Pak. J. Sci. Ind. Res. 2004 ***47*** *(4) 303-308*

LIPID CLASSES OF *HORDEUM VULGARE*

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(Received December 31, 2002; accepted June 28, 2004)

Two local varieties of *Hordeum vulgare Jao 87,* and *Jao 83* were studied for their lipid classes and fatty acid composition. Total barley lipids were solvent extracted and classified into their respective classes with the help of silicic acid gel column chromatography. Each lipid class was further fractionated by thin layer chromatography, and after methylation, its fatty acid composition was determined by gas chromatography. The lipid contents were 3-3.2% of the total barley weight in both the varieties. The percentages of neutral lipids, glycolipids and phospholipids, were 70, 10 and 20%, respectively. Gas chromatography showed the presence of fatty acids from series lauric ($C_{12:0}$) to arachidic ($C_{20:0}$) while linoleic acid ($C_{18:2}$) was the principal fatty acid in all the classes with a few exceptions.

Key word: *Hordeum vulgare,* Lipids, Fatty acids, Methyl esters, GC, TLC.

Introduction

Barly, *Hordeum vulgare,* of the family *gramineae,* (locally identified as *Jao*) is one of the oldest cultivated cereals. Barley is well-known for its nutritious and medicinal properties. It has been used as food by human beings and fodder for animals. It is also extensively used in pearling, malting and brewing.

Hordeum vulgare is six-rowed type barley variety, cultivated in rainfed and irrigated areas of Punjab. About 1,40,000 tonnes of barley per year is produced in Pakistan. Barley matures quicker than wheat and can be cultivated in climatic conditions which do not permit the survival of wheat. The caloric value of barley is lower than that of wheat and corn, the most efficient method of increasing this value with least disturbances in properties is to increase the lipid content, which can be achieved by growing healthier barley grains. Agriculture Research Institute, Faisalabad, has developed some barley varieties which give healthier barley grains and produce more crop/acre. These are well-adjusted to the local climatic conditions, absorb the fertilizer easily and have better resistance for diseases.

Most of the studies on barley concern distribution of lipids and the fatty acid composition in ripening and maturing grains (De Man and Cauberghe 1988), changes in lipid quality during malting and brewing processes (Narziss and Mueck 1986; De Varies 1990) identification of phospholipids of barley (Aylward and Showler 1962), identification of neutral and polar lipids (Parsons and Price 1974; Price and Parsons 1974) and effect of barley diet on lipid metabolism and diet digestibility in model animals (De Peters and Tayler 1985; Wang *et al* 1993).

The present work was undertaken with an idea to study the lipids of barley varieties cultivated in Pakistan. The improved barley varieties *Jao 83* & *Jao 87* were collected from Agriculture Research Institute, Faisalabad, and evaluated for their individual lipid classes, i.e neutral lipids, glycolipids and phospholipids to obtain detailed information about the fractions of each of these classes.

Materials and Methods

Extraction of lipids. The dried whole grain sample of barley *(Hordeum vulgare)* was crushed in an iron pestle and morter and then converted to a uniform powder with an electric grinder. The extraction of lipids was undertaken immediately to minimize any oxidative and enzymatic activity. The ground sample (50g) was placed in 1 litre beaker and stirred with 10 volumes of chloroform: methanol: water (1.0:1.0:0.9 v/v), with a magnetic stirrer for ½ an hour, using the method of Price and Parsons (Price and Persons 1974). The mixture was transferred to 1 litre separatory funnel, swirled for further solvent action, stirred with a glass rod to enhance solvent layering and allowed to stand overnight. The lipid charged chloroform layer was then collected in a round-bottomed flask, and restored to the original volume by chloroform. This was repeated twice. The pooled lipid extracts were evaporated to dry-

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Table 1
Percentage composition and R_f values of neutral lipid classes in *Jao 87* and *Jao 83*

Lipid class	*Jao 87*	*Jao 83*	R_f Value
Hydrocarbons	4.30	4.50	0.94
Sterol esters	3.20	3.60	0.91
Triglycerides	52.40	53.20	0.66
Free fatty acids	8.10	7.30	0.43
Diglycerides	11.30	10.60	0.30
Sterols	20.20	20.70	0.25
Monoglycerides	Traces	Traces	0.10

Table 2
Percentage composition and R_f values of glycolipid classes in *Jao 87* and *Jao 83*

Lipid class	*Jao 87*	*Jao 83*	R_f Value
Monogalactosyl diglyceride	27.40	26.50	0.81
Unknown (I	18.40	17.80	0.72
Unknown (II	17.40	17.20	0.65
Digalactosyl diglyceride	36.80	38.50	0.50

ness under vacuum, weighed, and stored under nitrogen in a refrigerator for further studies.

Classification of lipids. The lipids were separated into their classes by silicic acid column chromatography according to the methods of Rouser *et al* (1967). A glass column (45cm x2.5cm) was packed with slurry of 40g of silica gel 60 (E Merck 0.06-0.20 mm) in hexane. Lipid extract (2g in 10 ml n-hexane) was absorbed on the top of the column, and neutral lipids, glycolipids and phospholipids were eluted sequentially using diethyl ether, acetone and methanol (3x200 ml each). Solvents were removed from each fraction by rotary vacuum evaporator at 35˚C. The lipids were transferred to vials and weighed.

Thin layer chromatography. The lipid classes were separated by thin layer chromatography according to the techniques of Stahl (1969). Chromatographic plates of silica gel GF254 of 0.25 mm thickness were used for qualitative analysis and 0.5 mm thickness for quantitative work. The locating reagent used was 2,7 dichloroflourescein (Raie *et al* 1989). Lipid classes were quantified gravimetrically.

Neutral lipids. Separation of neutral lipids was carried out stepwise using different solvent systems, enrichment technique and derivative formation. Neutral lipid fraction was first separated in the solvent system hexane: diethyl ether

Table 3
Percentage composition and R_f values of phospholipid classes in *Jao 87* and *Jao 83*

Lipid class	*Jao 87*	*Jao 83*	R_f Value
Diphosphatidyl glycerol	2.20	2.50	0.95
Phosphatidyl glycerol	1.30	1.60	0.89
Unknown	2.00	1.90	0.82
Phosphatidyl ethanolamine	38.30	36.70	0.70
Phosphatidyl inositol	1.80	2.10	0.54
Phosphatidyl serine	16.80	14.70	0.47
Phosphatidylcholine	22.40	24.20	0.37
Lysophosphatidylcholine	15.20	16.30	0.10

Table 4
Fatty acid composition of total lipids of *Hordeum vulgare*

Lipid class	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}
Jao 87	1.7	4.04	20.9	Tr	2.4	10.2	53.3	5.4	0.08
Jao 83	1.2	2.6	23.08	Tr	2.6	10.15	53.6	4.61	0.07

(85:15v/v), and hydrocarbons,sterol esters and triglycerides were separated. The region below triglycerides was scratched off from the plate and after enrichment was developed in the solvent system hexane: diethyl ether: acetic acid (80:20:2v/v) (Chaudri *et al* 2000). This gave a separation of free fatty acids, diglycerides, sterols and monoglycerides. These fractions were purified by preparative thin layer chromatography and their infrared spectra were taken on a Hitachi 270-30 Infrared spectrophotometer. Infrared spectra of these fractions however, indicated an intermixing of sterols and diglycerides, which was most probably due to close R_f values of these two fractions. The two fractions were therefore collectively acetylated using pyridine and acetic anhydride (Hamiltan *et al* 1972), the lipid material was kept overnight with pyridine (6 ml) and acetic anhydride (2 ml) at ambient temperature. Later refluxed for 1 h, after cooling added to it 25 ml ether and then 50 ml ice cold water, stirred with a glass rod and transferred to a separatory funnel. The ethereal layer was washed three times with cold water and dired over anhydrous Na_2SO_4. Acetyl derivatives of sterols and diglycerides had quite different R_f values in the solvent system hexane: diethylether: acetic acid (80:20:2v/v) and were separated completely, the infrared spectra of the two fraction was again taken to substantiate the complete separation. Hydrocarbons and sterols esters were further separated using hexane: toluene (70:30 v/v) as the developing solvent. All the fractions were identified by comparing their R_f values with standards. The

Table 5
Fatty acid composition in neutral lipids of *Jao 87* & *Jao 83*

Lipid class	*Jao* type	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$
Sterol ester	87	Tr	1.20	22.80	2.00	3.10	14.20	34.40	4.30	17.00	2.00
	83	0.50	1.40	24.40	1.40	4.30	13.80	40.30	3.50	10.20	0.20
Triglycerides	87	1.80	2.80	20.60	0.10	0.60	15.60	54.20	4.30	Tr	-
	83	1.70	2.90	21.20	0.20	1.20	15.20	53.40	5.20	Tr	-
Free fatty acid	87	0.50	1.30	36.00	1.10	4.00	9.30	46.30	1.40	-	-
	83	1.20	1.80	30.20	0.10	3.50	8.70	51.70	3.40	-	-
Diglycerides	87	Tr	0.80	23.40	0.50	3.20	13.90	53.70	4.50	-	-
	83	1.30	1.60	17.90	0.20	2.30	15.20	56.20	5.40	-	-

Table 6
Fatty acid composition in glycolipids of *Jao 87* & *Jao 83*

Lipid class	*Jao* type	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Monogalactosyl diglyceride	87	1.20	1.70	22.80	0.50	2.50	6.80	58.40	5.80	Tr
	83	0.80	1.50	23.60	0.40	3.50	7.60	56.80	5.70	Tr
Digalactosyl diglyceride	87	0.80	1.40	24.20	0.25	3.60	6.40	56.20	6.20	Tr
	83	0.60	1.40	22.10	0.50	3.80	8.20	57.40	5.80	Tr

presence of sterols and sterol esters was also verified by spraying the plate with saturated solution of $SbCl_3$ in chloroform which gave blue violet to red voilet color on heating in an oven at 100-120°C for 10 min.

Glycolipids. Glycolipids were separated using solvent system chlorofrom; methanol: water (75:25:4v/v) (Price and Parsons 1974). Four spots were obtained, out of which two were identified by comparing their R_f values with reference standards. Glycolipids were also confirmed by spraying oricinol in concentrated sulphuric acid, which gave blue spots.

Phospholipids. Phospholipids were separated using the solvent system chloroform: methanol:water: ammonia (65:35:4:0.2) (Price and Parsons 1974). Eight spots were obtained, out of which seven were identified by comparing their R_f values with reference standards and confirmed by spraying the plates with molybdenum blue reagent (Amer and Lester 1964), which gave blue spots. Quantitative separation of all the classes was carried out using silica gel GF254 plates of 0.5 mm thickness. Fractions so separated were quantified gravimetrically and confirmed by infrared spectroscopy.

Methylations of lipid classes. Lipids fraction were converted to their respective methyl esters according to the method of Morrison and Smith (1964). The lipid fraction was heated with BF_3 methanol (1-2 ml) reagent in a test tube with Teflon lined screw cap in a boiling water bath for the recommended time and the methyl esters formed were extracted with hexane. Methyl esters obtained were purified by preparative TLC using hexane: ether (85:15v/v) solvent system.

Gas chromatography. Purified methyl esters of the lipid fractions were analysed by gas chromatography. A Schimadzu gas chromatogram, GC 14 A, with flame ionization detector, having a glass column (1.5m x 3mm) packed with 15% diethylene glycol succinate was used. The column temperature was programmed as 150°C-300°C with a rise of 5°C to 300°C, respectively. Nitrogen was used as a carrier gas with a flow rate of 40ml/min.

Results and Discussion

Total lipid content, determined by solvent extraction, was 3.0-3.2% for both barley varieties. Column chromatography revealed three major classes, neutral lipids 70%, glycolipids 10% and phospholipids 20% (approximately). Fractionation of the neutral lipids class yielded hydrocarbons, sterol esters, triglycerides, free fatty acids, diglycerides, sterols and monoglycerides. The results show that triglycerides constitute the major fraction, comprising more than 50% of the total neutral lipid content. This is in accordance with the usual pattern of vegetable oils i.e. they contain triglycerides as the major class (Gunistone *et al* 1986). Sterols constituted the next major fraction, almost 20%, such a high percentage is

Table 7
Fatty acid composition in phospholipids of *Jao 87* & *Jao 83*

Lipid class	Jaotype	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}
Diphosphatidyl glycerol	87	Tr	3.60	46.10	-	3.00	9.20	32.80	3.20	2.10
	83	Tr	3.80	44.60	-	3.20	10.00	36.60	3.80	1.80
Phosphatidyl glycerol	87	Tr	4.10	35.10	-	3.20	11.60	39.60	2.00	4.30
	83	Tr	5.20	34.30	-	3.00	11.60	39.60	2.00	4.30
Unknown	87	14.70	6.40	27.10	-	7.10	12.90	31.70	-	-
	83	10.60	7.50	30.20	-	7.10	12.90	31.20	-	-
Phosphatidyl ethanol	87	-	87.30	4.20	-	0.30	4.30	0.80	0.70	0.40
amine	8	Tr	82.40	8.50	-	0.60	6.40	1.20	0.60	0.30
Phosphatidyl inositol	87	5.20	-	24.30	-	-	11.70	56.20	2.50	-
	83	5.00	-	26.20	-	-	10.80	56.150	1.20	0.80
Phosphatidyl serine	87	29.70	-	25.70	-	6.20	8.80	20.80	-	8.80
	83	28.30	-	24.80	-	7.10	9.40	22.80	0.80	0.80
Phosphatidyl choline	87	3.40	-	25.10	13.10	-	24.20	31.40	2.90	-
	83	4.20	-	25.60	10.20	-	22.30	34.20	3.40	-
Lysophosphatidyl choline	87	1.20	0.90	30.60	-	6.30	17.81	37.50	2.50	2.90
	83	-	1.20	28.70	-	8.50	18.50	38.40	1.20	3.50

not usual for plant extracts (Bockenogen 1968). Other fractions, i.e. hydrocarbons, sterol esters, diglycerides and free fatty acids were found in small quantities while monoglycerides were found only in traces.

It was observed that simple thin layer chromatography of the neutral lipid class did not separate the subsclasses satisfactorily using one solvent system. It was indicated by infrared spectroscopy that the separated fractions showed an intermixing of other fractions, i.e. the fraction containing sterols showed a small peak due to (>C=O) of ester at 1740cm^{-1} alongwith proper peaks of sterol, whereas the fraction containing diglycerides showed a weak peak of -OH of sterol of 3600 cm^{-1}. To obtain pure fractions, the separation was carried out in three steps using the following:

i) Different solvent systems
ii) Enrichment technique
iii) Derivative formation

In this way a better separation was achieved (Fig.1a). The fractions obtained in this way were free of any intermixing that was confirmed by infrared spectroscopy. Hydrocarbon gave the specific peaks at 2850 cm^{-1} (-CH), 1460 ($-CH_2$) and 1380 ($-CH_3$); sterol esters, mono, di and triglycerides at 1740 (>C=O); free fatty acid at 1710 and 3000 (>C=O,-OH) and sterols at 3500 (-OH). Percentage composition of neutral lipid classes and their R_f values are shown in Table 1.

Glycolipid class was separated into four fractions out of which two fractions were identified to be monogalactosyldiglycerides and digalactosyldiglycerides. The other two spots remained unidentified (Fig.1b) and Table 2.

Phospholipids were separated by thin layer chromatography into eight fractions out of which seven fractions were identified to be (1) diphosphatidylglycerol, (2) phosphatidylglycerol, (3) phosphatidyl ethanolamine, (4) phosphatidylinositol, (5) phosphatidylserine, (6) phosphatidylcholine and (7) lysophosphatidylcholine (Fig. Ic) and Table 3. Phosphatidylethanolamine was the major fraction and phosphatidyl choline was the next major fraction found, these results are similar to the usual pattern of phospholipids found in cereals (Jones and Amos 1957).

Fatty acid composition of all the classes was determined by gas chromatography. The fatty acids found in barley lipids as given in Table 4 are similar to the general pattern found in cereal lipids. For neutral lipids, composition is given in Table-5. It was observed that the fatty acids from the chain length C_{12} to C_{20} were present. The major fatty acids found were palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) which were present in substantial amounts in all the classes. Linoleic acid was present in highest percentages in all the neutral lipid classes, palmitic acid was the next major fatty acid found followed by oleic acid. Other acids, C_{12}, C_{14}, C_{20}, $C_{16:1}$, $C_{20:1}$ were detected in minor amounts except C_{20} which was present in slightly higher amounts in sterol esters, the presence of $C_{20:1}$ in barley has not been reported in the past. Fatty acid composition of glycolipids as given in Table 6, was quite simi-

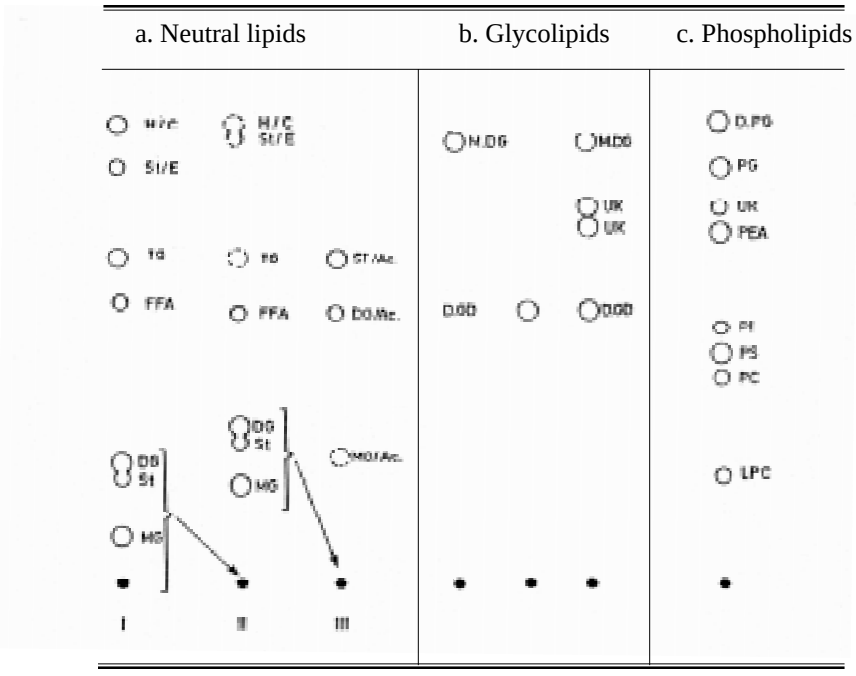

Fig 1. Thin layer chromatographic separation of (a) neutral lipids (b) glycolipids and (c) phospholipids.

a) Solvent system
i) Hexane:diethylether (85:15)
ii) Hexane:ether:acetic acid (80:20:2)

b) Solvent system
Chloroform:methanol:water (75:25:4)

c) Solvent system
Chloroform:methanol:water ammonia (65:35:4:0.2)

H/C Hydrocarbon
St/E Sterol esters
TG Triglycerides
FFA Free fatty acid
DG Diglycerides
ST Sterols
MG Monoglycerides
St/Ac Sterol acetate
DG/Ac Diglyceride acetate
MG/Ac Monoglyceride acetate

MGD Monogalactosyldiglyceride
DGD Digalactosyldiglyceride
UK - Unknown

DPG Diphosphatidylglycerol
PG Phosphatidylglycerol
PEA Phosphatidyl ethanol amine
PI Phosphatidyl inositol
PS Phosphatidyl serine
PC Phosphatidyl choline
LPC Lysophosphatidyl choline

lar to the neutral lipids. The fatty acids found were linoleic, palmitic and oleic acids in their order of abundance. Other fatty acids were found in minor amounts. The presence of fatty acids and their distribution in cereals has been reviewed by Bames (1983) from which it is evident that fatty acids of the cereal lipids are generally $C_{18:2}$, $C_{16:0}$, $C_{18:1}$, $C_{18:3}$ in order of abundance which agrees well with our results.

Linolenic acid, an essential fatty acid found to be predominant in neutral and polar lipids, makes barley a good source of essential fatty acids.γ-linoleic acid is the first intermediate in the bioconversion of linoleic to arachidonic acid, and therapeutic properties have been known for it. Arachidonic acid is of considerable importance as a precurser of prostaglandins, thromboxanes and leucotrienes. Prostaglandins have assumed increasing biological significance and they act as regulators in a wide variety of bodily functions (Wade 1991).

Although the fatty acids present in phospholipids (Table 7) were similar to those observed for neutral lipids and glycolipids but they show quite a different ratio here. Linoleic acid, which was the major fatty acid in the other two classes, was still found in large quantities in some fractions. In other fractions it was present only in minor amounts, whereas lower fatty acids (lauric and myristic) were found in large quantities in some fractions. In phosphatidylethanolamine, a major class found among the phospholipids, myristic acid (87.3 & 82.4% for *Jao 87* and *Jao 83*) was probably the only fatty acid occupying nearly all the positions, whereas all the other fatty acids were found in minor amounts. Similarly phosphatidyl-serine showed a relatively high percentage of lauric acid (29.7 & 28.3% for *Jao 87* and *Jao 83*, respectively). Fatty acid composition of phospholipids as reported by Parsons and Price (1979) contained a small percentage of lauric (Tr-1.5%)

and myristic acids (Tr-1.0%) and higher percentage of linoleic acid (45-53%) in phosphatidylserine and phosphatidylethanolamine respectively. This difference in fatty acid composition in the phospholipids of the foreign and local varieties can be related to the difference in the climatic conditions of these areas. An effect of climate, temperature and sowing time on fatty acid composition of barley grain was studied by Welch and De Man (1985). Persent research provides a basic information on the constituents of Pakistani varieties of barley and for improvement in the nutritional value of this cereal.

Acknowledgement

The barley varieties '*Jao 87* and *Jao 83*' were provided by Wheat Research Institute, PARC, Faisalabad.

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