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Intramolecular Fatty Acids Distribution in the Triglycerides of *Hordeum vulgare*

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Abstract. Triglycerides of a local variety of *Hordeum vulgare,* 'jao-87,' were separated from the lipids by column chromatography, purified on plain thin layer chromatography plates, and then fractionated by silver nitrate impregnated thin layer chromatography. Fatty acids composition of these fractions was verified by gas chromatography after their methylation. The distribution of fatty acids, attached at α, α'- and β-positions of the triglycerides and their fractions, was determined by lipolytic hydrolysis and subsequent gas chromatographic analysis of the 2-monoglycerides. The β-position was usually occupied by oleic, linoleic and linolenic acids, depending upon the comparative high percentage of the respective fatty acid in that triglyceride fraction.

Keywords: lipids, fatty acids, triglycerides, barley, *Hordeum vulgare*

Introduction

Hordeum vulgare (barley; 'vern. jao'), Family Gramineae, is a cereal of significant importance. Besides its use as food for human beings and feed for animals, it is a raw material for the malting and brewing industries. Barley contains about 3% lipids, in addition to starch, sugars, pectin, cellulose, proteins, lignin and tannins (Jones and Amos, 1957). Lipids are important nutritional components in cereal grains, as they contain higher energy than carbohydrates and proteins. They solubilize vitamins A, D, E and K, which are necessary for the proper maintenance of health and are a source of essential fatty acids, thus contributing to several metabolic functions (Gunstone *et al.,* 1986). Barley lipids are rich in linoleic acid, an essential fatty acid. γ-Linoleic acid is the first intermediate in the bioconversion of linoleic to arachidonic acid, which is known to have therapeutic properties, being a precursor of prostaglandins, thromboxanes and leukotrienes (Gunstone *et al.,* 1986).

There has been increasing interest in cereal lipids, as these are a potential source of significant amounts of dietary polyunsaturated fatty acids. Very little work has been done on the lipids of barley keeping in view these aspects. Most of the studies have been devoted to the identification and characterization of lipids, their fatty acids composition (Parsons and Price, 1974; Price and Parsons, 1974), and changes in lipids composition during malting and brewing processes (Price and Parsons, 1975; Walsh *et al*., 1965). Lipids of barley contain a number of neutral and polar constituents of which triglycerides is the major class comprising more than 50% of the total lipids content. Fatty acids composition includes palmitic, oleic and linoleic acids as the major fatty acids, whereas lauric, myristic, palmitoleic, stearic, linolenic and arachidic acids are also present, though in smaller quantities (Salma *et al.*, 2004). Knowledge of the composition and structure of triglycerides of barley is important for the understanding of its nutritional aspects,oil stability, and possible physiological effects.The fatty acids in a triglyceride molecule are distributed at α, α'- and β-positions. The present study reports for the first time the distribution pattern of fatty acids at α, α'- and β-positions in the triglycerides of barley. For this purpose, triglycerides and their fractions, obtained by argentation thin layer chromatography, were hydrolyzed selectively at α, α'-positions (Conacher *et al.*,1970; Subbaram and Youngs, 1964; Mattson and Volpenhein, 1961), whereas unhydrolized β-monoglycerides were separated by thin layer technique (Thomas *et al*., 1965). The fatty acids attached at the β-positions were identified after methylation by comparative gas chromatography. The distribution of fatty acids in the triglyceride molecules ranged from $C_{12:0}$- $C_{20:0}$ and the β-position was usually occupied by oleic and linoleic acids. The survey of literature reveals a usual distribution of oleic, linoleic and linolenic acids at the β-position in edible vegetable oils (Gunstone *et al.,* 1965; Mattson and Volpenhein, 1961).

Materials and Methods

Barley variety 'jao-87' was obtained from the Agricultural Research Institute, Faisalabad, Pakistan. Whole grains were crushed and lipids extracted in chloroform : methanol : water mixture as described by Price and Parsons (1974). Triglycerides (2 g), were separated from the total lipids by column chro-

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matography using a glass column (45 x 2.5 cm) packed with 40 g of silica gel G60 (.06-020 mesh) in hexane. The first eluate with pure hexane removed hydrocarbons; triglycerides were then eluted with 2% diethylether in hexane. Solvent was removed by distillation and fractions were checked by qualitative thin layer chromatography, using hexane : diethylether (85 : 15) as the solvent system. The eluted triglycerides were purified quantitatively using 0.75 mm thick silica gel plates with the same solvent system; 2 g lipids material yielded 1 g triglycerides.

The purified triglycerides were fractionated by by 20% $AgNO_3$-impregnated thin layer chromatography (Barrett *et al.*, 1963), using 0.25 mm thick plates. The solvent system used was benzene : diethyleither (9 : 1) and the bands were visualized under UV-lamp, after spraying the plates with 2,7-dichlorofluorescein (0.2% solution in methanol). Seven fractions were obtained, depending upon their unsaturation. The similar bands, after scraping, were combined and then extracted with chloroform. Seven fractions were obtained weighing 0.114, 0.272, 0.274, 0.200, 0.078, 0.041 and 0.012 g, respectively, from 1 g of triglycerides. The fractions were stored at 5 °C in refrigerator until further investigations.

Each triglyceride fraction (30 mg) was weighed in a 25 ml stoppered conical flask to which were added 0.5 ml di-isopropyl ether, 0.5 µl distilled water and 20 mg pancreatic lipase (Akhtar *et al.*, 1975). The reaction flask was stoppered and placed in a waterbath shaker at 50 °C for one h (Daneshrad, 1978; Luddy *et al.*, 1964). The mixture was cooled, diluted with 0.5 ml di-isopropylether and centrifuged at 2000 rpm for two min. The supernatant was separated and the solvent was removed from it under a stream of nitrogen. The hydrolyzed material contained 1-mono-and 2-monoglycerides, 1,3- and 1,2-diglycerides, free fatty acids, and unhydrolysed triglycerides, which were separated by 4.3% sodium tetraborate-impregnated thin layer chromatography (Thomas *et al.*, 1965), using solvent system benzene : diethylether : ethyl alcohol : glacial acetic acid (50 : 40 : 2 : 0.2) (Chaudri *et al.*, 1999). The band containing 2-monoglycerides was scraped off the plate and eluted with chloroform.

Pure triglycerides, fractionated triglycerides, and their respective 2-monoglycerides were converted to their methyl esters by heating with borontriflouride methanol reagent in a waterbath for one h (Raie *et al.*, 1989). Their fatty acids composition was determined by gas chromategraphy. The fatty acid methyl esters (FAME) were identified by comparison with known FAME. A Schimadzu GC 14A with flame ionization detector, having a glass column 1.5 m x 3 mm packed with 15% DEGS, was used. The column temperature was programmed at the range of 150 - 300 °C, with a rise of 5 °C/min. The injector and detector temperatures were kept at 250 and 300 °C, respectively. Nitrogen was used as the carrier gas with a flow rate of 40 ml/min.

Results and Discussion

The triglycerides separated by column chromatography were found to be 52.4% of the total lipids. These were fractionated by argentation thin layer chromatography into seven fractions as 11.2, 27.2, 26.8, 20.1,7.8, 4.1, and 1.4% of the total triglycerides contents. The fatty acids composition of whole triglycerides and its fractions, as determined by gas chromatography are shown in Table 1. The whole triglycerides fraction contained linoleic acid ($C_{18:2}$) as the major fatty acid, which was followed by palmitic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$), whereas lauric ($C_{12:0}$), myristic ($C_{14:0}$), stearic ($C_{18:0}$) and arachidic acids ($C_{20:0}$) were found in minor amounts. Palmitoleic acid ($C_{16:1}$) was also found in negligible amounts.

The $AgNO_3$ chromatography of triglycerides gave seven fractions, in accordance with the quantity of unsaturation present. Fraction-1 was rich in saturated fatty acids (89%), consisting mainly of $C_{16:0}$ (41.2%) and $C_{18:0}$ (40.8%), including minor amounts of $C_{12:0}$, $C_{14:0}$ and $C_{20:0}$. Fraction -1, therefore, was almost a saturated fraction. Fraction-2 showed a remarkably high percentage of $C_{18:1}$ (78.4%), whereas all the other fatty acids were present in minor amounts. Fraction-3 showed an increase in $C_{18:2}$ (44.2%) and a decrease in $C_{18:1}$ (27.8). Fractions-4, 5 and 6 showed a gradual increase in $C_{18:3}$, alongwith high concentrations of $C_{18:2}$ and $C_{18:1}$. The last fraction showed maximum quantities of unsaturation. Thus, the percentage of saturated fatty acids was maximum in Fraction-1, which was close to the solvent front, whereas in the following fractions this concentration decreased and that of unsaturated fatty acids increased gradually, such that the last fraction near the base-line contained maximum unsaturation. This increase in

Table 1. Fatty acids composition (%) of whole and fractionated triglycerides (TG) of *Hordeum vulgare* grains

Fraction	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Whole-TG	0.7	0.9	21.2	0.2	1.2	17.2	53.4	5.2	0.2
Fraction-1	1.3	3.9	41.2	1.5	40.8	7.6	1.9	nil	1.8
Fraction-2	2.8	3.2	1.7	1.3	2.5	78.4	7.2	1.3	1.6
Fraction-3	1.2	1.8	20.1	0.6	1.3	27.8	44.2	3.0	nil
Fraction-4	0.8	1.3	20.6	0.4	1.8	24.9	42.5	5.5	2.2
Fraction-5	0.5	0.6	19.6	nil	1.2	20.6	42.2	15.3	nil
Fraction-6	0.2	0.2	14.5	0.2	nil	16.4	44.1	24.4	nil
Fraction-7	nil	nil	10.6	nil	nil	15.8	46.7	26.8	nil

unsaturation from the solvent front towards the baseline is according to the typical pattern of $AgNO_3$ chromatography.

These result show that the proportion of unsaturated fatty acids was much higher, that is more than 75%, and that of saturated fatty acids was less than 25%. This is in accordance with the values reported for other vegetable oils (Raie *et al.*, 1992; Akhtar *et al.*, 1980; Zadernowski and Sosulski, 1978), which is characteristic for these oils.

Having found the fatty acids composition of triglycerides and their fractions, the nature of fatty acids attached at α, α'- and β -position of the triglycerides was then determined. Triglycerides and their fractions were hydrolyzed by pancreatic lipase having the capability to hydrolyze the triglycerides, preferentially at α, α'-, that is, 1,3-positions leaving 2-monoglycerides unhydrolysed. The fatty acids attached at position 2- of the monoglycerides was then determined by gas chromatography. Results of these determinations are shown in Table 2.

The recorded observations show that in the whole triglycerides fraction, only a small amount of saturated fatty acids was esterified at the 2-position. The percentages of unsaturated fatty acids, $C_{18:1}$ and $C_{18:2}$, esterified at the 2-position, were considerably higher. This indicates that the saturated fatty acids were preferentially esterified the at 1- and 3-positions and the unsaturated acids at the 2-position of the triglycerides.

It is also evident from these observations that concentration of saturated fatty acids was maximum in Fraction-1, which decreased, in the subsequent fractions. The percentage of unsaturated fatty acids, which was relatively low in Fraction-1 increased in Fraction-2, having the concentration of $C_{18:1}$ at its maximum level (53.2%). From Fraction-3 onwards to the next fractions, the concentration of $C_{18:1}$ gradually decreased alongwith a relevant increase of $C_{18:2}$, which reached its maximum value in the last fraction, Fraction-6. These results indicate that 2-position was usually occupied by unsaturated fatty acids, especially, $C_{18:1}$ and $C_{18:2}$. Similar results were obtained by Mattson and Volpenhein (1961) in their studies on plant lipids.

Table 2. Fatty acids composition (%) of 2-monoglycerides of *Hordeum vulgare* grains fractionated from whole triglycerides (TG)

Fraction	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Fraction-1	7.4	17.3	19.6	2.0	8.4	36.7	2.1	2.7	3.2
Fraction-2	1.6	1.3	14.6	2.6	9.6	53.2	8.7	3.2	4.3
Fraction-3	1.8	1.7	7.6	1.3	1.8	44.5	36.0	3.5	1.2
Fraction-4	1.5	2.4	9.5	3.0	4.8	31.4	42.7	2.3	1.3
Fraction-5	2.8	9.8	12.4	2.7	nil	24.4	39.9	6.4	nil
Fraction-6	1.2	5.8	8.6	2.8	nil	26.4	43.5	9.5	1.5
Whole-TG	3.2	2.9	11.8	0.8	2.6	34.2	37.4	5.7	0.5

The low concentration of saturated fatty acids and the high concentrations of unsaturated fatty acids at the 2-position in the monoglycerides obtained from triglycerides and their various fractions indicated that saturated fatty acids were preferentially esterified at 1- and 3-positions, and the unsaturated falty acids at 2-position of triglycerides. This relates to the general distribution pattern of fatty acids reported for other vegetable oils (Chaudri *et al*., 1999; Gunstone *et al.*, 1986).

The low percentage of saturated fatty acids and high percentage of unsaturated fatty acids at 2-position of the triglycerides and their fractions might be due to the possibility that high concentrations of saturated fatty acids at 1- and 3- positions force the unsaturated fatty acids towards the 2-position. It is, therefore, concluded that the distribution of fatty acids in the triglycerides of barley follows the 1,3-random, 2-random distribution pattern. this is in accordance with the suggestions made by Coleman *et al*. (1963), Coleman and Fulton (1961), Vander Wall (1960). This pattern is characteristic of vegetable oils, with a few exceptions. It could be argued on the basis of these results that both saturated and monounsaturated fatty acids were esterified at 2-position in the triglycerides of Fraction-1. The percentage of $C_{18:1}$ at the 2-position increased in Fraction-2. In the subsequent fractions, $C_{18:2}$ started sharing almost equally the 2-position with $C_{18:1}$, rather $C_{18:2}$ taking more share. It appears that $C_{18:1}$ and $C_{18:2}$ forced all the other fatty acids, saturated and unsaturated, towards 1- and 3- positions of the triglyecride moleules. This is in accordance with the Gunstone's distribution theory for fats. Unsaturated C_{18} fatty acids are acylated at 2-position, proportional to their total concentration in oil or fat, which also seems true in the case of present studies.

Keeping in view these results, the distribution pattern of fatty acids in different triglycerides fractions can be predicted. The abbreviations used in the subsequent discussion are given below:

S = saturated fatty acids ($C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20.0}$)
O = monounsaturated fatty acids ($C_{16:1}$, $C_{18:1}$)
L = diunsaturated fatty acids ($C_{18:2}$)
Le = triunsaturated fatty acids ($C_{18:3}$)

The structures of all the fractions can thus be predicted as follows:

Fraction-1 = close to the solvent front on TLC plate, least unsaturated or almost saturated, combinations may be SSS, SSO, SOS;

Fraction-2 = SOO, OSO, OOO, SOS, SSO, OOL;
Fraction-3 = OOO,SLO, OLO, OLL, SOL, SLL, OLS, LLL;
Fraction-4 = LOS, SLO,OLLe, OLL, LOL;
Fraction-5 = OLLe, OLeL, SLLe, LOLe;
Fraction-6 = LeOL, OLLe; SLeO, OLeL, LLL, LOL, OLO, OLS, LOS.

If separate symbols were used for each fatty acid, i.e., Lau, M, P, P O, O, L, Le, S, A, etc., a vast number of possible combinations can be arranged.

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