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Lipid Classes and Fatty Acids Comparison of *Oscar* and *Dunkeld* Variety of *Canola* Seeds

AMRAN WAHEED[1], ZEESHAN ALI[1,*], MUBEEN AKHTER[1] and KHALID MAHMOOD[2]

[1]Applied Chemistry Research Center, Pakistan Council of Scientific and Industrial Research, Laboratories Complex, Lahore-54600, Pakistan
[2]13-Dudley Place, Middlesex Hayes, United Kingdom

*Corresponding author: E-mail: drzeeshan2009@yahoo.com

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Canola seed varieties *Oscar* and *Dunkeld* grown by local cultivars of Pakistan, contained lipids (44.6, 42.2 %) of which neutral lipids (95.5, 96.8 %) and polar lipids (4.5, 3.2 %) respectively by preparative TLC. The neutral lipids of both varieties were further fractionated and identified as hydrocarbons (0.9, 0.7 %), wax ester (1.2, 1.5 %), sterol esters (1.2, 1.6 %) triacylglycerols (72.6, 73.3 %), free fatty acids (3.1, 3.4 %), 1,3-diacylglycerols (4.3, 4.1 %), 1,2-diacylglycerols (3.8, 3.7 %), fatty alcohols (1.5, 1.2 %), sterols (1.6, 1.5 %) and monoacylglycerols (5.3, 5.8 %) respectively. The fatty acids of whole extracted lipids and fractionated classes except hydrocarbons, fatty alcohols and sterols were analyzed through GC. The *Oscar* & *Dunkeld* contain low contents of total saturated fatty acid (8.2, 8.7 %) and higher contents of monounsaturated fatty acid (61.5, 60.8 %) respectively as compare to conventional vegetable oils. The fatty acids analysis reveals that the glycerides contain higher percentage of unsaturated fatty acid. Oleic acid is predominant amongst monounsaturated fatty acid while palmitic acid is the prominent saturated fatty acid in all the classes.

Key Words: Polar lipids, Neutral lipids, Fatty acids, Triacylglycerols, Canola.

INTRODUCTION

Botanical name of *Canola* is the same as that of mustard *i.e. Brassica campestris*. In Pakistan, it is also called as sweet Sarson. Mustard is among the oldest species, with Sanskrit record dating back to about 3000 B.C., Egyptian to 2000 B.C., Chinese beyond 1000 B.C. and an extensive literature from Greek and Roman times onward[1]. In Canada, the characters of genetic material with low or zero pungency and with zero erucic acid in the component are being work out. Similar work has been carried out in Australia to get Zem (zero erucic mustard) species. *Canola* is one of the attempts made in Australia and Canada. *Canola* oil is characterized by a low level of saturated fatty acid. It contains a relatively high level of oleic acid and an intermediate level of polyunsaturated fatty acids of which linoleic acid makes up approximately one third[2]. Diets containing *Canola* oil have been found equally as effective in reducing total plasma and LDL cholesterol as those containing corn oil[3], safflower oil[4], soybean oil[5] or sunflower oil[6].

The total production of edible oil in Pakistan is *ca.* 70 % less as the nation requires. In order to overcome this meagerness we are spending a lot of foreign exchange on importing oils[7]. In Pakistan mostly oil producing crop like mustard (Sarson, Rai) and rapeseed (toria) are being cultivated. The oil obtained from these crops is rich in sulphur containing fatty acids, erucic acid, which make it unfit for cooking. In previous years, different varieties of *Canola* like *Oscar*, *Dunkeld*, Rainbow, Range and Hyola (hybrid *Canola*) were imported in large quantities from Australia. The seeds were cultivated on large area, which gave encouraging results. *Canola* cooking oil has become favoured cooking medium in most advanced countries, parti-cularly in Canada and Japan where average life expectancy is the highest in the world. Fat profile comparison of *Canola* oil with different edible oil is well explained[8].

EXPERIMENTAL

Seeds of the two varieties of *Canola* (*Oscar* and *Dunkeld*) were procured from the local market. Solvents and reagents used were of analytical-grade mostly product of Merck-Darmstadt, Germany and Riedel-de-Haën, Germany. Silica gel HF_{254}, Merck Ref. 7739 was used for TLC and most of TLC standards are product of BDH, UK. BF_3-methanol complex (Merck-Schuchardt, Germany) used for esterifications of fatty acids. The standards; methyl esters of fatty acids were attained from Supelco®, USA for GLC analysis. The non-destructive locating reagent 2,7-dichlorofluorescein (Merck, Germany) used for coloured spots of lipid compounds under ultra violet light; λ 366 nm.

TABLE-1
R_f VALUES AND PERCENTAGES OF LIPID COMPONENTS IN SEEDS OF *Oscar* AND *Dunkle* VARIETIES

No	R_f values*	Lipid compounds	[%]	
			Oscar	*Dunkeld*
1	0.87	Hydrocarbons	0.9	0.7
2	0.83	Wax esters	1.2	1.5
3	0.79	Sterol esters	1.2	1.6
4	0.75	Triacylglycerols	72.6	73.3
5	0.54	Free fatty acids	3.1	3.4
6	0.32	Fatty alcohols	4.3	4.1
7	0.28	1,3-Diacylglycerols	3.8	3.7
8	0.23	1,2-Diacylglycerols	1.5	1.2
9	0.20	Sterols	1.6	1.5
10	0.16	Monoglycerides	5.3	5.8
11	–	Polar lipids	4.5	3.2

*R_f values of lipid classes are same for both varieties. The solvent systems: *n*-hexane-diethylether-acetic acid (80/20/2), TLC adsorbent: Silicagel, Chromatogram: 0.50 mm/20 × 20 cm, Coloring reagent: 2,7-dichlorofluorescein.

Preparation of seeds: The seeds were ground and weighed quantities of both varieties of seeds were dried in an oven at 105-110 ºC for 1 h, cooled in desiccators and weighed again. This process was continued till a constant weight of dry seeds was obtained.

Extraction of lipids: The lipids from ground seeds of *Oscar* and *Dunkeld* was extracted by using chloroform-methanol (2:1) mixture as the solvent. The process was repeated with half quantity of solvent for maximum extraction of lipids and extracts were pooled together in a round bottom flask. The solvent was removed under vacuum by rotary film evaporator (Hëidolph, Germany) at 40 ºC and purified the lipids by treating with a mixture of chloroform-methanol-sodium chloride solution (0.9 %): (3: 48: 47)[9].

Identification and quantification of lipid classes: Thin layer chromatograms (20 × 20 cm) of 0.5 mm thickness were prepared by coating silicagel; HF_{254}, Merck Ref. 7739 for the separation and identification of lipids. The plates were activated by heating at 105 ºC for 1 h. The solvent system used[10] for the separation of the different classes of neutral lipids was hexane-diethyl ether-acetic acid (80:20:2 v/v). The non-destructive locating agent 2,7-dichlorofluorescein was used, which gave purple yellow coloured bands under an ultraviolet light at λ (366 nm).

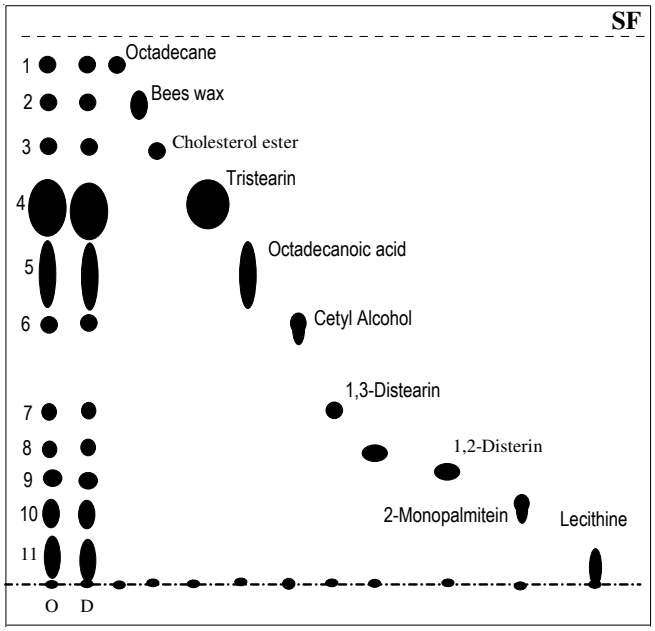
Esterification of different lipid classes: The methyl esters of total lipids and each lipid class except that of hydrocarbon, fatty alcohols and sterols were prepared by the use of boron trifluoride-methanol solution[11]. The methyl esters of the fatty acids of lipids were purified by thin layer chromatography (TLC), the solvent system *n*-hexane-diethyl ether (90:10)[12] was used prior to gas chromatography for the identification of fatty acids.

Gas chromatography: The fatty acid composition of total lipids and its different classes were determined on Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector and capillary column (25 m × 0.2 mm id) coated with polyethylene glycol. A temperature program for the column oven was 180 ºC-5ºC/min-220 ºC, while injector and detector temperatures were maintained at 230 and 250 ºC, respectively. The peaks were recorded on Shimadzu C-R4-A Chromatopac and identified by comparing their relative retention times with those of authentic standard run under the same parameters.

RESULTS AND DISCUSSION

The lipid contents (44.6, 42.2 %) in two varieties of *Canola* seeds; *Oscar* (O) and *Dunkeld* (D) respectively, were found out with polar solvent mixture; chloroform-methanol (2:1). There is also a slight difference in moisture contents in the seeds; 3.8 and 3.7 %, respectively. The lipids fractionation and characterization of fatty acids in different components have an important role in human metabolic functions. The detailed work on quantitative determinations of different lipid components and their corresponding fatty acids is carried out. The extracted lipids were fractionated by using solvent system; *n*-hexane-diethyl ether-acetic acid (80/20/2)[13]. The different lipid components *i.e.*, hydrocarbons (HCN), waxesters (WE), sterol esters (SE), triglycerides (TG), free fatty acids (FFA), diglycerides (DG), sterols (ST), monoglycerides (MG) and polar lipids (PL) were identified by comparing their R_f values with standards (Table-3). The standards; octadecane, bees wax, tristearin, octadecanoic acid, cetyl alcohol, cholesterol, 1,3-disterin, 1,2-disterin, 2-monostearin and lecithine are used for the identification of separated lipids as shown in Fig. 1. The different lipid components were extracted and recovered by rotary thin film evaporator. The lipid components are separated on polarity basis, the phospholipids are most polar then others so, they have the least R_f value. The both varieties; *Oscar* and *Dunkeld* contain higher contents of polar lipids *i.e.*; 4.5, 3.2 % respectively (Table-1). The previous work also reveals the presence of phospholipids in *Canola* seed oil[14]. The large and dense spot of triacylglycerols (Fig. 4) indicates the dominant class of extracted lipids. The percentage of total glycerides in *Canola* seeds of *Oscar* variety is; 83.2 % while *Dunkeld* variety contains; 84.0 % as shown in Table-1. Generally, the triglycerides comprises the major portion of extracted lipids in vegetable oils/fats and distributions of fatty acids in triglycerides play an important role for the characterization of different vegetable oils/fats[15]. The current work also showed higher percentage of triglycerides *i.e.*; *Oscar* (72.6 %) and *Dunkeld* (73.3 %). The current work indicates a minor difference in sterols of *Oscar* (1.6 %) and *Dunkeld* (1.5 %), while the percentages of sterol esters are; 1.2 % and 1.6 % respectively. The sterols play a vital role in human body, they may be in the form of free sterols and esterified sterols. Sterols are present in *Canola* oil as free sterols and esterified sterols in almost equal amounts[16,17]. However the work for mentioned varieties contradicts to the findings of Ackman[16] and Evershed *et al.*[17] on different varieties of *Canola* seed.

The fatty acids analysis of extracted lipids from both varieties; *Oscar* & *Dunkeld* was carried out by esterification and gas liquid chromatography (GLC). The fatty acids of total lipids and their corresponding lipid components *i.e.*, waxesters, sterols, free fatty acids, triglycerides, 1,2-diglycerides, 1,3-diglycerides, monoglycerides and polar lipids was determined by GC after transesterifications with BF_3-methanol. The analysis of total fatty acids (TFA) from whole extracted lipids reveals that both of the varieties contain lower values of total



O → *Oscar* variety, *D* → *Dunkel* variety

Fig. 1. Fractionation and identification of lipid components in seeds of *Oscar* and *Dunkle* varieties of *Canola*

saturated fatty acids (TSFA) and higher values of mono unsaturated (MUFA) and poly unsaturated fatty acids (PUFA). The oils containing higher values of MUFA are beneficial for heart and health[18]. The oleic acid ($C_{18:1}$) was found as the major fatty acid in *Oscar* and *Dunkeld* variety; 61.5 and 60.8 %, respectively. The percentage of oleic acid is comparable with previous work[19,20]. The oleic acid is beneficial to health and has cholesterol lowering effects[21,22]. The fatty acids composition of seed oils from both varieties are compared with conventional vegetable oils; sunflower, corn, soyabean, cotton seed and palm oil as shown in Table-2. Although palm, corn, soyabean, cotton and sunflower seed oils contain higher percentages of MUFA; 39, 26, 23, 19 and 18 %, respectively but they also have higher contents of TSFA; 51, 13, 15, 25 and 12 %, respectively as compare to *Oscar* & *Dunkeld* varieties of *Canola* seed.

The fractionated lipid classes *i.e.*, triglycerides, 1,2-diglycerides, 1,3-diglycerides, monoglycerides, waxesters, sterol esters and polar lipids polar lipids are comprised of fatty acids. The fatty acids are also identified in free forms (free fatty acids). The fractionated lipid classes were transesterified by BF_3-methanol reagent and the corresponding fatty acids were further analyzed and identified by GLC. The composition of fatty acids in whole extracted lipids (total lipid) and fractionated lipid classes of *Oscar* and *Dunkeld* varieties of *Canola* seeds are shown in Table-3.

The fatty acids; lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$) and arachidic acid

TABLE-2
COMPARISON OF TOTAL FATTY ACIDS (TFA) IN SEEDS OF *Oscar* and *Dunkeld* VARIETY OF *Canola* AND COMMON OIL CROPS

Fatty acids [%]*	*Canola Oscar*	*Canola Dunkel*	Sunflower[15]	Corn[15]	Soybean[15]	Cottonseed[15]	Palm[15]
Total saturated fatty acids	8.2	8.7	12	16	16	30	50
Unsaturated fatty acids	91.8	91.3	88	84	84	70	50
Monounsaturated fatty acids	61.5	60.8	19	31	23	19	39
Polyunsaturated fatty acids	30.3	30.5	69	53	61	51	11

*[15-Gunstone]

TABLE-3
PER CENT COMPOSITION OF FATTY ACIDS IN TOTAL LIPIDS AND LIPID CLASSES OF *Oscar* AND *Dunkeld* VARIETIES OF *Canola* SEEDS

Lipid class	Variety	Lauric acid ($C_{12:0}$)	Myristic acid ($C_{14:0}$)	Palmitic acid ($C_{16:0}$)	Stearic acid ($C_{18:0}$)	Oleic acid ($C_{18:1}$)	Linoleic acid ($C_{18:2}$)	Linolenic acid ($C_{18:3}$)	Arachidic acid ($C_{20:0}$)	Erucic acid ($C_{22:1}$)
TL	O	*T	T	5.8	0.9	61.5	21.3	8.9	1.5	T
	D	0.3	0.5	6.2	1.0	60.7	19.9	10.6	0.7	T
WE	O	0.5	11.0	9.8	2.0	41.8	34.8	T	ND	ND
	D	**ND	ND	17.0	3.2	50.2	26.2	3.3	ND	ND
SE	O	T	0.2	7.7	1.7	58.2	23.5	7.4	1.1	ND
	D	T	0.4	6.6	1.2	55.7	22.7	11.5	1.7	ND
TG	O	0.6	T	7.3	1.5	66.4	17.1	5.8	1.2	T
	D	1.3	1.2	13.2	1.5	71.5	8.7	2.5	ND	T
FFA	O	T	T	8.0	1.3	51.1	24.7	14.6	0.2	ND
	D	T	ND	17.8	2.9	52.9	24.2	2.1	ND	ND
1,3-DG	O	2.0	2.9	9.7	2.4	57.2	20.4	4.9	0.4	T
	D	0.5	T	14.9	1.4	58.1	21.6	3.4	ND	T
1,2-DG	O	0.7	1.6	11.1	3.6	60.4	18.9	3.3	0.3	T
	D	T	ND	16.5	3.5	56.7	22.1	1.1	ND	T
MG	O	0.8	2.2	5.6	0.5	61.3	22.1	6.2	1.2	T
	D	ND	ND	21.4	2.4	54.6	20.1	1.4	ND	T
PL	O	1.6	3.8	11.3	1.2	55.6	20.9	3.8	1.7	ND
	D	ND	ND	14.2	2.1	60.1	21.2	2.3	ND	ND

WE = Waxesters, SE = Sterol esters, TG = Triglycerides, FFA = Free fatty acids, DG = Diglycerides, ST = Sterols, MG = Monoglycerides, PL = Polar lipids; *T = Traces, **ND = Not determined; O = *Oscar*, D = *Dunkel*

($C_{20:0}$) have been found out in different lipid classes. However, variations in compositions are being observed in different lipid classes. All lipid class contains oleic acid (40.8-66.4 %) as major fatty acid and linolenic acid is the second prominent acid. The *Dunkeld* variety contains higher concentration of oleic acid (71.6 %) as compare to *Oscar* (66.4 %). The oleic acid percentage of glycerides (monoglycerides, 1,3-diglycerides, 1,2-diglycerides and triglycerides) in *Oscar* and *Dunkeld* variety are; 61.47 and 60.25 %, respectively. The oleic acid percentage of glycerides of both varieties are almost same to oleic acids in whole extracted lipids of both varieties *i.e.*, 61.5 and 60.8 %, respectively. The reason is that whole extracted lipids are mainly comprised of glycerides which is being found out by TLC. The percentages of palmitic acid are more then stearic acid in almost all the lipid classes of both varieties. The levels of $C_{16:0}$ (5.8-6.2 %) and $C_{18:0}$ (0.9-1.0 %) in the present analysis of canola seed oils are quite comparable with those for canola oil reported in the literature[23]. *Canola* oil is the preferred oil for health-conscious consumers because it is the lowest in saturated fat among the major edible oils[24]. The least contents of lauric acid, myristic acid and arachidic acid have been found out in both of the varieties. However traces of erusic acid are found in these varieties. In some of the fractionated classes these acids are present in traces and in some fractions they have not been determined as shown in Table-3.

The trend of fatty acids with respect to saturation in different lipid components of *Oscar* and *Dunkle* variety of *Canola* seed lipids is being summarized as shown in Table-4. The triacylglycerols being the major component of extracted lipids contain higher percentage of MUFA; 66.4 and 71.6 % as compare to TFA; 61.5 and 60.8 % present in seed lipids of *Oscar* and Dunkle variety, respectively. However, the total glycerides of *Oscar* and *Dunkeld* variety contain MUFA; 61.5 and 60.25 %, almost same to total monounsaturated fatty acids of both varieties. *Oscar* and *Dunkeld* variety contain lower contents of TSFA; 8.2 and 8.7 %, respectively.

The higher percentage of TSFA is present in *Dunkeld* variety as compare to TFA of this variety. The fractionated sterols are present as free sterols and esterified sterols in natural products, fatty acids profile of esterified sterols showed a slight difference of fatty acid composition. However, $C_{18:1}$ (58.2, 55.7 %) and have comparatively higher percentage of PUFA as compare to conventional vegetable oils. The work carried out on *Canola* seed oils by Gordon and Miller[24] showed higher contents of $C_{16:0}$ (17.5 %) in esterified sterols, however our findings about the selected varieties *i.e.*, *Oscar* and *Dunkeld* contradict to previous work, the current work showed $C_{16:1}$ (7.7, 6.6 %), respectively. The variations of result may be due to different genetically modified canola seeds and agronomical conditions for cultivation[25].

The polar lipid class *i.e.*, phospholipids analysis of *Oscar* and *Dunkeld* variety show higher percentage of TSFA; 19.7 and 16.3 %, respectively as compare to TSFA of total extracted lipids;8.2 and 8.7 %, respectively. While lower percentage of USFA; 80.3 and 83.7 %, MUFA; 55.6 and 60.2 % and PUFA; 24.7 and 23.5 %, respectively as compare to TFA of whole extracted lipids. The previous work[26,27] on fatty acids of Canadian variety of canola seed reveals that the fatty acid composition of the phospholipids in the low erucic acid rape seed (LEAR) variety from winter rapeseed cultivars and found TSFA: 17.77, MUFA: 47.36, PUFA: 34.85. So the current findings about fatty acids of phospholipids from selected varieties do not match with the previous work.

TABLE-4
SATURATED, MONOUNSATURATED AND POLYUNSATURATED FATTY ACIDS OF *Canola* SEED LIPIDS

Lipid class	Variety	TSFA [%]	USFA [%]	MUFA [%]	PUFA [%]
TFA	O	8.2	91.8	61.5	30.3
	D	8.7	91.3	60.8	30.5
WE	O	25.4	74.6	40.8	33.8
	D	20.3	79.7	50.2	29.5
SE	O	11.3	88.7	59.7	29.0
	D	9.8	90.2	62.5	27.7
TG	O	10.7	89.3	66.4	22.9
	D	17.2	82.8	71.6	11.2
FFA	O	9.5	90.5	51.1	39.9
	D	20.7	79.3	53.0	26.3
1,3-DG	O	17.4	82.6	57.2	25.4
	D	16.9	83.1	58.1	25.0
1,2-DG	O	17.3	82.7	60.5	22.2
	D	20.0	80.0	56.7	23.3
MG	O	9.9	90.1	61.8	28.3
	D	23.9	76.1	54.6	21.5
PL	O	19.7	80.3	55.6	24.7
	D	16.3	83.7	60.2	23.5

WE = Waxesters, SE = Sterol esters, TG = Triglycerides, FFA = Free fatty acids, DG = Diglycerides, ST = Sterols, MG = Monoglycerides, PL = Polar lipids; *T = Traces, **ND = Not determined; O = *Oscar*, D = *Dunkeld*

The work may be concluded that the selected varieties of canola seeds contain negligible contents of erucic acid in all fractionated classes of lipids as compare to traditional varieties of Mustard seed and Rapeseed of Indo-Pak region. The fractionated lipids classes of both varieties have comparable fatty acids profile and have considerable minor lipid contents *i.e.*, sterols, fatty alcohols and polar lipids. The seed oils of both varieties are a rich source of unsaturated fatty acids and essential fatty acids as compare to conventional vegetable oils. However, the contradiction of fatty acids in different lipid components might be due to different genetic modification of seeds and agronomical conditions for cultivation.

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