edible oil, the free fatty acid contained in it is less than 1.15%.The peroxide value of the oil was determined to be 3.14. The peroxide value is an indication of unsaturation of fats or oils. The oil under investigation contains fair amount of unsaturated fatty acids, The facts support the relevant findings by other authors (Jahan *et al*. 2003).

Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fats or oil. The shorter the average chain length in the fatty acids, the higher is the saponification number (Jain 1995). The amount of saponification value of the oil was found to be 189.24. The value has a close similarity to that of groundnut oil (188-196), sunflower oil (190-198) and corn oil (187-193). The saponification equivalent of the seed oil was found to be 296.44. It is directly proportional to the average chain length of fatty acid present. Higher value indicates the presence of appreciable quantity of higher fatty acids. The result clearly indicates that the oil contained mainly fatty acids of C_{18} molecular weight along with some palmitic acid. The ester value of the oil was found to be 185.55. In general if a fixed oil or fat has unsaponifiable matter present in excess of about 2% there is reason to suspect adulteration. The amount of unsaponifiable matter of the oil was calculated to be 1.24%. The acetyl value of the oil was found to be 3.78 which is similar to that of cotton seed oil and linseed oil. This result indicates low content of free hydroxyl groups in the oil sample. The acetyl number is thus a measure of the number of OH groups in the fat. The

Reichert Meissel value of the oil was estimated to be 0.719, which has close similarly with those of olive oil, sunflower oil, cotton seed oil and soabean oil. The result indicates that the seed oil contained low amount of water soluble volatile fatty acids.

The Polenske value of the oil was measured to be 0.945. This value represents the measure of the volatile fatty acid insoluble in water but soluble in alcohol. The amount of cholesterol, determined by spectrophotometric method, was found to be 26.59 mg per 100 g of oil. Chemical characteristics of the seed oil and other related oils have been furnished in Table- 1. Physico-chemical properties of the seed oil revealed that the oil contains more unsaturation than other seed oils. It contains higher amount of unsaturated fatty acids. Appreciable amount of long chain fatty acids is present in the oil. It contains small amount of unsaponifiable matters. Low amount of short chain fatty acids is found in the oil. The oil is less rancid owing to the fact that the free fatty acid contained in it (0.87%) is less than 1.15%. Chemical characteristics of the oil have close similarity to those of groundnut oil, sunflower oil and corn oil. The oil is suitable for edible purpose as it contains low level of cholesterol.

Table 1. Chemical characteristics of *T. belerica* seed oil and other related oils.

Name of sample	Iodine value	Saponification value	Saponification equivalent	Acid value	Free fatty acids (%) (as oleic)	Ester value	Unsaponifiable matter (%)	Acetyl value	Peroxide value
T. belerica seed oil	107	189.24	296.44	3.69	0.87	185.55	1.24	3.78	3.14
Coffee oil	89-101	180-192	-	-	-	5.5-6.5	-	-	-
Palm oil	49-57	197-207	-	-	2-85	-	0.5-2.0	-	-
Butter oil	26-38	208-230	240-270	0.45-35.5	-	-	-	-	-
Linseed oil	175-200	189-195	287-296	6.0	0.5-0.75	-	1-1.5	4.0	-
Soybean oil	129-137	190-195	282-295	1.27-1.54	0.35-0.85	-	0.7-16	-	-
Cotton oil	103-111.3	194-196	283-292	0.6-0.9	0.4-0.9	-	1.0	0.7-12	-
Coconut oil	82-96	225-260	210-250	2.5-10	-	-	0.15-0.7	-	15-17
Katio seed oil	64	192.0	-	1.4	0.50	-	-	-	-
Rape seed oil	94-100	175	-	-	-	-	0.80	-	-
Grape oil	94-143	178-190	-	0.08-	-	-	-	-	-
Castor oil	85	180	-	-	-	-	-	-	0.7
Sunflower oil	125-140	190-194	287-295	0.5-5.0	1.15-0.45	-	0.3-0.9	-	-

The fatty acid methyl esters mixture derived from the oil of *T. belerica* was subjected to TLC examination. Its constituent fatty acids were identified by comparing the R_f values of methyl esters of standard fatty acids. The results are summarized in Table 2. By comparing the R_f values of the spots obtained from seed oil methyl esters with those of known standards, it was concluded that the oil contained myristic acid, stearic acid, palmitic acid and oleic acid.

Table 2. Separation and identification of fatty acids in seed oil by thin layer chromatography.

Fatty acid methyl esters (known standard)	R_f value in different solvent system				
	Solvent system A	Solvent system B	Solvent system C	Solvent system D	Solvent system E
Methyl myristate	0.714	0.808	0.792	0.598	0.829
Methyl palmitate	0.838	0.876	0.772	0.731	0.768
Methyl oleate	0.811	0.871	0.723	0.786	0.772
Methyl stearate	0.849	0.868	0.761	0.767	0.761
Methyl linoleate	0.832	758	0.768	0.849	0.845
Methyl arachidate	0.799	0.810	0.877	0.808	0.812
Methyl palmitoleate	0.416	0.540	0.729	0.816	0.427
Methyl Linolenate	0.661	0.489	0.778	0.702	0.610
Fatty acid methyl esters mixture derived from seed oil (unknown)	0.722	0.801	0.796	0.592	0.818
	0.830	0.887	0.761	0.736	0.761
	0.818	0.879	0.729	0.760	0.778
	0.842	0.869	0.768	0.769	0.767

Fatty acid analyses of the oil samples by GLC were carried out after converting the fatty acids mixture to corresponding methyl esters mixture. The identification of fatty acid components from GLC analyses was carried out on the basis of relative retention time and was quantified by measuring the peak area in comparison with standard chromatogram which was presented in Table 3.

Table 3. GLC analysis of the standard fatty acid methyl esters mixture.

Peak No.	Name of the standard fatty acid and structural formula	Retention time	Relative retention time with respect to palmitic acid	Log_{10} relative retention time	Peak area
1	Capric acid $CH_3(CH_2)_8COOH$	1.04	0.084	-1.076	220
2	Caprylic acid $CH_3(CH_2)_6COOH$	2.87	0.231	-0.636	670
3	Lauric acid $CH_3(CH_2)_{10}COOH$	5.89	0.473	-0.325	765
4	Myristic acid $CH_3(CH_2)_{12}COOH$	9.22	0.741	-0.137	826
5	Palmitolic acid $CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	11.96	0.961	-0.017	498
6	Palmitic acid $CH_3(CH_2)_{14}COOH$	12.45	1.000	0	1166
7	Oleic acid $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	14.95	1.201	0.080	1744
8	Stearic acid $CH_3(CH_2)_{16}COOH$	15.37	1.235	0.092	980
9	Arachidic acid $CH_3(CH_2)_{18}COOH$	18.00	1.446	0.160	1086
10	Behenic acid $CH_3(CH_2)_{20}COOH$	20.50	1.647	0.207	1131
11	Lignoceric acid $CH_2(CH_2)_{22}COOH$	22.77	1.829	0.262	1115

The fatty acid compositions of the oil samples are presented in Table -4 and gas liquid chromatograms are given in Figure-1. It was evident from the Table that *T. belerica* seed oil contained 17.70% myristic acid, 21.67% palmitic acid, 45.67% oleic acid and 14.93% stearic acid. A large quantity of unsaturated fatty acid (45.67% oleic acid) in the oil indicates its effectiveness to reduce the risk of cardiovascular problems in human beings.

Table 4. Fatty acids composition of the seed oil as obtained by GLC analysis.

Peak No.	Retention time	Relative retention time with respect to palmitic acid	Log_{10} relative retention time	Area	Relative Percentage	Remark
1	9.27	0.745	-0.128	473	17.70	Myristic acid
2	12.43	0.998	$-6.9? 10^{-4}$	579	21.67	Palmitic acid
3	14.91	1.198	0.078	1220	45.67	Oleic acid
4	15.26	1.226	0.088	399	14.93	Stearic acid

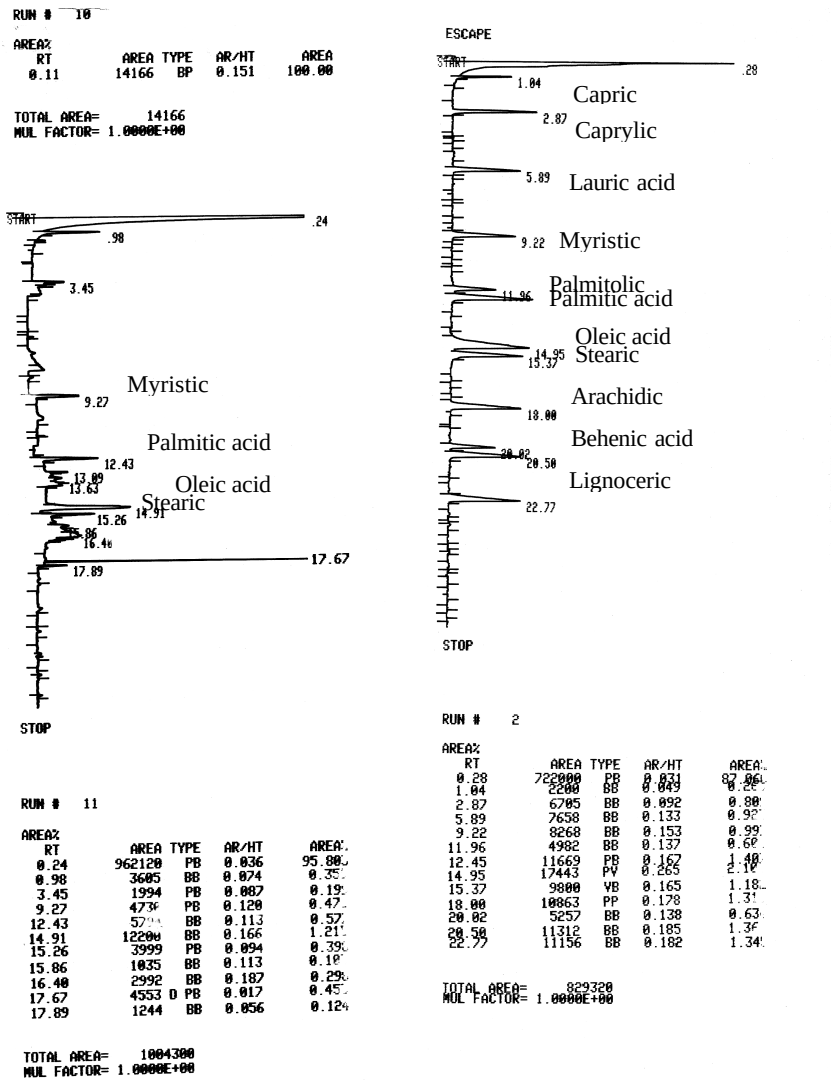

Fig. 1. Gas liquid chromatogram of fatty acid methyl esters obtained from *T. belerica* seed oil and standard fatty acid methyl esters.

The micronutrients and other related substances in seed kernel along with seed oil were estimated. The results indicated that kernel contained 0.79% vitamin C; 0.19 mg vitamin B_1 per 100 g; 0.45 mg vitamin B_2 per 100 g, 1104 ?g vitamin A (?-carotene) on wet weight basis per 100 g; 22.57% total lipid; and 8.38% protein. The quantity of Ca, Mg, K, Na, P, Fe, Mn, Zn and Cu contents in seed oil and seed kernel were recorded as 0.30% and 0.12%; 0.02% and 0.05%; 0.20% and 1.15%; 0.20% and 0.18%; 0.01% and 0.45%; 23 ppm and 204 ppm; 1 ppm and 4 ppm; 12.00 ppm and 54.00 ppm and 12 ppm and 50 ppm, respectively. The results cited above revealed that the seed kernel along with seed oil contained in it expresses its high nutrition values. These indicate the important roles these substances must play in the living organism. The foregoing evidences clearly demonstrate that *T. belerica* seed oil and seed kernel are highly suitable for edible purpose. It is more *suitable than other edible seed oils.*

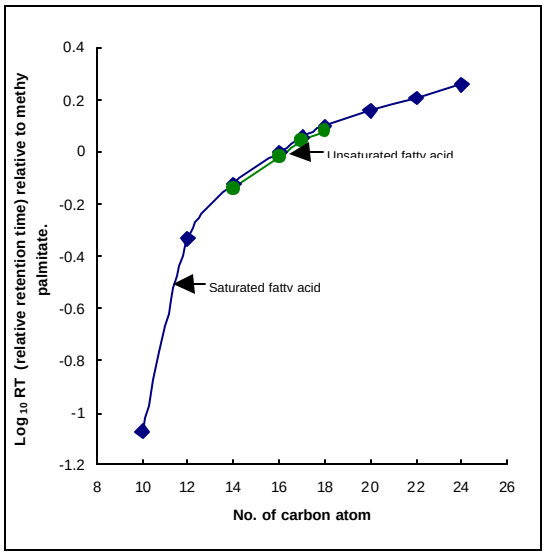

Figure 2. Relationship between $\log_{10}$ relative retention times (RT), relative to methyl palmitate and the number of carbon atoms of fatty acid methyl esters.

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