Studies on Fatty Acid Composition of Fixed Oil Extracted from *canola* and *rapeseeds* for Erucic Acid Content

[1]S. HAMID, [2]OBAID-UR-REHMAN, [2]A. B. JASRA AND [2]A. A. MUGHAL
[1]*Applied Chemistry Research Centre*
PCSIR Laboratories Complex, Lahore-54600-Pakistan
[2] *Govt. College, Sargodha, Pakistan*

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Summary: The fixed oils from the seeds of *canola* and rape plants (N.O. Cruciferae) have been characterized and studied for their fatty acid composition by GLC. The two oils were compared and it was found that mustard oil (rape seed oil) samples contained 41.5-45.7% erucic acid other than palmitic acid (1.8-4%), stearic acid (0.34-11%), oleic acid (15.6-24.3%), linoleic acid (12.0-13.7%), linolenic acid (1.9-2.1%), arachidic acid (2.0-2.9%) and lignoceric acid (4-4.7%). The canola oil contained only 0.0-1.7% erucic acid other than myristic acid (0.1-0.4%), palmitic acid (5.5-11.5%), stearic acid (1.6-3.7%), oleic acid (48.9-60.6%), linoleic acid (3.7-26.8%), linolenic acid (6.4-9.7%), arachidic acid (2.0-2.9%), and behenic acid (0.34-0.81%).

Introduction

Both the *canola* and *rapeseed* plants (N.O. Cruciferae) are shrubs, with herbaceous stem. These species of family cruciferae (Brassicaceae) are believed to be Eastern Mediterranean and spread to Europe, Africa, Iran, Indo-Pak subcontinent and the Far-East. In Pakistan four varieties of Brassica plants are grown widely for oil, vegetable and fodder purposes. They are *Brassica oleraceae* (cabbage or cauliflower); *B.junceae* (Brown mustard); *B.nigra* (Black mustard); and *B. campestris* (rapeseed). Rapeseed oil, to a small extent, is used in cooking[1,2]. Canola oil comes from a rapeseed plant variety that is grown in North America and has been carefully developed by Canadian scientists to provide an oil with ideal fatty acids contents and desirable cooking properties. The name "Canola" is derived from the words "Canadian oil", in recognition of the plant's beginning.

Oil from canola seeds is being used for edible purposes and now canola industry is well-established in the world. The canola seed is grown and processed into food products round the world, particularly in Canada, Japan and China where average life expectancy is the highest in the world [3-5]. Canola oil is helpful in the treatment of cancer . Diets containing canola oil have been found effective in reducing plasma total and LDL-cholestrol as canola oil is abundant in monounsaturated fatty aids, which have several advantages over poly unsaturated fatty acids [6-9].

While breeding in good mono-unsaturated fatty acids, the Canadian plant geneticists also bred out erucic acid, a fatty acid that can make up as much as 45% of the oil content of conventional rapeseed [10-12]. Standard rapeseed's high erucic acid content prevents the oil use in the United States and Canada because of concern for potentially harmful health effects [11]. Researchers working with laboratory rats suggested that high concentrations of erucic acid in the diet could damage rat heart tissue [13-15].

The Canadian variety of mustard is the low-erucic acid rapeseed (LEAR). The canola oil produced from LEAR plants typically contain less than 0.5% erucic acid. This amount is so low that it eliminates any potential concern for negative effects [16].

Pakistan is deficit in the production of oils and fats. About 75% of oil consumed by the Ghee Industry in Pakistan is imported [17] and a large amount of foreign exchange is drained every year for the import of oils and fats to meet the local demands. Pakistan Oil Development Board has stressed the need of research on oil seed crops. In 1999-2000 the production of Canola and rapeseed oils in Pakistan was 251.4 thousand tons [18-19].

The studies were undertaken to compare the physico-chemical characteristics of rapeseed and Canola oils. Particularly emphasis was laid to determine any significant differences in the fatty acid composition with special reference to erusic acid of the two cooking media.

Results and Discussion

The studies on the fixed oil extracted from the seeds of canola and rapeseeds collected from three different places in the Punjab and the Sindh

Provinces, were undertaken to investigate the %age yield, physico-chemical characteristics and fatty acid composition of the oil. These seed oils have been subject of the studies by various workers at different times in different countries in order to make its chemical evaluation. Since no systematic studies were reported and a noticeable difference appeared in various results with the published ones in literature, and canola oil is costly as compared to rape seed oil, it was therefore taken necessary to present our findings based on most modern techniques available in PCSIR Laboratories Complex, Lahore. The results of our studies on the oil of the seeds, collected from three different places of the Punjab and The Sindh Provinces are presented in Tables (1), (2) and (3).

Oil content is an important criterion used as a commercial measure of the quality of the oil bearing seeds. The rapeseeds collected from Khushab (1A*), Jhang (2B*) and Sukkhar (3C*), were recorded to yield 29, 33 and 35% oil, containing 0.81, 0.99, and 0.89% unsaponifiable matter, respectively.

The presence of unsaponifiable matter in all the samples of the oil was well with in limits and did not impair the quality of the oil. The unsaponified fraction of each oil as identified by comparative TLC was found to be composed of a mixture of hydrocarbons, aldehydes, ketones, sterols, as well as pigments. The physico-chemical constants of the three oils were not unusual except the acid and

Table-1:Physico-Chemical Properties of the Oil Samples from *canola* and *rapeseeds*

Physico-chemical Constants		Khushab Sample 1		Jhang Sample 2		Sukkhar Sample 3	
		A	A*	B	B*	C	C*
Moisture and volatile matter(%)		0.079	.041	0.091	0.050	0.063	0.039
Colour index in 1" cell	Yellow	3.5	3.1	3.6	2.0	4.0	2.5
	Red	0.5	0.9	0.5	1.5	1.0	1.0
Specific gravity		0.920	0.937	0.920	0.936	0.925	0.938
Refractive index		1.4675	1.4300	1.4660	1.4245	1.4595	1.4240
Acid value (40°C)		0.40	0.95	0.50	0.81	0.39	1.0
Saponification value		120.7	130.0	125.0	135.0	128.5	131.0
Iodine value		101.17	113.5	109.0	115.0	106.71	111.09
Peroxide value		1.45	1.92	1.50	1.81	1.20	1.63
Ester value		120.30	129.05	124.50	134.19	127.61	130.0

Here A, B, C for canola seed oil samples and A*, B*, C* for rapeseed oil samples

Table: 2 Oil Composition

Oil Composition	Sample 1		Sample 2		Sample 3	
	A	A*	B	B*	C	C*
Unsaponifiable matter (%)	0.68	0.81	0.78	0.99	0.80	0.89
Sponifiable matter (%)	99.32	99.19	99.22	99.01	99.20	99.11
Saponifiable Matter						
Free fatty acids (%)	0.14	0.27	0.19	0.21	0.11	0.29
Glycerides (%)	99.86	99.73	99.81	99.79	99.89	99.71
Glycerides						
Glycerol (%)	8.41	10.01	8.71	10.31	9.31	10.24
Fatty acids (%)	91.59	89.99	91.29	89.61	90.69	89.76

Table: 3 Fatty Acid Composition of Fixed Oil from *canola* and *rapeseed*

Fatty Acids	Sample 1		Sample 2		Sample 3	
	A	A*	B	B*	C	C*
Myristic acid	0.3	--	0.4	--	0.1	--
Palmitic acid	8.71	4.0	5.5	3.81	11.5	1.8
Stearic acid	3.7	0.34	2.81	10.9	1.6	11.0
Oleic acid	57.6	24.3	48.9	15.6	60.6	15.7
Linoleic acid	3.7	13.7	26.8	12.41	14.1	12.0
Linolenic acid	9.7	1.9	7.3	2.1	6.4	2.0
Arachidic acid	2.9	2.9	2.7	2.8	2.0	2.0
Behenic acid	0.81	--	0.70	--	0.34	--
Lignoceric acid	--	4.1	--	4.0	--	4.7
Erucic acid	--	43.4	0.9	41.5	1.7	45.7

peroxide values indicating that the oil extracted from the seeds from different places were partially hydrolysed and got rancid. The presence of free fatty acids in all the three samples of the oil were confirmed by IR spectrum of the oils, which besides characteristic bands, also showed absorbance at 3350-3450cm^{-1} and 1710cm^{-1}. The absence of any band at 950cm^{-1}in the IR spectrum of these oils indicated the absence of any trans-isomers of fatty acids. The oils indicated iodine values to be classified as non-drying oils. The fatty acid composition of the oils of the rapeseeds showed that they were composed of myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, behenic and lignoceric acid. On the other hand the canola oil contained myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic and behenic acids. The oil which was found to be mainly composed of unsaturated fatty acids and a minor amount of saturated fatty acids, a small percentage of long chain fatty acids was also noticed on GLC analysis. These studies indicated that the seeds were a good source of non-drying oil. The oils were also rich in essential fatty acids.

The presence of erucic acid (41.5-45.7%) a mono-unsaturated C_{22}-acid (cis -13 decosenoic acid) [20,21] in rapeseed poses the main problem for edible purposes. Erucic acid is alleged to cause nutritional problem and various food regulations restrict erucic acid content to 10% at the most.

These facts make the rapeseed oil unfavourable for human consumption as compared to canola oil. Studies are needed to be conducted for reducing the effects of erucic acid by changing its chemical nature, so as to render it less harmful. Thus Pakistan will be able to exploit this potential source of oil for edible purposes.

Experimental

Plant Material Collection and Extraction of Oil

Three samples of seeds each for canola and rapeseed plants were collected from different places of the Punjab and Sindh Provinces of Pakistan, i.e., Khushab, Jhang and Suakkhar. The seeds were dried and crushed separately in an iron pestle and mortar. The oil was extracted from each sample by Soxhlet apparatus using n-hexane as the solvent and percentage yields were determined.

The oils were subjected to physico-chemical investigations and fatty acid composition. The specific gravity, refractive index, acid value, saponificaiton, perxide and iodine values were determined using the standard methods [22-24] while ester, free fatty acid and glycerol contents were derived from the above determined values [25]. See (Table 1, 2).

Saponification of the Oil and Liberation of Fatty Acids

The oil (3g) of each sample was refluxed on water bath with 70ml alcoholic potassium hydroxide solution (0.5N) separately for three hours. The solvent was distilled out under reduced pressure and the residual soap was washed thrice with petroleum ether to remove the unsaponifiable matter. The soaps were dissolved separately in water. They were acidified with 20ml sulfuric acid (2N) and were refluxed on water bath for one hour. The liberated fatty acids were extracted with diethyl ether and dried over anhydrous sodium sulfate. After the removal of the solvent, a mixture of fatty acids of each sample was obtained [22,26].

Preparation of Methyl Esters of the Fatty Acids

The fatty acid mixture obtained from each sample was refluxed on water bath for three hours with absolute methanol (40ml) and a few drops of concentrated sulfuric acid. After the distillation of excess methanol, the mixture of methyl esters of fatty acids was dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The absence of peak at 3450cm^{-1} and shifting of C=O peak from 1710cm^{-1} in the infra-red spectrum of the methyl esters of each sample indicated complete esterification of the fatty acids which was further confirmed by T.L.C of each sample [22].

Resolution and Identification of Methyl Esters by GLC

Chemical composition of the oil of each sample was determined by the GLC of the methyl esters, with flame ionization detector and helium as the carrier gas. The samples were injected at 200°C using a glass column (1.5x 4mm) containing 10% polyethylene glycol succinate coated on diatomite support maintained at 190°C. The samples gave peaks of myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, behenic, lignoceric, and erucic acid methyl esters which were confirmed by running a standard mixture under identical condition [22] as shown in (Table 3).

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